MICROBIOLOGY

AND INFECTION CONTROL FOR HEALTH PROFESSIONALS

GARY LEE AND PENNY BISHOP

5TH EDITION

ALWAYS LEARNING

PEARSON

MICROBIOLOGY

AND INFECTION CONTROL FOR HEALTH PROFESSIONALS

I dedicate this book to my father and friend Phillip
Gary Lee

For my daughters Karin, Christine and Genevieve
Penny Bishop

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CONTENTS

LISTS

LIST OF CASE HISTORIES

LIST OF SPOTLIGHT ON BOXES

PREFACE

The first edition of *Microbiology and Infection Control for Health Professionals* was written in response to the need for a text suitable for use in degree programs in nursing and the health sciences. Since then, the text has been widely adopted in medical and health science courses in universities throughout Australia and New Zealand and is found in many clinical and professional libraries. The first edition of the book received an award for Excellence in Educational Publishing in 1997 as the best Australian tertiary textbook, and the second edition received the same award in 2002. The authors were also the recipients of the 1998 Excellence in Teaching Award from the Australian Society for Microbiology. We have been gratified by the continued response of students and our colleagues in academic and clinical areas of microbiology and we are pleased to be able to present a revised and updated text for this fifth edition.

The preparation of a new edition involves a complete review of all chapters in the book in order to ensure that the information about infectious diseases and infection control is as current as possible. Some of the core material does not need to be changed, but when new diseases have been identified, or the prevalence of existing diseases has altered, or when the principles of patient management have changed, the information needs to be updated.

The role of the health professional in today's society is complex, and the level of knowledge and skill required to work effectively is very high. Changing patterns of healthcare, new technologies, the emergence of new pathogens and increasing concerns about the incidence of hospital-acquired infections, all highlight the need for health professionals to have a thorough understanding of the microorganisms responsible for infectious diseases, a knowledge of the way they are spread, and the current methods for their prevention and control.

The incidence and spread of infectious diseases in different parts of Australia are affected by socioeconomic conditions as well as geographical factors. There is a wide variation in climatic conditions, in the distribution of insect vectors and also in people's access to health services. Because Australia is geographically isolated from the rest of the world, it is free of some of the diseases that are prevalent in other countries. However, world travel creates opportunities for these diseases to spread to Australia. There is thus a risk of the global spread of new and recurrent diseases. In this text there is a comprehensive discussion of emerging threats to the human population, the appearance of new and old diseases such as influenza, tuberculosis and mosquito-borne diseases, and the increasing problem of the development of antibiotic-resistant bacteria.

In this edition, we maintain our approach of presenting a comprehensive text on the subject of microbiology and infection control, and, as before, there is an emphasis on Australian data throughout the book.

Microbiology is a field that is continually and rapidly changing as a result of new research, the discovery of new diseases and pathogens, and changes in clinical practice. This edition again represents a comprehensive analysis of the current literature in the fields of medical microbiology and infection control. Chapter 1 presents an updated history of medical microbiology and a global perspective on the occurrence of infectious diseases in the world today. The chapter on diagnostic techniques and point of care diagnosis has been updated, and the section on immunology substantially revised. Unit Three, which deals with prevention and control of infectious diseases, has been revised to include current guidelines and to highlight the importance of the increase in antibiotic-resistant organisms.

We have an increased emphasis on new and emerging diseases, important public health issues and the impact of globalisation on the spread of disease. The incidence rates, methods of diagnosis, treatment and prevention of specific diseases reflect current research and clinical practices. Health professionals need a thorough understanding of microbiology in order to provide safe, effective healthcare, and this book provides current information with applications to clinical situations wherever possible. The central theme throughout the book is the relevance of microbiology to patient care.

The book is designed to enable information to be accessed in a number of ways. For students, it can be used at different levels depending on their science background and the number of hours of microbiology in their course. The content has been arranged so that educators can use the book according to the way they prefer to teach the course. For example, the major groups of microorganisms could be covered, followed by applications to transmission and control. Alternatively, the nature of infectious diseases could be covered using the 'body systems' approach and then relating it to transmission and prevention. The content of the book is selected specifically for health professionals, and many of the chapters can be used for self-directed learning activities.

There are 21 chapters, divided into four units. Chapter 1, 'The invisible world', is designed to introduce students to the importance of microbiology and presents an overview of the issues confronting the health professional. It contains the historical background to our current knowledge of medical microbiology and a discussion of the impact of new and emerging diseases. The other chapters in the first unit contain an overview of microbial metabolism and general information about the different types of microorganisms (bacteria, viruses, fungi and parasites) and the types of diseases they cause.

The second unit deals with host–microbe relationships. A description of types of infection and disease is followed by a discussion of the ways in which microbes gain access to, and exit from, the human body. General principles of epidemiology and the value of epidemiological studies are presented, with an increased emphasis on the value of surveillance and the need for evidence-based practice. The balance between pathogenic mechanisms and host defence mechanisms is discussed. Chapter 9 deals with the non-specific and specific immune defences of the human body, as well as other immunological reactions. Special emphasis is placed on the increasing occurrence of opportunistic infections in immunodeficient patients and the roles of the immune system in both preventing infection and contributing to certain disease conditions.

Unit Three addresses the methods of disease prevention and control, with a special emphasis on guidelines recommended for use in Australia and New Zealand. It covers principles of sterilisation and disinfection and provides information on currently recommended procedures. Antimicrobial agents and their use are described, and the problems associated with the development of resistant organisms are discussed. The chapter on healthcare-associated infections details the incidence of infection in Australian hospitals, describes methods of infection control, and refers to the current guidelines for the control of transmission of infectious diseases.

A unique feature of this unit is Chapter 14, 'Issues in Public Health', which addresses topics of particular interest to Australian students in the health professions. These include notifiable diseases, community health services, primary healthcare, Australian immunisation schedules, infection control in childcare centres, Aboriginal health and infectious diseases in remote areas of Australia. There is also a description of the health scene in New Zealand, including a comparison of the health of different ethnic groups.

Unit Four is intended as a reference section for students and scholars of medical microbiology and uses the 'body systems' approach to give a detailed description of individual diseases. It includes a chapter on methods of specimen collection and techniques for identification of particular pathogens. The major diseases that affect each body system are described, together with relevant data, where appropriate, on their incidence in the world and Australia, diagnosis, treatment and prevention.

The fifth edition of *Microbiology and Infection Control for Health Professionals* is intended to continue to provide a comprehensive guide to microbiology, infectious diseases and infection control, suitable for students in health professions, clinicians, educators and scholars of microbiology. Wherever appropriate, the emphasis is placed on Australian data, the incidence of diseases in Australia, and CDNA guidelines for infection control practices.

Features

The major pedagogical features of the book have been retained. The Spotlight boxes found throughout the text deal with incidents or events that are of general interest. The number of case histories has been expanded, with questions designed to test student understanding.

Each chapter has an introductory list of focus questions, a chapter summary and questions at two levels: revision and 'test your understanding'. Each chapter ends with a list of material for further reading.

Where relevant, website addresses have been included to enable students to access the latest information for themselves.

The book contains a comprehensive glossary and is fully indexed. Extensive use is made of diagrams and colour figures to illustrate the text.

We hope that students and clinicians will continue to find this book a valuable addition to their professional libraries.

Penny Bishop

Gary Lee

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ABOUT THE AUTHORS

Penny Bishop BSc (Hons), MSc, PhD, MASM

Dr Penny Bishop was a Senior Lecturer in the Department of Clinical Nursing in the Faculty of Nursing at the University of Sydney for 20 years, where she was responsible for the development and integration of science courses into the undergraduate nursing curriculum. Her main area of teaching was microbiology and infection control in the pre- and post-registration program for nursing students, and also in the Master of Nursing program. She is currently an Honorary Associate in the Faculty of Medicine at Sydney University.

Dr Bishop graduated with Honours in Biochemistry from the University of Sydney and obtained her MSc for research at the Children's Medical Research Foundation, Sydney. She was awarded her PhD from the University of Sydney for studies on the biochemical properties of bacterial cell membranes and has a number of research publications in microbiology and molecular biology to her credit.

Dr Bishop spent five years in research laboratories overseas, first in the Department of Bacteriology and Immunology at Harvard Medical School, and then at the Karolinska Institute in Stockholm.

Prior to joining the Faculty of Nursing she taught across a wide range of courses in the faculties of Science and Medicine at the University of Sydney and the University of New South Wales, and spent six years doing research in the Chemistry school at Macquarie University.

Penny Bishop is a member of the Australian Society for Microbiology (MASM), the Australian Society for Biochemistry and Molecular Biology (ASBMB) and the Australian Infection Control Association (AICA). Her interests include the use of techniques of molecular biology to study the epidemiology of hospital-acquired infections. In 1998 she was the recipient of the Excellence in Teaching Award from the Australian Society for Microbiology (ASM) with Dr Gary Lee. She also served for three years as editor of *Microbiology Australia*, the journal of the Australian Society for Microbiology.

Gary Lee BSc (Hons), MSc, PhD, MASM

Gary Lee is an Honorary Senior Lecturer in the Sydney Medical School, University of Sydney. In over 30 years in the tertiary education sector Gary has taught microbiology, immunology, physiology and pathophysiology at undergraduate and postgraduate level in a broad range of health professional courses including nursing, medical radiation technology, occupational therapy and physiotherapy. He also had a key role in the development and delivery of off-shore, allied health degree courses (undergraduate and postgraduate) in Singapore. Gary Lee had several years experience in diagnostic pathology laboratories in microbiology, haematology, blood banking and serology.

Gary Lee received his BSc in microbiology and immunology from the University of New South Wales, and obtained a PhD for his work on infection and immunity to *Salmonella typhimurium*. He completed an MBA at the University of Central Queensland.

Gary Lee is a member of the Australian Society for Microbiology. His research interests focus on the epidemiology of community-acquired MRSA, infection control and antibiotic usage in hospitals, and evidence-based education. Dr Lee led the development of a generic Health Sciences degree program that was first offered in the University of Sydney in 2002, and which is now a foundation program for graduate entry courses in the University. From 1995–1997, Dr Lee was Associate Dean and Chair of the Faculty of Health Sciences Undergraduate Studies Committee, and was Head of the School of Biomedical Sciences from 1998 to 2001. In 1998 he received, in conjunction with Dr Penny Bishop, the Australian Society for Microbiology Excellence in Teaching Award.

UNIT

I

Fundamental microbiology

CHAPTER

1

The invisible world

CHAPTER FOCUS

- Why is the study of microbiology essential for the health professional?
- How are microorganisms classified?
- Why are microorganisms important in the environment?
- What are the major groups of microorganisms that cause disease in humans?
- What have been the major discoveries in microbiology in the last 100 years?
- How have these discoveries contributed to the prevention and treatment of infectious diseases?
- What are the major challenges posed by emerging infectious diseases in the world today?
- How will infectious diseases impact on our health in the future?

INTRODUCTION: THE MICROBIAL WORLD

Each day humans are exposed to countless numbers of microorganisms (microbes)—tiny, invisible organisms that are present everywhere in our environment, in our homes, on our bodies, on the things we touch and on the food we eat.

These microorganisms affect every part of our daily lives: some play an essential role in the environment; some are used to produce food; some are used in industry; some live in or on the human body; and some cause disease (**pathogens**). The interaction between humans and microbes determines the state of our health and the quality of the environment in which we live. For this reason an understanding of the microbial world is essential for every health professional.

The science of microbiology is the study of microorganisms, their properties, classification, growth requirements, method of reproduction and distribution in nature. Microorganisms are living organisms too small to be seen with the naked eye. They include viruses, bacteria, protozoa, algae and fungi, as well as some of the microscopic stages in the life cycles of parasites such as worms (helminths).

The smallest viruses are about 20 nm (a nanometre is equal to one-billionth of a metre, or 10^{-9} m) or 0.2 μ in size. (A micron, μ, is one-thousandth of a metre, or 10^{-6} m.) Bacteria range in size from about 1 μ in length up to several microns. Protozoa and fungal spores are larger—50 to 200 μ—but are still invisible to the naked eye, which has a resolution of about 0.2 mm (200 μ). Bacteria are visible under a light microscope, but viruses require the high resolution of an electron microscope to be seen.

THE IMPORTANCE OF MICROORGANISMS IN OUR ENVIRONMENT

In nature, microorganisms carry out a number of important functions. They contribute to the decomposition of organic matter and the recycling of nutrients that help to maintain the balance of chemicals in the soil. Special nitrogen-fixing bacteria live symbiotically with certain plants, absorbing nitrogen from the air and converting it into compounds that can be used by the plant for growth. Some algae carry out photosynthesis, using energy from the sun to convert carbon dioxide in the air into carbohydrates. Marine microorganisms form the basis of the food chain in lakes, rivers and oceans. One of the most important groups of microorganisms are the **normal flora**, the microbes that reside on the human body in various symbiotic relationships (see Chapter 7, page 138).

As well as their role in nature, microorganisms are of benefit to humans in many other ways. Edible fungi such as mushrooms provide food; yeasts are used to make bread, wine and beer; bacteria are used in the production of cheese and yoghurt. Other microorganisms are used in the manufacture of certain pharmaceuticals and drugs, and in various industrial processes. They are a valuable tool for scientific research, and their use in laboratory experiments has been fundamental to many of the breakthrough discoveries made in biochemistry and molecular biology. These include the understanding of metabolic pathways and the discovery of the genetic code.

The science of microbiology includes the study of the relationship between microorganisms and their environment (which includes the human host). In many circumstances this interaction is beneficial; however, if the balance between microbe and nature is disturbed, a situation can arise where microbial growth becomes uncontrolled and the microorganisms cause disease. Some microorganisms cause disease in plants, while others infect animals and/or humans. Very few of the thousands of different microorganisms in our environment actually cause disease although, in some parts of the world, infectious diseases are still a major cause of illness and death.

MICROORGANISMS AS PATHOGENS

From earliest times, infectious diseases such as smallpox, plague (the 'Black Death'), tuberculosis ('consumption'), cholera and malaria, and epidemics such as the 'Spanish' flu of 1918, have posed a threat to human life and created fear and superstition. This was mainly because people did not know what caused the diseases or how they were spread. A century ago, more than 60 per cent of all deaths were due to infectious diseases. The discovery of antibiotics and the successful development of vaccines led many people to believe that infectious diseases were a thing of the past. In Western countries, less than 5 per cent of deaths are now directly attributable to infectious disease; however, similar advances have not occurred in developing nations, where millions of people are affected: around 60 per cent of deaths are from infectious diseases, many of which could be prevented by better health programs.

Throughout the world, we are now faced with the emergence of new diseases, the recurrence of 'old' diseases and an increase in the numbers of drug-resistant microorganisms. Worldwide, nearly 15 million (25 per cent) of the estimated 57 million deaths each year are caused by infectious diseases.

Infectious agents are also being implicated as important determinants, not just complications, of chronic diseases. For example, hepatitis B infection can explain the occurrence of chronic liver disease; and the discovery that *Helicobacter pylori* infection induces gastric inflammation, leading to ulcers, has transformed traditional thinking that this was a chronic condition linked to stress. (This subject is addressed more fully in Chapter 7.)

In this chapter we will introduce the student to the importance of microbiology and discuss the scientific discoveries that have disproved the old myths and superstitions surrounding infectious diseases. Most of the microorganisms (pathogens) that are the cause of infectious diseases have now been identified. Their method of transmission is known and it has been possible to devise strategies for the prevention and control of infections. However, over the last 20 years a number of new infectious diseases have emerged and old ones have re-emerged. This has serious implications for human health in the future and is examined later in this chapter.

The main focus of this book is on medical microbiology, the study of the microbes that are responsible for infectious diseases, the nature of the diseases they produce in humans and the way in which they are spread. Infectious diseases have a great impact on human lives, and knowledge of this topic is essential for all health professionals.

The public health issues that are of particular importance in Australia are discussed in Chapter 14.

THE NATURE OF LIVING ORGANISMS

In the 18th century, as scientists began to study the complex world of living organisms, it became necessary to develop a system of classification. The Swedish botanist Carl Linnaeus devised the earliest classification scheme that put all living organisms into two major groups—animals and plants. With the development of the microscope the world of cells and cell structure was revealed, and it became accepted that all living matter is made up of cells. It also became clear that some organisms consist of only one cell, whereas others are made up of many cells. Complex, higher organisms are made up of many parts, or organs, that are differentiated into various types of cells according to the function they perform. In the 19th century, improvements in microscopes enabled scientists to study the 'invisible' organisms—that is, those too small to be seen with the naked eye—and to show that some of these microscopic creatures were responsible for the infectious diseases of higher organisms.

The distinctive properties of each group of organisms are discussed in later chapters, together with descriptions of the types of diseases they cause.

CLASSIFICATION OF LIVING ORGANISMS

The classification of living organisms is a kind of shorthand used to describe groups of organisms which:

- have a similar genetic composition
- have characteristics in common
- have developed in similar ways
- have certain growth requirements, or
- are found in certain locations.

Early scientific observations of living organisms were confined to a description of their **morphology** (external appearance). In the 20th century, new techniques and instruments enabled scientists to study the different life forms in more detail. As more microorganisms were discovered, and more was learnt about their structure, habitats and nutritional requirements, further refinements in classification were made.

The three domains

Recent developments in molecular biology have allowed scientists to compare the nucleic acid composition (DNA and RNA) in different organisms. In 1978, based on ribosomal RNA studies, Carl Woese proposed that all organisms should be grouped in one of three domains. The domain **Archaea** contains the archaebacteria, the most primitive of all organisms. A second domain, the **Bacteria**, contains the eubacteria (true bacteria). A third domain, the **Eucarya**, contains Protista, Fungi, Plants and Animals (see Figure 1.1). It may be that, in the future, further revisions of the system of classification will be required.

Naming of microorganisms

Carl Linnaeus devised a careful naming system for each species within the kingdoms, a system still widely used today. He proposed that each group of organisms should be placed into a **genus** (plural: genera), the name of which is always spelt with a capital letter. Within the genera there are different **species**, which have discrete but related characteristics. A species can be further divided into different **strains** that exhibit specific properties. For example, under the Linnaean system a bacterium would be named *Escherichia* (genus) *coli* (species), O157 (strain).

The names assigned to microorganisms do not follow any system; they may reflect the place where the organism is commonly found (e.g. coli = of the colon), the name of the researcher who first described the organism (e.g. Escherich) or sometimes the disease the bacterium causes (e.g. tetani) or its shape (e.g. bacillus, meaning rod-shaped).

A further refinement in the classification of microorganisms has come with the development of serological techniques that enable the differentiation between strains of organisms on the basis of their antigenic properties or **serotypes** (i.e. the unique structure of the chemical molecules on the outside of the cell). The development of nucleic acid probes (see Chapter 15) has enabled some organisms (viruses, in particular) to be classified and named according to their type of nucleic acid (RNA or DNA).

In the higher kingdoms of plants and animals, the genera are grouped into families, orders, classes and phyla. These are required to classify the huge diversity of plants and animals, but are of less importance in the microbial world.

PROCARYOTIC AND EUCARYOTIC CELLS

The basic structural and functional unit of life is the cell. Cells contain a complex mixture of chemicals, consisting of proteins, lipids, nucleic acids and carbohydrates suspended in a watery fluid called **cytoplasm**, which is enclosed by the plasma membrane and, in some cells, by an outer cell wall. The development of the electron microscope enabled the fine structure of cells to be examined in detail and revealed two distinct types of cells—**procaryotic** and **eucaryotic**—which are distinguished on the basis of their subcellular

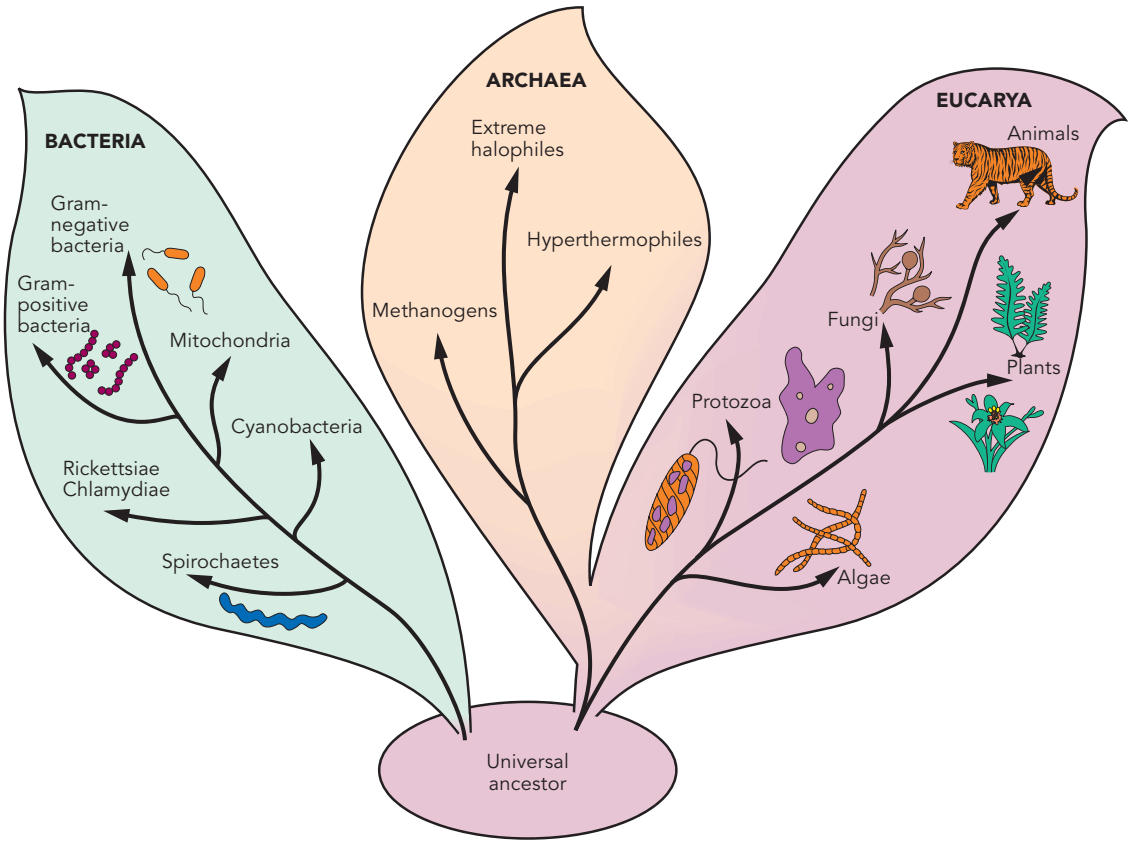


FIGURE 1.1

The three-domain system of classification

structures and function. This is a useful distinction for the medical microbiologist and, for the purposes of this text, we shall continue to refer to them in this way.

Bacteria are procaryotes and all other living organisms are eucaryotes. Eucaryotic cells have structures that are not found in procaryotes, and these differences can be exploited in the development of drugs to treat microbial infections (see Chapter 12). All bacteria of medical importance are classified as true bacteria. The eucaryotic microorganisms of medical significance include some of the protozoa, fungi and helminths (worms). Viruses are not capable of independent reproduction so are not included in this system of classification. Grouping microorganisms in this way provides a useful tool for understanding their different properties and assists in devising methods for their control.

Table 1.1 summarises the principal characteristics of procaryotic and eucaryotic cells.

VIRUSES

According to the classification systems described above, viruses are not considered to be living cells because they are unable to reproduce or carry out any metabolic reactions on their own. However, viruses are very important pathogens and, together with bacteria, are the cause of many infectious diseases (see Chapter 5).

DISCOVERY OF THE CAUSES OF INFECTIOUS DISEASES

Our understanding of the relationship between microorganisms and infectious diseases dates back only to the late 19th century. Before then, people viewed infectious diseases and epidemics with fear and superstition. It was believed that infectious diseases were due to exposure to bad air or 'miasmas'. From the 15th century onwards, voyages of exploration around the world and the development of trade routes were responsible for carrying not only goods, but also diseases from one part of the world to another. Travellers from the East brought diseases such as plague and cholera to Europe, where they were easily spread in the crowded, unsanitary and unhygienic conditions that existed in many cities

Various practices were followed to try to prevent the spread of diseases; for example, in Italy travellers were isolated for 40 days to prevent the spread of plague. (The Italian word for 40, *quaranta*, is the origin of our word 'quarantine'.) However, these measures were not particularly effective because, although people realised disease could be spread from person to person, they had no real knowledge of its cause.

Our current understanding of the nature of infection and transmission of infectious diseases is the outcome of the painstaking work of a number of scientists and doctors,

TABLE 1.1 Characteristics of procaryotic and eucaryotic cells

CHARACTERISTIC	PROCARYOTIC	EUCARYOTIC
Size	0.4 to 2.0 μ diameter	5 to 100 μ diameter
Cell wall	Always present	Bounded by wall or membrane
Nucleus	No defined region	Contains DNA, bounded by double membrane
Genetic material	One circular chromosome of double-stranded DNA; plasmids in some cells	Double strand of DNA associated with proteins (histones) to form pairs (of chromosomes); DNA also present in mitochondria and chloroplasts
Membrane-bound subcellular organelles	Absent	Present—includes mitochondria, chloroplasts, Golgi apparatus, lysosomes, endoplasmic reticulum
Motility	Some are motile, use flagella	Some are motile, e.g. protozoa; most are non-motile
Plasma membrane	Present—selectively permeable	Present—contains sterols; selectively permeable
Cytoplasm	Contains all enzymes and chemicals; no defined structures	Has cytoskeleton, exhibits streaming (moving of cytoplasmic fluid)
Endoplasmic reticulum	Absent	Present
Ribosomes	Present, smaller 70S	Present, larger 80S
Reproduction	Mainly asexual binary fission	Sexual or asexual

which led to significant discoveries. Some of these researchers are listed in Table 1.2.

'Seeing is believing'

Until the 17th century there were no instruments that could magnify sufficiently to make microorganisms visible to the human eye. The first person to use lenses for magnification was the Dutchman Antony van Leeuwenhoek, who observed microorganisms (or 'animalcules', as he called them) suspended in a drop of pond water in front of a carefully blown glass hand lens (see Figure 1.2). His paper to The Royal Society in 1673 showed pictures of small creatures which look very like the familiar bacteria observed in laboratories today. In 1665, Robert Hooke had built a simple compound microscope and was able to observe the structure of a thin layer of cork. He used the term 'cells' to describe the orderly arrangement of units that he saw. However, these researchers' observations were largely forgotten for almost 200 years.

Spontaneous generation versus biogenesis

In the second half of the 19th century there was considerable discussion among scientists as to the origin of living matter. One theory was that living cells could arise from non-living matter, the theory of **spontaneous generation**. This theory arose from the observation that food left out in the air was soon found to contain millions of microscopic organisms. Other scientists contended that life could only arise from pre-existing living cells, the theory of **biogenesis**.

The arguments for each point of view became quite heated, but the debate was finally settled by Louis Pasteur who, with a series of ingenious experiments, demonstrated the presence of microorganisms in air and in liquids. Figure 1.3 describes Pasteur's experiment.

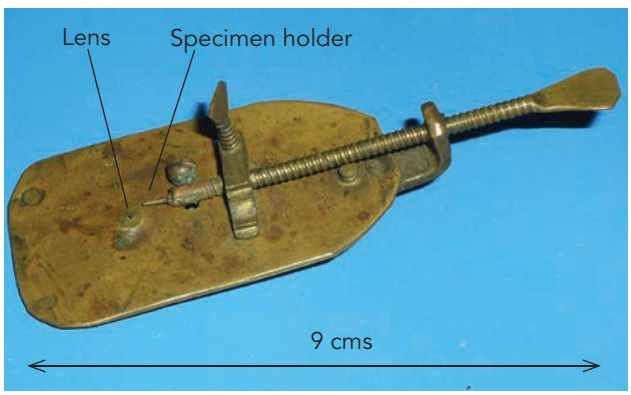


FIGURE 1.2

One of Leeuwenhoek's microscopes from the University museum, University of Utrecht, Netherlands

The tiny (9 cm) microscope contains a finely ground aspherical lens about 2 mm in diameter. The specimen is mounted on the tip of the holder and held up to the light. The screws are used to adjust the position of the specimen and to focus. Magnification is 295x.

Source: Dr Penny Bishop.

Pasteur demonstrated that microorganisms are present in and on all kinds of non-living matter—solids, liquids and air. He went on to show that organisms can be destroyed by heat, and he developed methods to prevent microbes from gaining access to solutions that have been heat-treated. The knowledge that there are microorganisms everywhere in the environment and that, given the right conditions of temperature, water and nutrients, they can reproduce and multiply is of great significance medically, scientifically and in everyday life.

During the second half of the 19th century, a number of scientists and doctors were working on a variety of medical

TABLE 1.2 Scientific discoveries

YEAR	SCIENTIST	DISCOVERY
1665	Robert Hooke	Light microscope
1673	Anton van Leeuwenhoek	Lens-observed microbes
1796	Edward Jenner	Smallpox vaccine
1840	Ignaz Semmelweis	Childbed fever
1854	John Snow	Epidemiology of cholera
1857	Louis Pasteur	Fermentation
1861	Louis Pasteur	Disproved spontaneous generation
1864	Louis Pasteur	Pasteurisation
1867	Joseph Lister	Use of disinfectants in surgery
1876	Robert Koch	Discovery of anthrax; Germ theory of disease
1880	Louis Pasteur	Immunisation for cholera
1884	Elie Metchnikoff	Phagocytosis
1884	Hans Christian Gram	Gram staining method
1888–1910	Various scientists	Bacteria responsible for most diseases isolated and characterised
1898	Ross, Grassi	Malaria: mosquitoes
1900	Walter Reed	Yellow fever: mosquitoes
1910	Paul Ehrlich	Chemotherapeutic drugs
1928	Fleming, Chain, Florey	Penicillin
1953	Watson and Crick	Structure of DNA

and scientific problems in different parts of Europe. They each contributed ideas and observations, which together formed a picture of how microorganisms survive, reproduce and cause disease. Some of the discoveries they made were in response to economic pressures and had a commercial basis. For example, in France, wine merchants were concerned because the wine they made sometimes turned sour when stored or shipped. Pasteur, from his work on the growth of microorganisms in liquid cultures, showed that the conversion of sugar to alcohol in the absence of air (a process called **fermentation**) was due to the activity of small organisms called yeasts. The presence of other organisms (bacteria) caused further reactions that produced acid and led to souring of the wine. He showed that the troublesome bacteria could be destroyed by heating the wine to 56°C for 30 minutes (a procedure now called **pasteurisation**), thus preventing the souring of the wine while still retaining its quality. This method was later extended to treat milk in order to remove undesirable organisms, some of which caused souring of the milk and others that were the cause of diseases such as tuberculosis. Pasteurisation is still widely used to preserve milk products and destroy any pathogens that may be present.

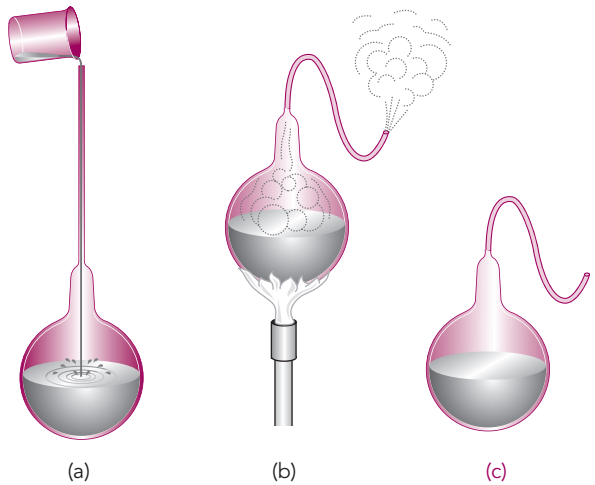


FIGURE 1.3

Pasteur's experiment

In order to disprove the theory of spontaneous generation, Pasteur devised the following experiment. He poured nutrient broth into a flask with a long thin neck, then heated the neck and bent it into an S-curve, a 'swan-necked' flask. He then boiled the broth in the flask, thus killing all the bacteria present in the flask, and expelling all the air. On cooling, air re-entered the flask, but the bacteria were trapped in the S-bend in the tube. Even after a long period of time, no growth of microorganisms occurred in the flask, showing that new cells could only arise from pre-existing living cells.

The observation that microbial growth was linked to food spoilage was important as it provided a basis for the idea that microorganisms might also be responsible for infectious diseases.

Transmission of infection

In the 19th century people had little, if any, knowledge about the cause of infections or how they were spread. In Europe, the Hungarian physician Ignaz Semmelweis was concerned at the high incidence of **puerperal** (**childbed**) **fever**. He thought it might be due to the transfer of an 'infectious agent' from one patient to the next by the midwives. He also observed that many doctors were in the habit of going from patient to patient, or from an autopsy to a delivery, without washing their hands. He instituted methods of handwashing in chlorinated solutions for all physicians and nurses. This resulted in a significant reduction in infections which we now know are caused largely by streptococci. However, Semmelweis's ideas were ridiculed by his colleagues. His abrasive personality led to his dismissal from the hospital, and he spent some time in an asylum. Ironically, he is said to have died from a streptococcal infection.

At about the same time, an American, Oliver Wendell Holmes, also noticed that women suffered fewer infections when they gave birth at home rather than in hospital, and he also concluded that the infection was carried from person to person on the hands of midwives and doctors.

Other ways in which infections could be transmitted were still not well understood. John Snow, an English physician who worked in London in the mid-1800s, was intrigued by the fact that some of his patients contracted cholera while

others did not. Although the causative agent of cholera was not known at that time, Snow suspected that it was being transmitted in water. He surmised that the agent responsible for cholera was being excreted by people suffering from the disease into the sewage which was dumped in the River Thames, contaminating the water used by other inhabitants. His work was confirmed by ROBERT KOCH in Germany. The work of these two scientists laid the basis for our modern epidemiological ideas of common vehicle transmission and water purification (see Chapter 8).

In England, a surgeon named JOSEPH LISTER had heard of Pasteur's work on bacterial fermentation. He wondered if the incidence of infections, such as gangrene, in surgical wounds might be reduced if microbes were prevented from gaining access to the wounds. He knew that phenol (carbolic acid) could kill bacteria, so he began to treat surgical wounds with carbolic acid and developed procedures for surgery that were quickly adopted by other surgeons. These included boiling the instruments, soaking linen and bandages in carbolic acid, and spraying a fine mist of the disinfectant into the air during surgery. Carbolic acid is quite corrosive and its use must have caused severe pain and discomfort, but the incidence of post-surgical infections (and deaths) was dramatically reduced.

The germ theory of disease

The stage was set for the next important discovery—that bacteria are the cause of many of the known infectious diseases. In the middle of the 19th century, in Europe, the disease anthrax was killing large numbers of cattle. Both Pasteur and the German microbiologist Robert Koch were trying to find the cause of the disease. In 1876, Koch examined a sample of blood from an animal that had died from anthrax and observed rod-shaped cells (bacilli) under the microscope. He transferred a drop of blood to a flask of nutrient broth in the laboratory, allowed the bacteria to multiply, and then injected the bacteria into otherwise healthy animals. The animals developed anthrax and died (see Figure 1.4). Koch then isolated the same bacteria from the blood of the dead animals. Based on these results, Koch proposed the **germ theory of disease**, which states that each infectious disease is caused by a particular microorganism.

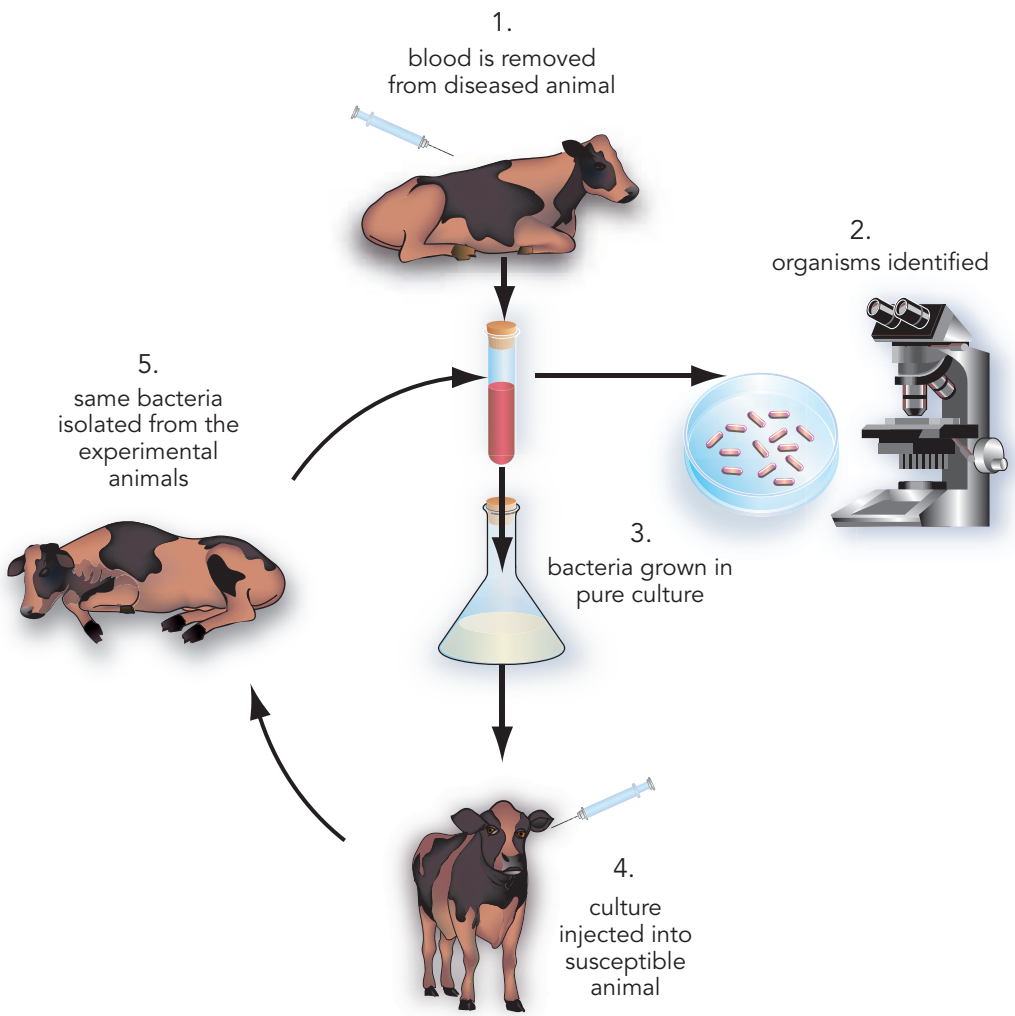


FIGURE 1.4

Koch's experiment showing that anthrax is caused by a particular bacterium

The experimental procedure he developed was referred to as **Koch's Postulates**, and it formed the basis of a set of principles to be followed in order to determine whether an organism was the causative agent of a particular disease. The organism has to be isolated from the diseased patient, grown in culture away from the patient, and then produce the same disease when introduced into a susceptible host. It was further required that the same organism could then be isolated from the new host (see Figure 1.4). These principles were eagerly adopted by other scientists and during the next 30 years were used successfully to isolate and identify the causative organisms of most of the then known diseases of bacterial origin (see Table 1.3). To a large extent these principles are still valid today, although they require some modification in light of our current understanding of the nature of infectious agents. They are discussed further in Chapter 7.

The methods developed by Koch were applicable only to diseases caused by bacteria. Viruses, which can replicate only inside living cells and cannot be seen under a light microscope, could not be isolated and identified in this way, so the viral nature of some diseases was not established until much later.

Another important contribution made by Koch was the development of a technique for growing bacteria in the laboratory on plates of nutrient medium, solidified with agar. This technique is still the basis for the isolation and identification of bacteria in diagnostic microbiology laboratories (see Chapter 15).

Insects as carriers of disease

A further important discovery that occurred about this time was that diseases could be spread by insects. Two important diseases that were shown to be carried by mosquitoes were malaria and yellow fever. Scientists in several countries had been studying malaria, which was originally thought to be acquired by drinking contaminated water. By 1895, due mainly to the work of the French doctor Charles Laveran, the protozoan that causes malaria had been identified and the different forms of the parasite in the human body had been described. However, there was no agreement on how the infection was acquired or transmitted from person to person.

In 1898 the work of the English army physician Ronald Ross in India, and of Giovanni Grassi in Italy, finally demonstrated that the malaria parasite was spread by a particular type of insect, the *Anopheles* mosquito. There were many arguments about these findings and it was not until many years later that it was accepted that control of mosquitoes was the most effective way to limit the spread of malaria.

Yellow fever had long been a problem in America. In 1793, Philadelphia had to be evacuated because of an outbreak of yellow fever that even threatened the life of the newly elected president George Washington. The way the disease is spread was not understood until 1900, when it was

TABLE 1.3 **Identification of bacteria responsible for disease**

DATE	DISEASE	ORGANISM	SCIENTIST
1876	Anthrax	*Bacillus anthracis*	Koch
1878	Skin infection	*Staphylococcus*	Koch
1879	Leprosy	*Mycobacterium leprae*	Hansen
1880	Gonorrhoea	*Neisseria gonorrhoeae*	Neisser
1880	Typhoid	*Salmonella typhi*	Eberth
1880	Pneumonia	*Pneumococcus*	Pasteur, Sternberg
1882	Tuberculosis	*Mycobacterium tuberculosis*	Koch
1883	Cholera	*Vibrio cholerae*	Koch
1883	Diphtheria	*Corynebacterium diphtheriae*	Klebs
1885	Cystitis	*Escherichia coli*	Escherich
1885	Tetanus	*Clostridium tetani*	Nicolaier
1886	Pneumonia	*Streptococcus pneumoniae*	Fraenkel
1887	Meningitis	*Neisseria meningitidis*	Weichselbaum
1887	Brucellosis	*Brucella melitensis*	Bruce
1889	Tetanus	*Clostridium tetani*	Kitasato
1892	Gangrene	*Clostridium perfringens*	Welch, Nutall
1894	Plague	*Yersinia pestis*	Yersin, Kitasato
1897	Botulism	*Clostridium botulinum*	van Ermengem
1898	Dysentery	*Shigella dysenteriae*	Shiga
1905	Syphilis	*Treponema pallidum*	Schaudinn, Hoffmann
1906	Whooping cough	*Bordetella pertussis*	Bordet, Gengou

shown by US Army surgeon Walter Reed that yellow fever is also transmitted by mosquitoes. Although the viral nature of the disease was not known at the time, the discovery was important as it was the first time that the transmission of a viral disease by an insect was recorded.

In the 1880s the French government had started to build the Panama Canal, but the project had to be abandoned due to a number of factors, a major one being the number of deaths of workers from malaria or yellow fever. When the US government took over the project, officials had the benefit of knowing how these diseases were spread. They undertook drainage of the swamps and a spraying program that successfully eliminated mosquitoes from the area, dramatically reducing the incidence of the disease and allowing the completion of the canal.

The pandemic of bubonic plague that occurred at the end of the 19th century resulted in the deaths of 12.5 million people in India and Hong Kong alone. It coincided with Koch's work on the Germ theory of disease and the isolation of the bacterium responsible for plague, *Yersinia pestis*, in 1894. However, the mode of transmission of the disease was not understood. Although rats had been implicated, it was only in the early 1900s that the role of the rat flea as the carrier of the disease was established. Plague is still endemic in wild animal populations in many parts of the world, and fleas act as the carrier from infected animals to humans.

Epidemic typhus (also called 'spotted fever', 'trench fever' or 'gaol fever') is now known to be carried by the human body louse that thrives in crowded, dirty environments. It was responsible for millions of deaths in World War I and postwar Europe, but improved hygiene, a vaccine and antibiotics have now reduced the mortality rate of the disease.

The increasing importance of insects as carriers of emerging viral diseases is discussed later in this chapter.

Treatment and prevention

By the early part of the 20th century the causative organisms of many diseases had been identified, and in many cases it was known how they were spread from person to person. The work of Semmelweis and Lister had introduced the concept of transmission by human hands or contaminated instruments. Insects had also been implicated as carriers (vectors) of disease. However, effective methods for the treatment and prevention of infectious diseases had not yet been developed. To a large extent, treatment for infectious diseases was merely supportive, using procedures that relieved the symptoms but did not cure the disease or prevent its transmission. Florence Nightingale was an English nurse who was sent to the Crimean war in 1854. Shocked by the filthy conditions in the hospitals and the numbers of soldiers who were dying of infection, she instituted a system of cleaning and hygiene that resulted in a marked drop in infections. Her careful observations of the effects of her methods led to her ideas about hygiene being adopted back in England, although right up to her death in 1910 she never accepted that living microorganisms caused infection.

Discovery of antimicrobial drugs

The worldwide influenza epidemic of 1917–18 resulted in the deaths of between 25 and 40 million people, more than three times the number who died during World War I. Scientists such as Paul Ehrlich began the search for a drug, 'a magic bullet', that could destroy pathogens in the human body without harming the patient. This resulted in the discovery of the first chemotherapeutic agent, salvarsan, which was active against *Treponema pallidum*, the bacterium responsible for syphilis. The discovery of sulfa drugs followed, and then the discovery of penicillin by Alexander Fleming in 1928. Penicillin was developed as a therapeutic agent during World War II by Howard Florey and Ernst Chain. Since then, many other antimicrobial drugs have been isolated and synthesised and used to treat bacterial infections, especially in hospitals (see Chapter 12).

Immunology

The work of other scientists expanded our knowledge of the disease process and the way in which the body responds to invasion by foreign organisms. As far back as 1796, Edward Jenner observed that milkmaids who suffered from cowpox, a relatively mild disease that produced pustular lesions similar to smallpox, appeared to be immune to smallpox. He tested his theory by inoculating a child with material taken from a cowpox lesion and subsequently exposing him to smallpox (see Figure 1.5). The child appeared to be immune. This procedure laid the basis for our modern methods of vaccination (named after the pox virus *Vaccinia*). At that time, the viral nature of the disease was not known.

Almost 100 years later, Pasteur was experimenting with chicken cholera and accidentally used an old culture of the bacteria to inject some chickens. They did not become sick

FIGURE 1.5

Jenner inoculating a child

Source: The Granger Collection, New York.

and were subsequently shown to be immune when exposed to a fresh culture. Pasteur realised that he had found an 'attenuated' or weakened strain of the pathogen which was able to protect the chickens against the virulent (disease-causing) strain in the same way as Jenner's cowpox had protected against smallpox. This concept was carried further by Pasteur when he developed a weakened strain of anthrax which provided protection against that disease.

A number of scientists attempted to produce effective vaccines by treating infectious organisms in various ways. Some of these were successful, but others—such as the tuberculin preparation against *Mycobacterium tuberculosis* developed by Koch—had disastrous consequences. Several of Koch's patients developed tuberculosis, and the vaccine—and vaccination as a preventative method—was discredited. Many modern vaccines consist of attenuated strains of viruses and are particularly effective because they mimic the disease, producing an immune response without any symptoms of the disease. Others consist of killed microorganisms or fragments of bacteria or viruses which are able to provoke an immune response without producing symptoms of disease (see Chapter 9). Vaccination is the main method of protection against viral diseases, as there are few effective antiviral drugs that do not also damage the host cells (see Chapters 5, 12).

Although these early scientists had some success in producing vaccines, they had little understanding of how the immune system functions. In the late 19th century many scientists believed that immunity was due to the presence of special components in the blood. The Russian zoologist Elie Metchnikoff discovered that certain blood cells could ingest microbes. He called these cells **phagocytes**, meaning 'cell-eating'. We now know that phagocytes play an important role in the non-specific defences of the body. However, an understanding of the specific immune response whereby the body produces antibodies to specifically inactivate the invading organism, and memory cells to maintain immunity, did not come until the second half of the 20th century. The discovery in the 1980s of the AIDS virus, which attacks the T lymphocytes of the immune system, has generated a huge amount of research in that area and contributed significantly to our knowledge of the functioning of the cell-mediated immune system.

The Australian contribution

Although Australia has a relatively small population, the standard of scientific research and medical knowledge has always been in the forefront of world achievement. A number of Australian scientists have made major discoveries in microbiology and related disciplines, working alone or in groups, or collaborating with researchers in other countries. Some of the more important ones are mentioned here. For a more complete description the reader is referred to Fenner (1990).

Perhaps the most famous Australian researcher is Howard Florey, who was educated in Adelaide but worked mainly in England. Based on Alexander Fleming's earlier observations, Florey and Ernst Chain developed penicillin as a therapeutic agent. The three scientists shared the Nobel Prize in 1945.

One of the most significant discoveries was the recognition in the early 1940s by Norman Gregg, a paediatrician at the Royal Alexandra Hospital for Children in Sydney, that rubella infection in the first trimester of pregnancy was the cause of congenital defects, including deafness, heart and brain lesions, and cataracts. The successful development of a vaccine for rubella has greatly reduced the incidence of congenital rubella syndrome.

Australians have been world leaders in the fields of immunology and viral research. Another Nobel Prize winner, in 1960, was Frank Macfarlane Burnet (see Figure 1.6). His early work was in the fields of viral, rickettsial and bacterial infections, but he gained most recognition in the field of immunology for his work on immunological tolerance, the biology of self-recognition, and his development of the theory of clonal selection and antibody response.

Throughout his lifetime, Frank Fenner made major contributions to an understanding of the nature of viral infections and played a major role in the successful campaign by the World Health Organization (WHO) to eradicate smallpox.

In 1996, Peter Doherty (see Figure 1.7) together with the American Rolf Zinkernagel received the Nobel Prize for his work on the role of the major histocompatibility complex (MHC) antigens in T cell recognition of virus-infected cells. The work was first published in 1972, but the significance of the observations has become more apparent as knowledge of the nature of viral infection and the immune response has increased.

In 1982, Perth doctors Robin Warren and Barry Marshall (see Figure 1.8) proposed that gastric ulcers were caused by the bacterium *Helicobacter pylori*, and not by stress or excess stomach acid as previously thought (see

FIGURE 1.6
Nobel laureate Sir Frank Macfarlane Burnet

FIGURE 1.7

Nobel laureate Professor Peter Doherty

Source: Professor Peter Doherty.

Chapter 18, page 458). In 2005 they were awarded the Nobel Prize for medicine for their discovery. Thousands of people have been cured of gastric ulcers since antibiotic treatment for *Helicobacter* infection began.

The human papillomavirus has been linked to the occurrence of cervical cancer, which is responsible for about 1000 deaths in Australia each year (see Case History 5.2: Cervical cancer, Chapter 5, page 100). Researchers in Brisbane led by Ian Frazer have contributed to the successful development of a vaccine that should save the lives of many women.

FIGURE 1.8

Nobel laureates Dr Robin Warren (left) and Dr Barry Marshall

Source: Frances Andrijich.

A number of disease-causing microorganisms were first identified in Australia and found to have worldwide significance. In 1973, Ruth Bishop and Ian Holmes described a virus responsible for severe gastroenteritis in children. It was later named *rotavirus* and found to be a major cause of infant diarrhoea and mortality throughout the world. A vaccine to protect against rotavirus has been developed and is now included in the Australian immunisation schedule. Its use should lead to reduced infant mortality, especially in developing countries. Yvonne Cossart demonstrated the presence of human parvovirus B19 in blood donors, and Ian Gust worked on the classification of hepatitis A. Another important organism first described by Australians is the rickettsia responsible for Q fever, *Coxiella burnetii* (Macfarlane Burnet and Edward Derrick).

The bacterium *Mycobacterium ulcerans*, which causes necrotising ulcers (Bairnsdale ulcers), was identified by Jean Tolhurst and Glenn Buckle. Although not common in Australia (see Chapter 16, pages 393–4), these ulcers are a major problem in Africa where they are called Buruli ulcers.

A number of viral pathogens that are found mainly in Australia and New Guinea have been identified by other Australian groups. They include many of the arboviruses transmitted by mosquitoes, such as Ross River virus (Ralph Doherty), Murray Valley encephalitis virus (Eric French) and Barmah Forest virus. In 1994 a new virus was discovered which could cross the species barrier and infect animals as well as humans—the equine morbillivirus—now renamed Hendra virus. This was followed soon after by the discovery of the rabies-like bat lyssavirus and the Menangle virus (see Chapter 5, page 99 and Spotlight box: Viral zoonoses linked to flying foxes, Chapter 8, page 160).

INFECTIOUS DISEASES IN THE 21ST CENTURY

Advances in the prevention and treatment of infectious diseases

During the last 100 years, our knowledge and understanding of the microbial causes of infection has increased dramatically. We now know the cause and method of transmission of many diseases and can implement strategies to deal with outbreaks when they occur. Improvements in hygiene and sanitation in the developed world have significantly reduced mortality rates from infectious diseases. The discovery of antibiotics and the development of vaccines have allowed us to develop methods to treat and prevent disease.

Public health

The area of disease prevention and health maintenance called Public Health is of vital importance when developing strategies to deal with outbreaks of disease. A number of international agencies monitor outbreaks of infectious diseases around the world, publish reports online, develop policies

and advise on management of outbreaks (see Chapter 14). Electronic communication between health authorities in different countries enables rapid dissemination of information about an outbreak, the reporting of cases, and sharing of experience for treatment and control.

Antibiotics

One of the most important advances of the 20th century was the development of antimicrobial drugs. Since the first antibiotic, penicillin, was discovered and developed in the 1940s, a number of other antimicrobial drugs have been produced to treat or cure disease. These have had a significant impact on the level of morbidity and mortality due to infectious diseases. Antibiotics have been used successfully to treat community-acquired infections as well as more serious life-threatening infections in hospital patients. However, the effectiveness of these drugs is now threatened, as many organisms are becoming resistant to the available drugs and there have been no new classes of antimicrobials discovered in recent years (see Chapter 12).

Vaccination

Many infections can now be prevented by the administration of a vaccine. The WHO's vaccination campaign against smallpox led to the worldwide eradication of this disease in 1977. Polio, which once crippled thousands of people annually, has almost been eradicated from Western countries including Australia, which was declared free of polio in 2000. The WHO launched its Global Polio Eradication Initiative (GPEI) in 1988. By 2006, transmission of indigenous wild poliovirus (WPV) was interrupted in all except four countries (Afghanistan, Pakistan, India and Nigeria). Subsequently, 39 previously polio-free countries experienced outbreaks following importation of WPV. Worldwide, only 1291 WPV cases were reported in 2010, a 19 per cent decrease from 2009. The target is complete eradication by 2014.

Currently, the WHO also has a campaign to eradicate measles, but this is proving more difficult, partly because the rate of measles vaccination has fallen in many Western countries (see Spotlight box: MMR vaccine and autism, Chapter 14, page 341).

The incidence of congenital rubella syndrome, which causes serious birth defects in babies whose mothers are infected in the first trimester of pregnancy, has been dramatically reduced by the development of a vaccine.

As well as vaccines to prevent the childhood diseases of diphtheria, whooping cough, tetanus, polio, measles, mumps and rubella, new vaccines have been developed for chickenpox, meningococcal disease, pneumococcal disease, human papillomavirus and rotavirus. A combined vaccine is available to protect against hepatitis A and B. Vaccines against influenza are developed each year to combat new strains of the virus. However, it is essential that a high level of vaccine coverage is maintained in the community to prevent the recurrence of vaccine-preventable diseases (see Chapter 14).

Diagnostic techniques

In the last 50 years there has been a rapid expansion of knowledge in the fields of virology, immunology and molecular biology. The new science of molecular biology allows us to understand the basis of inheritance at a molecular level—how information can be carried from one generation to the next and translated into functional cell components.

Microorganisms can now be identified by their genetic material, the DNA or RNA, using specific techniques to distinguish between closely related strains. This testing is often quicker than conventional techniques and enables 'real time' testing by diagnostic laboratories to identify microbial pathogens and follow their transmission in the clinical environment (see Chapter 15).

These techniques have also been used to identify 'new' viruses that are not able to be cultured in the laboratory.

THE GLOBAL HEALTH SCENE

The advances described above have not been uniform throughout the world. Whereas developed nations generally have a high standard of living and many of the infectious diseases have been controlled or eliminated, people living in developing regions face very different problems. The challenges facing healthcare workers in these countries are quite different from those found in other parts of the world. Socioeconomic factors such as poverty and malnutrition, as well as a high incidence of disease, lack of health programs, low vaccination rates, wars and famine, all contribute to a lower standard of health. Most striking is the disparity in the incidence of HIV/AIDS between countries such as sub-Saharan Africa and the Western world. These disparities are summarised in Table 1.4.

Today the world is faced with a number of newly identified microorganisms responsible for serious infectious diseases as well as the re-emergence of old diseases in a resistant or more virulent form. In addition, many of the common bacteria responsible for skin and wound infections have become resistant to antibiotics and now pose a serious threat to critically ill patients in hospital (see Chapter 12).

NEW AND EMERGING INFECTIOUS DISEASES

There are currently around 1400 microorganisms that are known to be able to infect humans. Approximately 90 of these can be considered to be 'new' or 'emerging' infections, having first been reported after 1980. The majority of the newer infectious agents are viruses and most are **zoonoses**—diseases that originate in animals. Many of these, including SARS, have originated in wildlife such as bats or monkeys (see Chapter 8).

These infections vary considerably in terms of their effects on public health and the economies of affected countries. For example, HIV/AIDS has killed an estimated 25 million people with another 40 million currently infected, whereas Menangle virus is known to have infected only two people, causing a mild febrile illness. However, even with a low morbidity and mortality, an outbreak of a new disease and the response to

TABLE 1.4 The global health scene: major issues affecting health

DEVELOPED NATIONS	DEVELOPING COUNTRIES
■ Improvement in patient survival due to advances in treatment and medical interventions	■ Famine, lack of clean water, poor sanitation
■ Susceptibility of immunocompromised patients to infection	■ Poverty, malnutrition, high susceptibility to infection
■ Increased resistance of hospital strains of bacteria to antibiotics	■ Lack of drugs
■ Changes in lifestyle, increase in sexually transmitted infections, injecting drug use	■ Lack of health services
■ Travel—globalisation	■ Gastrointestinal diseases
■ Non-compliance with vaccination	■ Parasitic diseases—helminth infestations
■ Emerging viral infections—SARS	■ Vaccines not available due to expense or lack of transport
■ Viral mutation—influenza A	■ HIV/AIDS
■ Arboviruses	■ Blood-borne and haemorraghic diseases
■ Multi-drug-resistant TB (MDRTB)	■ Malaria
■ Bioterrorism	■ Tuberculosis
	■ Civil war
	■ Natural disasters

it can have substantial economic outcomes. For example, the 2003 SARS epidemic caused fewer than 1000 deaths but cost the Asian economy an estimated US$18 billion. Similarly, variant CJD (Creutzfeldt-Jakob disease), or 'mad cow disease', which has so far caused fewer than 250 deaths worldwide, has cost the UK around US$6 billion.

AIDS

By far the most significant new disease is **acquired immune deficiency syndrome** (**AIDS**), caused by the human immunodeficiency virus (HIV-1). It first appeared in the 1980s, attacking the immune system, destroying T cells and leaving the patient susceptible to opportunistic infections. The precursor to the human virus probably occurred in chimpanzees in Africa and moved into the human population. Although the number of new cases in Australia has been contained, largely due to a public education campaign and needle exchange program, this is not the case in other parts of the world, especially the developing nations (see Figure 1.9).

In parts of sub-Saharan Africa, AIDS is endemic in the heterosexual population and there is a lack of government support for education to prevent transmission, and insufficient money to provide drugs to treat everyone. In these areas, it is estimated there are over 20 million people living with HIV. The Caribbean is the second-worst affected area.

The most recent world figures estimate that over 3 million people are newly infected annually. There are over 7400 new infections every day; of those, 97 per cent are in low- and middle-income countries. About 1200 are in children, and 6200 in adults aged 15 and over, and 48 per cent are women. The most recent report can be accessed at <www.unaids.org/int>.

The largest increases in HIV infections are occurring in South-East Asia, Eastern Europe and Central Asia, with injecting drug use contributing to a significant share of infections. In Europe, heterosexual transmission is increasing and women account for an increasing proportion of new HIV-positive diagnoses. At the end of 2010 it was estimated there were 34 million people living with AIDS, of whom nearly 50 per cent are women. There have been more than 25 million deaths since the beginning of the epidemic. Treatment with a combination of antiretroviral drugs has been effective in prolonging life in Western countries, but the cost of drugs is very high and fewer people in developing countries are able to access treatment. The greatest challenge is the development of a vaccine.

On World AIDS Day, November 2011, the United Nations adopted a policy to strengthen the measures to combat AIDS, especially recognising the high percentage of women and girls who are infected in developing countries and the consequent socioeconomic impact. There have been concerted efforts to halt the spread of AIDS, with the report stating that, at the end of 2010, 6.6 million of the estimated 14.2 million eligible people in low- and middle-income countries (i.e. 47 per cent) were receiving antiretroviral treatment. UNAIDS estimates that a total of 2.5 million deaths have been averted in low- and middle-income countries since 1995 due to the roll out of antiretroviral therapy. However, the cost of retroviral therapy runs into billions of dollars annually and there is still a need to focus on preventative measures.

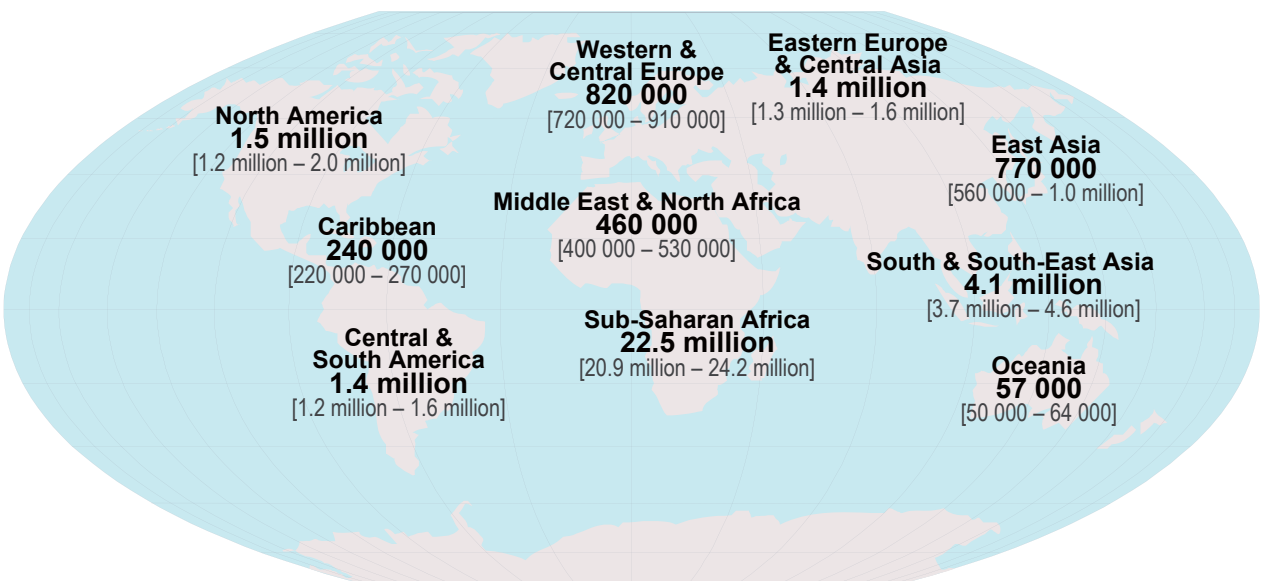
SARS

In 2003 there was an outbreak of a pneumonia-like illness named Severe Acute Respiratory Syndrome (SARS). A single person infected with the virus travelled from China to Hong Kong, stayed in a hotel and spread the infection to other guests, who subsequently carried it to almost 30 other countries (see Case History 8.1: SARS, Chapter 8, page 166). The speed with which the world community reacted to this new disease prevented the occurrence of a potentially serious pandemic. The causative organism was identified as a new coronavirus and the outbreak was contained in just a few months.

UNAIDS
JOINT UNITED NATIONS PROGRAMME ON HIV/AIDS
UNHCR UNICEF WFP UNDP UNFPA UNODC ILO UNESCO WHO WORLD BANK

UNAIDS 2011 World AIDS Day report

	2001	2005	2008	2009	2010
People living with HIV	**28.6 million** [26.7-30.9 million]	**31.0 million** [29.2-32.7 million]	**32.3 million** [30.4-33.8 million]	**32.9 million** [31.0-34.4 million]	**34 million** [31.6-35.2 million]
New HIV infections	**3.15 million** [2.96-3.33 million]	**2.81 million** [2.63-2.97 million]	**2.74 million** [2.52-2.93million]	**2.72 million** [2.48-2.93 million]	**2.67 million** [2.46-2.90 million]
AIDS-related deaths	**1.85 million** [1.67-2.16 million]	**2.22 million** [2.07-2.48 million]	**2.04 million** [1.87-2.21 million]	**1.89 million** [1.72-2.05 million]	**1.76 million** [1.59-1.91 million]
New infections in children	**550 000**	**540 000**	**460 000**	**430 000**	**390 000**



Total: 33.3 million
[31.4 million – 35.3 million]

FIGURE 1.9

Adults and children estimated to be living with HIV/AIDS at the end of 2009

(a) Table of HIV infections and AIDS deaths, 2001–10; (b) global incidence of HIV, 2009.

Sources: World Health Organization; Reproduced by kind permission of UNAIDS/ONUSIDA, 2010, <www.unaids.org>.

The **SARS-CoV** (SARS–coronavirus) story is a striking example of the way in which a network of doctors, scientists and public health experts from all parts of the world worked together to identify the causative organism and contain the disease. The economic cost of the outbreak was high—in terms of losses in trade, travel and tourism. In Hong Kong alone, the SARS outbreak is estimated to have cost the country 4 per cent of its gross domestic product. Recent research has identified the coronavirus in horseshoe bats, which are thought to be the natural reservoir. Exposure to civet cats infected with the bat virus is thought to have been responsible for transfer of the virus to humans. Live animal markets in Asia are common and allow infected animals to come in contact with humans.

Re-emergence of 'old' diseases

Tuberculosis

A number of diseases that had almost been eradicated have re-emerged, often in a form exhibiting multi-resistance to existing antimicrobial drugs. Among these, **tuberculosis** (**TB**) is one of the most significant, occurring in one-third of the world's population. Eight million new cases develop each year and it is now the greatest single cause of death due to infection throughout the world, with an estimated 3–4 million deaths each year. Patients can be treated with a combination of drugs, but poor compliance with the therapy has led to the emergence of new strains, MDR-TB, which is resistant to two or more drugs, and an extremely drug-resistant strain, XDR-TB, that is resistant to all the usual drugs and is virtually untreatable (see Chapter 14, pages 356–7, and Chapter 17, pages 435–40).

Malaria

Malaria is an ancient disease but is still widespread in tropical regions. Attempts to control it by eradicating the mosquitoes that transmit infection have not been successful. It is estimated that there are about 3 million new cases of malaria each year, with between 1 and 2 million deaths.

Children and pregnant women are particularly vulnerable. In some areas, strains of malaria have appeared that are resistant to the usual antimalarial compounds, chloroquine and primaquine. Artesunate compounds have been used in recent years, but resistance is also developing to these drugs. A vaccine is urgently needed but is difficult to develop because of the complicated life cycle of the malaria parasite (see Chapter 19).

Haemorrhagic fevers

There have been recurrent outbreaks of **Ebola fever**, a disease caused by a deadly haemorrhagic virus that occurs mainly in sub-Saharan Africa. It attacks every tissue and organ in the body, causing death in most patients within a few days. The patient is highly infectious and the virus is spread by contact with infected blood or other body fluids. Since the patient is bleeding from the skin and body orifices, it is very easy for the virus to spread, especially in the unhygienic conditions that prevail in developing countries. Another virus in this group is the **Marburg** virus. Both viruses are thought to have a natural wildlife reservoir.

These viruses are not currently present in Australia and are unlikely to become established because of the short incubation time, easily identifiable symptoms and Australia's isolation.

Influenza: Swine flu and Avian flu

In 2009 a highly infectious strain of influenza A, **H1N1** (**swine flu**), appeared in Mexico. It was very similar to the strain of influenza responsible for the 1918 pandemic, which cost at least 20 million lives. Although it was highly contagious from person to person, the disease symptoms were not as severe as first feared and a vaccine was produced quickly to contain the disease. The main concern was the potential of H1N1 to cross with the **avian flu virus** (**H5N1**), which has affected millions of birds and chickens in all parts of Asia. H5N1 virus is classed as endemic in the bird population and has been spread by migratory birds to Europe and other parts of the world. The virus has been responsible for a number of infections in humans who have had close contact with infected birds, causing illness and sometimes death, but at present there is no evidence of human-to-human transmission. If H1N1 and H5N1 were to cross, there is the potential to produce a strain of influenza virus that is both highly transmissible and causes serious illness (see Spotlight box: Influenza A, Chapter 5, page 96).

The spread of viral diseases

The last few years of the 20th century and the start of the 21st century were marked by reports of an increased number of newly identified viral diseases, and the spread of others from their country of origin to other parts of the world. Australia, because of its isolation, used to be relatively free of many of these diseases, but with the increase in speed and frequency of air travel it is possible for new diseases to be introduced. For example, the introduction of multiple strains of dengue fever by travellers can give rise to cases of the haemorrhagic form of dengue fever (see Chapter 19, page 495).

Japanese encephalitis

This mosquito-borne viral encephalitis is common throughout Asia, and some cases have been reported in the Northern Territory and Torres Strait Islands. Since the mosquito vector is present in Australia, this disease has the potential to spread to the southern, more populous states of Australia. Viruses transmitted by insect vectors are of increasing importance in Australia (see Chapter 5, page 101, and Chapter 14, page 358).

Hendra virus

In 2011, outbreaks of the Australian **Hendra virus** affected several rural properties in northern NSW, causing the deaths of at least 20 horses, while others had to be destroyed. Hendra virus was first identified in 1994 in Queensland when it killed 14 horses and their trainer. It has a mortality rate

of over 50 per cent in horses and is transmitted to humans by close contact with infected body fluids. Since 1994 there have been seven cases of infections in people who cared for infected horses, and four deaths. Fruit bats have been implicated as the reservoir for Hendra virus, and also for the rabies-like bat **lyssavirus** and the **Menangle** virus, which affects pigs and humans (see Figure 1.10). These are all examples of new viruses which appear to be able to cross the species barrier (see Spotlight box: Viral zoonoses linked to flying foxes, Chapter 8, page 160).

Nipah virus

The **Nipah** virus caused an outbreak of infection in Malaysia in 1998–99, as a result of deforestation. Flying foxes (fruit bats), which are the natural reservoir of Nipah virus, migrated from deforestation areas to nearby fruit orchards. Pigs on these and nearby farms are believed to have ingested bat saliva from half-eaten dropped fruit containing the virus. The virus spread to humans, with 250 pig farmers becoming infected; the mortality rate was 40 per cent. The virus was contained by culling of a million pigs, but a similar virus has emerged in India and Bangladesh.

Chikungunya virus

This virus was first recognised in East Africa and is endemic to Africa and several South-East Asian countries, including Thailand, Indonesia and the Philippines. Since 2006 there have been reports of its spread worldwide. It is transmitted to humans by mosquitoes and causes severe fever and joint pain, which may persist for weeks. There were eight cases reported in Melbourne in 2007, all acquired overseas.

Hantaviruses

These are another group of viruses that can produce haemorrhagic symptoms. An outbreak of disease with influenza-like symptoms, in which the lungs filled with fluid, leading to death by respiratory failure, occurred on an Indian reservation in New Mexico in 1993. It was found to be caused by a hantavirus, and epidemiologists linked the outbreak to a recent plague of deer mice. Many of these viruses are maintained in nature in a rodent reservoir. Humans acquire the diseases by direct contact with the animals or with viruses shed in urine or saliva. As yet, there have been no reports of hantavirus infections in Australia.

FIGURE 1.10

Flying fox

Source: Dr Raina Plowright.

West Nile virus

This virus, which originated in North Africa, can also infect humans, causing encephalitis. The first cases appeared in North America in 1999. It is carried by migratory birds and is now classified as endemic in North America. There have been no reports of the virus in Australia.

It is probable that many more viruses will emerge or be identified in future. This is due to factors such as global travel, the movement of humans into previously uninhabited areas, and the advances in techniques for identification of new viruses.

Prion diseases

Prions are unusual infective particles consisting only of protein. They are responsible for a group of fatal neurological disorders called **transmissible spongiform encephalopathies** (**TSEs**) (see Chapter 20, page 530). The appearance in England of cases of variant Creutzfeldt-Jakob disease (vCJD), a new prion disease with evidence of a link to 'mad cow disease' (bovine spongiform encephalopathy, BSE), raised concerns about the number of people who may have been infected by eating contaminated beef. The cows were infected by being exposed to a TSE called scrapie in high-protein feed supplements. The use of this feed was banned and affected cows had to be slaughtered. People who had eaten infected meat developed symptoms some years after exposure. The number of cases of vCJD seems to have peaked, with a total of around 250 deaths worldwide, and the risk of transmission in blood or body products or by medical procedures is now considered very low.

Because of the long incubation time for the disease, Australia, which is currently free of BSE, banned blood and organ donations from any person who lived in England for six months or more between 1980 and 1996 (see Spotlight box: Prions, Chapter 11, page 247). The importation of beef and beef products was also banned.

REASONS FOR THE EMERGENCE AND RE-EMERGENCE OF INFECTIOUS DISEASES

There are a number of different factors that have influenced the emergence and re-emergence of the diseases described above. These include genetic changes in microorganisms, an increase in the susceptibility of some people to infectious diseases, and an increase in the exposure of humans to the animal reservoirs or insect vectors involved in the transmission of infectious agents. It is estimated that 60 per cent of emerging diseases have originated from animals—mainly wild animals in areas such as sub-Saharan Africa, India and China.

Genetic changes: mutation and adaptation

Microbes are, generally, highly adaptable organisms. They have a very short generation time and a variety of genetic mechanisms that allow them to rapidly evolve and adapt to changing environments. This has proved to be a significant factor in the development of more virulent or resistant strains of bacteria and viruses.

Development of resistance to antimicrobial drugs

The over-use and misuse of antimicrobial drugs has contributed to the emergence of resistant bacteria that pose a serious problem, especially in healthcare facilities. Repeated minor mutations in bacteria, and transfer of genetic material between bacteria, enable them to gradually become increasingly resistant to antimicrobial drugs, as has occurred with *Staphylococcus aureus* and a number of other important pathogens such as highly invasive strains of group A streptococci (GAS), sometimes referred to as 'flesh-eating bacteria' (see Chapter 12). This poses a serious problem in the hospital environment and threatens the advances in treatment that have been made since the discovery of antibiotics in the mid-20th century.

In South-East Asia, over-use of the artemisin drugs to treat malaria has led to the development of resistance in some regions.

Viral mutation

Viruses are generally 'species specific'—that is, they infect only one type of animal or cell (see Chapter 5). An important factor in the occurrence of epidemics of 'new' viral diseases is the ability of viruses to mutate and change their outer structures. This can have the effect of altering the specificity of the virus so that it can infect a human cell as well as an animal cell. Changes to the outer structure can also modify the virus in such a way that existing vaccines are no longer effective. This is best illustrated by the influenza A virus, which has undergone repeated changes to its outer envelope during the last century. As a result the influenza vaccine has to be modified each year to take into account the variations in the virus structure. Most new strains of influenza A emerge from Asia, where farming practices allow close contact between humans and animals. The influenza virus is able to infect domestic animals, particularly pigs and ducks, where it undergoes genetic recombination and acquires new genetic characteristics, giving rise to a new and more virulent strain of the virus. The swine flu pandemic that occurred in 2009 is an example of viral mutation, the virus moving from pigs to humans (see Spotlight box: Influenza A, Chapter 5, page 96, and Chapter 17, pages 424–7). The influenza virus has also developed strains that are resistant to the limited number of drugs available to treat it.

Various species of bats appear to be the natural reservoir for a number of the viruses described above. Animals exposed to bats or their droppings may become infected and passage of the virus through the animal may then produce a virus type that can infect humans.

The emergence of the virus responsible for SARS—a virulent coronavirus related to the human cold virus—is also thought to have involved the passage of the virus from a bat through an animal (in this case, a civet cat).

Persistence of viral infections

Some of the emerging viruses that have been identified have been shown to persist in the human body in a latent form, able to be reactivated if the immune system is depressed (see Chapter 5, page 97). Others, such as hepatitis B, hepatitis C and human papillomavirus (HPV), persist in a chronic, infective carrier state that can lead to cancer. It is thought that other viruses may play a part in the development of cancer, but the link is not yet proven. Some viruses that persist in the body have been linked to emerging diseases such as chronic fatigue syndrome (CFS). A large number of CFS sufferers show evidence of previous exposure to viruses such as Epstein-Barr (EB), cytomegalovirus (CMV), coxsacksie B virus, human herpes virus 6 (HHV6) and human T cell lymphotrophic virus 1 (HTLV1).

There is still very little understanding of how persistent viral infections affect the immune system, but there is some evidence that viruses also play a part in autoimmune diseases.

Increased human susceptibility

Advances in medical treatments in developed countries mean that there are increasing numbers of people whose immune system is compromised to some extent—because of age, premature birth, major surgery, or the medical treatment they have received. Many patients have underlying illnesses that make them more susceptible to infection. Chronic diseases such as diabetes, autoimmune diseases, malignancies and renal failure can suppress host defences and increase the likelihood of infection. Many patients have reduced immunity due to infection with HIV/AIDS, or immunosuppressive therapy following organ transplants or chemotherapy for cancer.

These patients are particularly vulnerable to a range of otherwise harmless microorganisms. They also act as reservoirs for some of the more serious pathogens (e.g. multi-drug-resistant tuberculosis, MDR-TB, in AIDS patients). In particular, susceptible patients are at risk from opportunistic pathogens (microorganisms that would not cause disease in healthy individuals).

The hospital environment may contain antibiotic-resistant microorganisms and other opportunistic pathogens. Fungal infections in immunocompromised patients are becoming more common. Building works that disturb the environment, create air currents and release fungal spores can also pose a significant risk to susceptible patients in a healthcare facility (see Chapter 13).

Lifestyle factors

Certain human behavioural factors, particularly sexual activity and recreational drug use, contribute to an increased prevalence of certain infectious diseases. The re-emergence and increased incidence of some sexually transmitted diseases, such as gonorrhoea and AIDS, are at least partly related to

male homosexual activity. Abuse of intravenous recreational drugs has contributed significantly to the increased incidence of some infectious diseases such as hepatitis B, hepatitis C and AIDS. Substance abuse is also a major challenge to controlling outbreaks of diseases such as TB.

Recreational water sports can expose participants to water-borne zoonoses such as leptospirosis.

Economic development and land use

With changes in land use and encroachment into native forests, humans have been exposed to previously unrecognised microbes and disease-carrying animals and insects (especially mosquitoes, ticks and sandflies). For example, the outbreak of Nipah virus described above can be related to deforestation. Flying foxes (fruit bats) migrated from areas of deforestation, and infected pigs. Ultimately, the virus spread to humans. Farming practices in Asian countries that allow close contact between humans and animals are a major contributor to the emergence of infectious diseases that have crossed the species barrier.

Often the changes that humans make to the environment create more favourable conditions for the animal reservoir or insect vector of the organism. For example, the planting of grain crops can encourage the rodents that carry viruses (e.g. hantavirus in deer mice). Irrigation may establish breeding grounds for mosquitoes or sandflies that transmit diseases such as leishmaniasis.

In some cases, our modern lifestyle facilitates the spread of an organism. For example, in tropical regions, strains of cholera can be found in coastal waters. When this water is used as ballast for shipping, it is sometimes discharged into harbours or rivers in other parts of the world. The cholera bacteria are thus spread, contaminating the water and entering the food chain.

Technological changes

Some advancements in technology have been unexpectedly associated with the emergence of certain infections. The widespread use of air-conditioning systems has been blamed for spreading organisms around a building. They have been linked to the occurrence of Legionnaires' disease, an atypical pneumonia caused by the organism *Legionella pneumophila*, that is spread in the fine aerosol vapour associated with air-conditioning cooling towers.

In the food industry, major changes have occurred in how food is produced, preserved and processed. Fresh food is now routinely transported all over the world, carrying with it the risk of the introduction of disease to farms and the domestic market (see Chapter 14). Globally, there has been an increase in the incidence of haemolytic uraemic syndrome (HUS) related to toxin-producing strains of *Escherichia coli*. This bacteria is found mainly in cattle, sheep and goats, but is spread to humans either directly or by contamination of meat with animal faeces during processing. It can also be spread on salad vegetables. HUS is a serious, sometimes fatal complication of infection by these bacteria (see Chapter 18, pages 448–9 and Case History 8.4, page 176).

Salmonella species are a major cause of gastrointestinal infections. The organism is present in poultry and animals, and is spread when products such as raw salad vegetables are contaminated during processing at huge central facilities. There is an increasing emphasis on 'fresh' foods preserved only by refrigeration. This has provided conditions favourable to *Listeria*, an organism that grows at refrigeration temperatures.

In agriculture, changes in feeding practices to promote faster growth of food animals contributed to the emergence of 'mad cow disease' (variant Creutzfeldt-Jakob disease).

Environmental changes

Global warming is predicted to have significant implications for many aspects of human life, including infectious diseases. It is probable that the numbers and distribution of insect vectors of human disease will be affected. The prevalence of mosquito-borne diseases such as malaria may extend southwards from tropical regions where they are now endemic. In the past decade, mosquito-borne infections (e.g. dengue fever and yellow fever) have been reported at higher altitudes in South America, Asia and Central Africa than previously recorded.

In Australia there has been an increase in reports of infections with mosquito-borne viruses such as Ross River virus, Barmah Forest virus and Murray Valley encephalitis virus. These are often related to rainfall and climatic conditions, such as the severe flooding that occurred in 2011. Global warming and changes in climate also affect the environments where microorganisms can thrive.

International travel

The history of infectious diseases is also the history of exploration and the spread of disease by travellers carrying organisms around the world. In the early days of white settlement in Australia, infections such as influenza, measles and smallpox had a devastating effect on the Aboriginal population, which had never been exposed to these diseases before and therefore had no immunity.

As an island continent, Australia has been for many years free of diseases that are endemic in other parts of the world (e.g. plague and rabies). Our isolation and stringent quarantine regulations combined to prevent these diseases from entering the country or becoming established in animal reservoirs. However, international travel, complacency, lack of compliance with quarantine and health regulations, and the emergence of resistant strains of organisms are rapidly creating a situation where no area can be considered free of a particular infectious disease.

The recent increase in international travel has contributed to the spread of infectious diseases and the emergence of some new ones (see Spotlight box on the next page). Travel that once required months has been reduced to hours, and infections can reach any part of the world in a very short time. The speed of travel also means that travellers can spread an infection during the incubation period of the disease—that is, before exhibiting any signs or symptoms of being ill. The effect of travel is starkly demonstrated by

the SARS outbreak in 2003. People from Singapore, Canada and Vietnam became infected while visiting Hong Kong in March 2003, and carried the SARS virus back to their countries. Subsequent travel by other infected people resulted in the spread of the disease to 30 countries within a month.

Inadequate/reduced public health measures

Some infectious diseases have re-emerged because the public health measures introduced to control them have been reduced or have broken down. Although vaccines are available for many infectious diseases, there is widespread complacency in developed countries and many people do not have their children immunised. As a result, epidemics of diseases such as measles, diphtheria and whooping cough still occur—even in Australia, where all these vaccines are freely available. If the level of vaccine coverage falls below a critical value, the protective effect of 'herd immunity' is lost and there is a real risk of a recurrence of epidemics of diseases such as measles (see Chapter 14). For example, polio has become endemic again in Nigeria because the government suspended its immunisation program.

In developing countries, people are still suffering and dying from infectious diseases such as gastroenteritis, measles and polio, which would occur much less frequently if better hygiene, vaccines and medical treatment were available. Poverty, lack of education and lack of government finance for effective medical programs are largely to blame.

As discussed above, diseases that had almost been eradicated, such as tuberculosis, have re-emerged, often in a form exhibiting multi-resistance to existing antimicrobial drugs. Tuberculosis is endemic in many developing countries and is now the greatest single cause of death throughout the world. The incidence of malaria in developing countries has also increased since spraying with DDT to control mosquitoes was suspended.

Wars and natural disasters

Man-made and natural disasters can intensify the risk of epidemics, as can conditions in crowded refugee camps. Inadequate surveillance systems, destroyed infrastructure, and disruption of disease control and infection control programs contribute to the emergence of infectious diseases. Refugee camps may be overcrowded and lack adequate clean water and sanitation. Serious outbreaks of infectious diseases with high case fatality rates are often the result. For example, in the aftermath of the Rwanda crisis in 1994, outbreaks of cholera caused at least 48 000 cases and 23 800 deaths in one month in the refugee camps in the Congo. The earthquake in Haiti

SPOTLIGHT ON

International travel and the spread of disease

The outbreak of swine flu that originated in Mexico in March 2009 spread rapidly around the world, despite efforts to contain it. It first reached Australia on a plane from the United States in May 2009. When the Health Department was notified that a passenger on the plane had been diagnosed with swine flu, it issued directions that all plane travellers arriving from the United States should stay in quarantine in their homes for a week. The Australian government has a strategic plan to deal with pandemics of infectious disease and this was put into place with people being quarantined and supplied with anti-influenza drugs. Thermal imaging equipment was used at many airports around the world to try and identify passengers who had an elevated temperature, which might indicate they had swine flu (see Figure 1.11).

The risk of diseases being spread on international flights is not new. In 2007 a passenger on an international flight was diagnosed with multi-drug-resistant tuberculosis (MDR-TB), necessitating a widespread search for everyone who had been in contact with him in airports, planes and other facilities on two continents.

There is always concern about the potential for the spread of microorganisms when a large number of people are in a confined space such as an aeroplane, for a long period (eight hours or more). According to the WHO, commercial aircraft have 20 exchanges of air per hour and the air is passed through a HEPA filter which will remove particulate matter such as bacteria. Air is re-circulated within the same section rather than throughout the whole aircraft.

These incidents have raised public awareness of the need to educate health officials and infectious patients about the risks of travel, travel safety and how to deal with infectious passengers.

FIGURE 1.11

Thermal imaging

Source: AFP Photo/Byun Yeong-Wook.

that killed 220 000 people and left 1.2 million homeless was followed by an outbreak of cholera which affected at least 300 000 people and resulted in 4500 deaths. The UN report into the outbreak concluded that the disease was unknowingly imported by a foreign aid worker, as there had been no cholera in Haiti for over a century. Because of the devastation from the earthquake, sanitation and water services were disrupted and the water supply easily became contaminated.

Often the disruption may result in an increase in insect carriers or an animal reservoir and lead to the re-emergence of a disease that had been previously under control. For example, in post-war Kosovo (1999–2000) an outbreak of tularaemia occurred among the displaced people. Food stores and wells had become contaminated by the increased rat population in the area. The outbreak of Marburg haemorrhagic fever in Angola in 2004–05, which affected 374 people with a case fatality rate of 88 per cent, followed years of civil war in that country.

Outbreaks of infection often occur following extreme climatic conditions such as floods and cyclones. The devastating tsunami that hit South-East Asia on Boxing Day 2004 caused widespread flooding, with a resulting increase in vector-borne diseases, malaria and dengue fever (see Figure 1.12). Many injured tourists returned home carrying unusual tropical infections.

Bioterrorism

Since 11 September 2001 the threat of bioterrorism has been discussed constantly in the media. **Bioterrorism** is the fear created by the threat of biological warfare. Biological weapons (microorganisms) are popular with terrorists because they are easy to obtain, easy to produce in large quantities and easy to disseminate.

The damage that can be done by microorganisms, although real, is limited by the nature of the organisms, their stability, the way they are transmitted and environmental conditions. The Centers for Disease Control and Prevention in the United States has ranked the most dangerous microorganisms. In the highest category are anthrax, smallpox, plague, botulism, tularaemia and the haemorrhagic fevers. Among these, the smallpox virus is one of the most important because there is no treatment for it. Since it was eradicated globally (the last natural case occurred in 1977), and vaccination was discontinued, most of the world's population are no longer immune. Stocks of the virus are supposed to be held only in Russia and the United States, but it is possible that during the dissolution of the Soviet Union stocks of the virus could have fallen into the hands of terrorists. It is a serious life-threatening disease and easily transmissible. However, there is a vaccine available, which could be used if an outbreak occurs.

FIGURE 1.12

Devastation and flooding following the 2004 tsunami in Indonesia

Infections in trauma wounds were common.

Source: Photo courtesy of Kay Withnall.

Anthrax spores are very stable and can be easily disseminated as a fine powder or aerosol. Anthrax is treatable with antibiotics unless resistant strains are used, and a vaccine is also available. It does not spread easily from person to person. In the United States, the anthrax powder that was sent by mail after 11 September 2001 infected 22 people, of whom five died.

The food supply is a major area where bioterrorism is effective. **Food biosecurity** is the prevention of the intentional contamination of food and water with hazardous agents, including pathogens and toxins. The main risks are incurred during production and processing, and controls are needed over the importation and transportation of food. The main agents are *Salmonella*, toxigenic strains of *E. coli* (which cause haemolytic uraemic syndrome), *Listeria* and botulinum toxin.

In agriculture, infectious diseases can have devastating economic effects; for example, the naturally occurring outbreaks of foot and mouth disease and avian flu have cost farmers all over the world billions of dollars and made a significant impact on the economies of affected countries. Australia is very vulnerable to the importation of these diseases and this could be exploited by terrorists. Bioterrorists can cause physical, economic, social and political damage with minimal effort. The actual loss of life may not be great: the main aim of bioterrorism is to create panic and cause civil disorder, and this can have a major impact on the economy and political stability of a country.

CONCLUSION

The chapters in this book cover basic information about the microorganisms that can adversely affect humans. In this chapter we have introduced the microorganisms that are responsible for many of the important infectious diseases affecting our lives. We have traced the discovery and development of knowledge about these organisms and discussed the reasons for the re-emergence and spread of the diseases responsible for millions of deaths around the world. Later chapters deal with the properties of the microorganisms that cause infectious diseases, their transmission and control, as well as methods for the prevention and treatment of infection. Finally, Unit Four presents a systematic approach to the infectious diseases of the various body systems.

SUMMARY

- Microorganisms are present everywhere and play an essential role in many processes that maintain life. Very few microorganisms actually cause disease.

THE IMPORTANCE OF MICROORGANISMS IN OUR ENVIRONMENT

- Microorganisms carry out many essential processes in the environment.
- The major groups of organisms of medical importance are viruses, bacteria, protozoa and fungi, and some multicellular parasites.

THE NATURE OF LIVING ORGANISMS

- Classification is the grouping together of organisms that have characteristics in common.
- Carl Woese proposed a three-domain system of classification, consisting of the Bacteria, the Archaea, and the Eucarya, which contains the kingdoms of Protista, Fungi, Animals and Plants.
- Microorganisms are named according to their genus, species and strain; they can also be classified according to their serological properties or type of nucleic acid.

PROCARYOTIC AND EUCARYOTIC CELLS

- All cells can be described as either procaryotic or eucaryotic.
- Procaryotic organisms are unicellular with a simple structure consisting of a cell wall located outside the plasma membrane. The cytoplasm contains enzymes, nutrients, chemicals, ribosomes and a nuclear area containing a single circular chromosome.
- All bacteria are procaryotic.
- Procaryotic cells reproduce asexually by binary fission.
- Eucaryotic organisms comprise all organisms other than the bacteria.
- Eucaryotic cells have a nucleus surrounded by the nuclear membrane. Other structures in the cytoplasm include the endoplasmic reticulum, ribosomes, the Golgi complex, mitochondria, lysosomes in animal cells, and chloroplasts in plant cells.

VIRUSES

- Viruses are not able to reproduce independently of a living cell. They cause infection in animal, plant and bacterial cells and are quite specific for the cell type they attack.

DISCOVERY OF THE CAUSES OF INFECTIOUS DISEASES

- Until the end of the 19th century it was thought that infectious diseases were due to miasmas.
- In the past, explorers spread diseases from the East to Europe, and later to the Americas and Australia.
- During the last 100 years, there has been a dramatic increase in knowledge and understanding of the microbial causes of infection.
- The development of suitable magnifying instruments was necessary in order to see microorganisms.
- Pasteur disproved the theory of spontaneous generation and showed that microorganisms were responsible for food spoilage.
- Scientists such as Semmelweis and Lister showed how transmission of infection could be prevented.
- In the 19th century Robert Koch proposed the germ theory of disease; he and other scientists isolated and identified the causative organisms of most of the then known diseases of bacterial origin.
- Work on malaria and yellow fever showed that some diseases are transmitted by mosquitoes. Plague is transmitted by fleas.
- The search for drugs to treat infectious diseases led to the synthesis of chemotherapeutic agents and the discovery of antibiotics.
- Edward Jenner used cowpox to prevent infection by smallpox; this laid the basis for modern vaccination programs.
- Some diseases have been eradicated (smallpox), are preventable (polio) or are controllable (plague).
- Research in immunology and molecular biology has increased our understanding of how infectious diseases affect the body and can be treated.
- A number of Australian scientists have made significant discoveries in microbiology.
- Improvements in public health have contributed to successful control of outbreaks of disease.
- The discovery of antibiotics and the introduction of vaccines have reduced mortality from infectious diseases.
- New diagnostic techniques help to quickly identify and track the organisms responsible for an outbreak of disease.

NEW AND EMERGING INFECTIOUS DISEASES

- The successful treatment of infectious diseases in the future is threatened by the emergence of new and resistant organisms.
- HIV/AIDS is the most serious of the 'new' diseases.
- A number of new viral diseases such as SARS and new strains of influenza A will pose a challenge in the future.
- Outbreaks of infectious diseases may occur as an outcome of a natural disaster.
- Bioterrorism is the use of biological (microbial) weapons.

STUDY QUESTIONS

1. Why is the study of microbiology important for the health professional?
2. On what method does Woese base his system of classification?
3. What are the major differences between eucaryotic and procaryotic cells?
4. Which organisms are procaryotic?
5. Describe some of the contributions made to microbiology by Louis Pasteur.
6. What is meant by the 'theory of spontaneous generation'?
7. Name the scientist responsible for proposing the germ theory of disease.
8. What contribution did Semmelweis make to our understanding of disease transmission?
9. Which important diseases are transmitted by insects?
10. Who discovered penicillin?
11. What is the origin of the word 'vaccination'?
12. Which infectious diseases are responsible for most deaths in the world today?

TEST YOUR UNDERSTANDING

1. What are the major scientific achievements that have contributed to a reduction in deaths from infectious diseases?
2. How do resistant strains of TB arise? Which groups of people are most vulnerable to TB?
3. How has our modern lifestyle contributed to the spread of infectious diseases?
4. What are some of the major successes in the treatment and control of infectious diseases?
5. Identify factors that contribute to the emergence of new infectious diseases.
6. How can bioterrorists achieve their goals?

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CHAPTER
2

Biological reactions in microbial cells

CHAPTER FOCUS

- Why is an understanding of biological reactions important for the health professional?
- How does the structure of a biological compound relate to its function?
- What is the role of enzymes in biological processes?
- What are the principal pathways of energy production in microorganisms?
- How are different classes of biological molecules interconverted?
- What metabolic processes carried out by microorganisms are of use to humans?

INTRODUCTION

All living cells, from the smallest microorganisms to the most complex animal cells, undergo continual processes of breakdown, synthesis, replication and repair. **Metabolism** is the overall term used to describe these processes and it refers to all the different reactions that must occur in a cell in order for it to grow and reproduce. The pathways of metabolism are remarkably similar in all living cells. The differences that do occur usually reflect the availability of nutrients or the need for a cell to carry out a specialised function—which may be secretion, storage, structural support, energy for movement, or reproduction. Much of our knowledge of the processes that occur in human cells has been derived from research on the major metabolic pathways that occur in microbial cells.

Catabolism is the process of breakdown of complex molecules, usually with the release of energy. **Anabolism** refers to the synthesis of new or replacement molecules. This is an energy-requiring process. A **metabolic pathway** is a series of reactions in the process of metabolism.

Although metabolic processes are complex, consisting of thousands of chemical reactions, an understanding of the basic concepts of metabolism does not require an extensive knowledge of chemistry. The reactions are logical and can be understood in a simplified descriptive form. Many students are unnecessarily deterred from attempting to study the biochemistry of cells, as they do not see its relevance to their work as health professionals. However, a knowledge of the processes that occur in all living cells will enable students to understand the chemistry underlying many of the phenomena they observe every day. For example:

- how pathogens invade, replicate and cause disease in a host
- why adequate nutrients are needed to provide energy for cellular activity, growth and repair
- why some microorganisms need a particular environment to survive
- how cells replicate and repair themselves
- the nature of the immune response
- the therapeutic use of drugs
- why some infections can be treated with antibiotics and others cannot
- how microorganisms are identified by biochemical tests
- how microbial reactions are used to produce compounds of use to humans.

This chapter assumes a basic knowledge of atoms and molecules, of ionic and covalent bonds, and some carbon chemistry. The metabolic pathways are described using diagrams, words and simple chemical formulae. Students can therefore study the reactions at a level suited to their chemistry background.

STRUCTURE OF BIOLOGICAL MOLECULES

All living matter is made up of a number of complex molecules containing the elements carbon, hydrogen, oxygen, nitrogen, phosphorus and sulfur, with lesser (trace) amounts of other elements. The way in which the atoms of these elements are arranged determines the structure and unique function of each molecule. The most important element is carbon, a small atom with four electrons in its outer shell, capable of forming four covalent bonds. Compounds containing carbon are called **organic compounds**. When carbon combines with other atoms it forms a molecule with a particular shape (**stereospecificity**) (see Figure 2.1). The configuration of atoms in the molecules of carbon-containing compounds gives rise to the enormous diversity we observe in biological molecules; it is also responsible for the specific shape and function of these molecules.

Functional groups

A number of specific structures are found joined to carbon in various organic compounds and are essential for the activity of these compounds. They are called 'active', or 'functional', groups. They include:

- Carboxylic acid—COOH in fatty acids (lipids), acetic acid (vinegar), amino acids (proteins)
- Amino—NH_2 in amino acids (proteins)
- Hydroxyl—OH in alcohols and glycerol (carbohydrates)
- Sulfhydryl—SH in proteins
- Organic phosphate—R CH_2 OPO_4 in phospholipids and nucleic acids
- Ester linkage—RC = OO R in triglycerides.

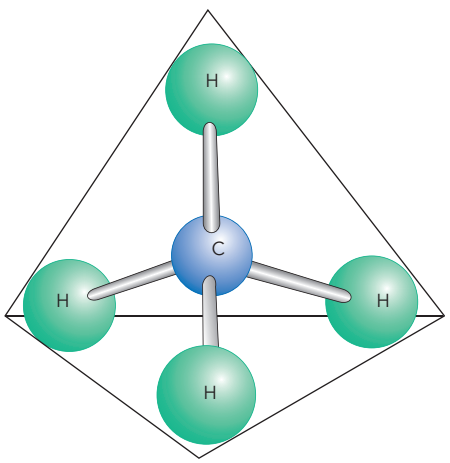


FIGURE 2.1

Structure of carbon-containing compounds

A carbon atom with four single bonds has this shape because of the direction of the bonds. The tetrahedral molecule of methane, CH_4, shown here, contains carbon bonded to four hydrogen atoms.

Another property conferred on biological molecules containing carbon is **stereoisomerism**. Because the carbon atom is linked to four other atoms, it often forms compounds which, although they have the same formula (composition), do not have the same structural configuration; they are mirror images and cannot be superimposed on each other (see Figure 2.2). This can be likened to a pair of gloves, where the right-hand glove cannot be substituted for the left because it has a different configuration.

Specificity

One of the most fascinating properties of biological molecules is their *specificity*—that is, the ability of a particular structure in a molecule to recognise and fit together with a complementary structure in another molecule. You may have experienced the frustration of having different pieces of electrical equipment that do not fit together—for example, having the wrong lead for a machine, or a plug and socket that do not 'match'. A similar situation occurs in living cells. Biological molecules contain particular chemical groups in unique structures that confer certain properties on the molecules. The way in which these molecules interact forms the basis for the regulation of all life processes. If the structures of two molecules are compatible, then those molecules can combine and interact in the same way that an electric plug will fit into a compatible socket (see Figure 2.3). To take this analogy further, sometimes an 'adaptor' is needed to join two pieces of equipment together. In biochemical terms, this adaptor molecule is called a **cofactor** or **coenzyme**.

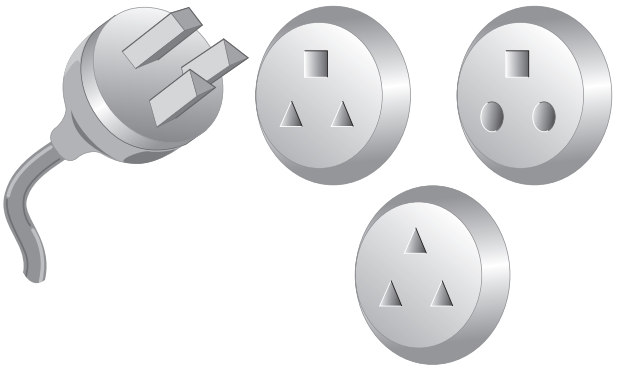


FIGURE 2.3

Specificity

The need for biological molecules to be the right shape in order to interact with each other is illustrated by this electrical plug and three different sockets.

The specific interaction between two compatible biological molecules determines which reactions can take place. It explains the unique mechanism of many important processes such as DNA replication, enzyme reactions, antigen–antibody interactions and the specificity of viral attack.

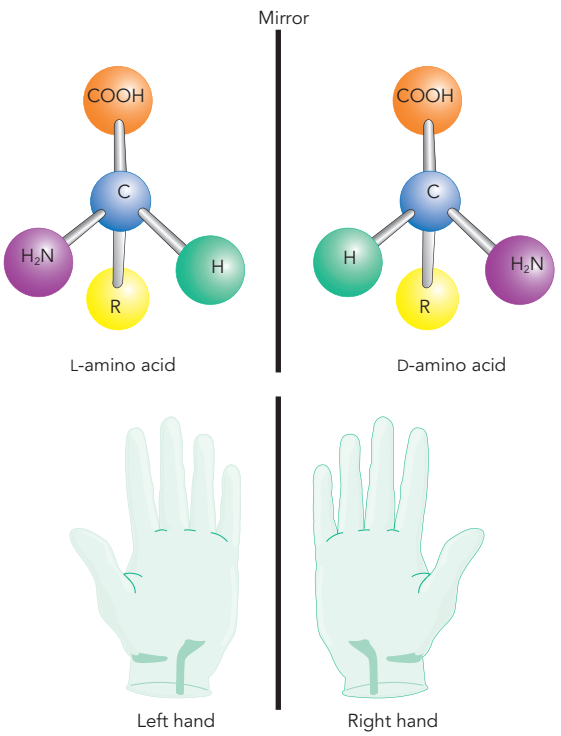


FIGURE 2.2

Stereoisomerism

Models of the L- and D- forms of an amino acid are mirror images and cannot be superimposed, just like the right- and left-hand gloves illustrated.

ENZYMES AND CHEMICAL REACTIONS

Metabolic processes involve thousands of chemical reactions. But what makes these reactions occur? A **catalyst** is defined as a substance that speeds up a chemical reaction by lowering the activation energy required for the reaction to take place. It takes part in the reaction but is itself unchanged.

What is **activation energy**? The simplest definition is that it is the amount of energy required to make a reaction occur. For example, if two substances such as hydrogen and oxygen are mixed, a reaction will not occur at a measurable rate. However, if some energy is applied (in the form of a spark or heat), the reaction occurs very rapidly. The necessary input of energy is called the activation energy. In industry, the amount of energy in the form of heat that needs to be added is often very great, but the addition of a catalyst (e.g. a metal) will allow the reaction to occur with a much lower energy input. In other words, *the catalyst lowers the activation energy*.

In biological systems the addition of large amounts of heat would destroy the cellular proteins, so an alternative way of providing the necessary energy has to be found. Living cells use **enzymes** which act as biological catalysts and lower the amount of energy (activation energy) required to get a reaction started. This is why enzymes are sometimes described as 'speeding up a chemical reaction' (see Figure 2.4).

Enzymes enable reactions to occur that might not otherwise happen; they give the reaction a 'push' so that it acquires a momentum of its own. They do this by providing a surface or site on which the reaction can take place, thereby allowing the reactant molecules to be held in close proximity to each other and increasing the efficiency of their interaction. All

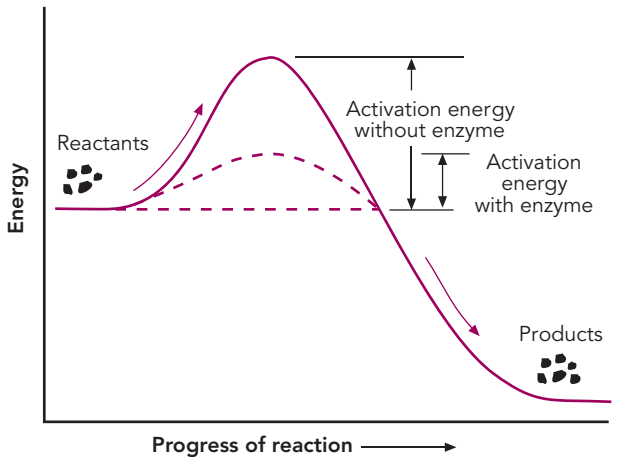


FIGURE 2.4

Activation energy

A chemical reaction cannot take place unless a certain amount of activation energy is available to start it. Enzymes lower the amount of activation energy needed to initiate a reaction. They thus make it possible for biologically important reactions to occur at the relatively low temperatures that living organisms can tolerate.

this is done at a temperature compatible with the normal activities of the cell.

Structure of enzymes

Enzymes are proteins produced in cells in response to the metabolic requirements of the cell. The genetic information needed for the synthesis of each particular enzyme is coded for in the DNA of the cell, together with information that enables the cell to synthesise all its other protein requirements (see Chapter 4). Each enzyme is able to act on a particular type of chemical compound, its **substrate**. The name of the enzyme is usually derived from the substrate it uses and the type of reaction that occurs. Most enzyme names end in *-ase*. For example, lactic dehydrogenase is an enzyme that removes hydrogen from its substrate, lactic acid.

Enzymes are proteins and are therefore made up of chains of amino acids. Each enzyme has its own unique sequence of amino acids and is folded in a certain way to give it a specific shape. Within this shape there is a particular location or area on the molecule called the **active site**. This site is rather like an electrical socket. It allows the attachment of a correspondingly shaped molecule or substrate. Once attached, this substrate can be modified or split; that is, it undergoes a metabolic reaction. It is then released and another substrate molecule takes its place and the reaction continues (see Figure 2.5).

There are hundreds of different enzymes in each cell. Every reaction that occurs in a living cell is controlled by a specific enzyme. Each enzyme has an active site specific for the shape of a particular substrate. Some enzymes contain both a protein and a non-protein component. The non-protein part, called a **cofactor** or **prosthetic group**, is necessary for enzyme activity. If the cofactor is an organic molecule, it is called a **coenzyme**. These coenzymes fit into the active

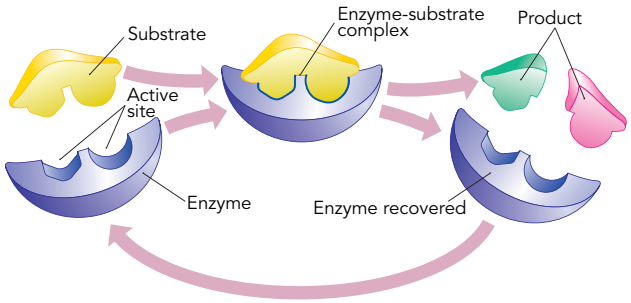


FIGURE 2.5

Enzyme-substrate complex

site like an adaptor (see Figure 2.6). Some coenzymes can be synthesised by the cell. Others must be obtained from the food supply and are considered **essential nutrients**. Some enzymes require the presence of divalent metal ions as cofactors for activity, such as magnesium (Mg^{++}), calcium (Ca^{++}), manganese (Mn^{++}) and zinc (Zn^{++}).

Bacteria are usually able to synthesise all the coenzymes they require, whereas most animals need to receive them in their diet. They are called essential nutrients or **vitamins** (e.g. folic acid). These metabolic differences can be exploited when developing drugs to selectively inhibit the bacteria causing an infection without harming the host (human) cells. This topic is discussed further in Chapter 12.

Factors influencing enzyme activity

As mentioned before, enzymes usually react preferentially with a particular substrate. Sometimes, however, they may bind to a closely related compound, or **analogue**. When a compound with a structure similar to the substrate binds reversibly or irreversibly to the active site and prevents the real substrate reaching the enzyme, the activity of the

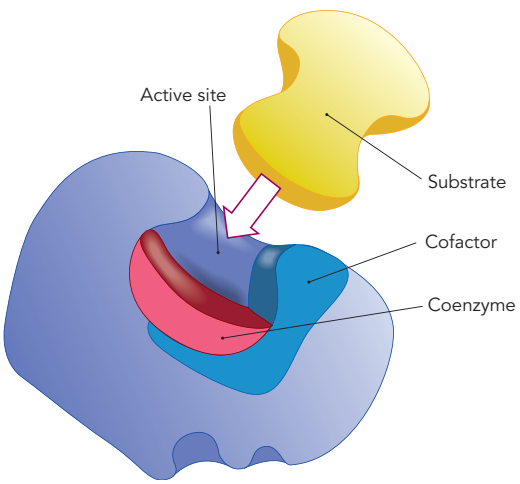


FIGURE 2.6

Components of an enzyme

Many enzymes require the protein portion of the enzyme as well as a cofactor (non-protein) portion for activity. Cofactors may be metal ions or organic molecules called coenzymes.

enzyme is inhibited, or an inactive product is formed. This property has been used to design nucleotide analogues, which are effective as antiviral drugs (see Chapter 12).

Enzymes have been found to function optimally at certain pH values and salt (ionic) concentrations. This is because a change in pH or ionic concentration may alter the electric charge on the protein molecule and therefore its shape. If the shape of the active site is altered, the substrate may not be able to attach. Temperature also affects the rate at which enzyme reactions take place. Bacteria therefore reproduce fastest at their optimum temperature.

It is important to remember all these factors when considering the metabolic reactions described below. Environmental factors can also exert some influence but, in general, microorganisms have shown themselves to be particularly adaptable to changes in their environment and can utilise a range of nutrients depending on what is available.

ENERGY PRODUCTION IN BIOLOGICAL SYSTEMS

The basic metabolic process of life is the production of energy for use in other cellular processes. Initially, all energy comes from the sun, the radiant energy being trapped by the chlorophyll in the cells of green plants. In a process known as **photosynthesis**, the basic molecules of carbon dioxide (from the air) and water are converted to a simple carbohydrate molecule, glucose (containing six carbon atoms). In the process, oxygen is released. The removal of carbon dioxide from the atmosphere and the release of oxygen enables the survival of oxygen-requiring organisms such as humans, other animals and many microorganisms. This can be written as:

Photosynthesis

$$6\,CO_2 + 6\,H_2O + \Delta E \rightarrow C_6H_{12}O_6 + 6\,O_2$$

carbon dioxide + water + energy (from sun) → glucose + oxygen

The energy from the sun that has been trapped and used to produce glucose is released when a molecule of glucose is broken down during metabolism. This complex process is called **oxidation**, or **respiration**, and consists of a number of interrelated biochemical pathways, including those of **glycolysis**, **fermentation** and **aerobic respiration**. Reactions that occur in the presence of oxygen are described as **aerobic**. Glycolysis and fermentation, which can occur in the absence of oxygen, are described as **anaerobic**. Many of these reactions require the involvement of various cofactors as well as enzymes, and result in the production of energy, which is trapped in a small molecule called **adenosine triphosphate** (**ATP**). ATP is termed an 'energy-rich storage molecule' because the subsequent breakdown of ATP releases energy for use in other reactions in the cell.

In aerobic respiration (e.g. in muscle cells), glucose is completely broken down in a series of steps to form carbon dioxide and water, with the release of considerable amounts of energy. The reaction can be written as:

Respiration

$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O + ATP$$

glucose + oxygen → carbon dioxide + water + energy

It is obvious that this is the reverse process to photosynthesis.

There are many other reactions that take place, depending on the needs of the cell and the availability of oxygen. The reactions described in this chapter are of importance in microbial cells.

The energy released during the breakdown of the glucose molecule in living cells is used to form ATP. ATP is a small, water-soluble, biological energy-storage molecule which can diffuse around the cell and provide energy for other reactions in the cell. ATP consists of a molecule of the purine base, adenine, joined to the pentose (5-carbon) sugar, ribose, and then to three phosphate groups (see Figure 2.7). Each of these phosphate groups has a different 'bond energy'—that is, the amount of energy required to form or break the bond. The third phosphate bond, the 'triphosphate', is very labile; that is, it is readily broken with the release of a large amount of energy. It also requires a large amount of energy to form it. The triphosphate bond is therefore referred to as a 'high-energy bond'. The base-ribose unit is called a **nucleoside**, and the base-ribose-phosphate is a **nucleotide**. ATP is also one of the building blocks of the nucleic acids, RNA and DNA.

For a molecule of glucose to be broken down or metabolised, it must first acquire some energy, or be 'activated'. This occurs through the removal of one phosphate group from a

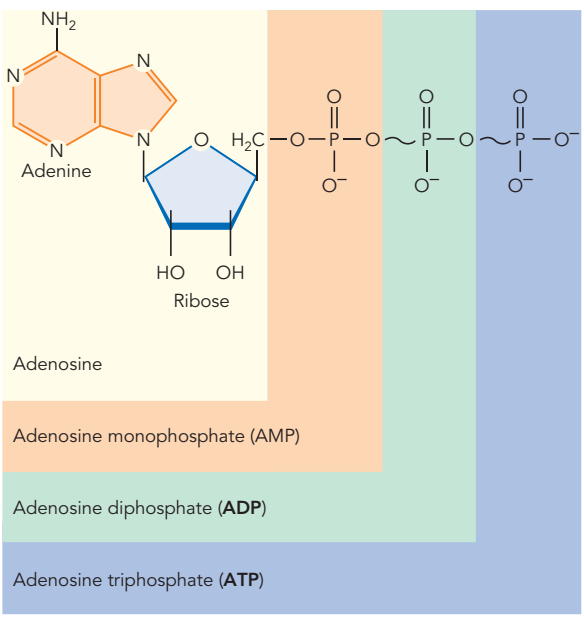


FIGURE 2.7

Structure of ATP

ATP is composed of the purine base, adenine, joined to the sugar, ribose and three phosphate groups. The addition of the third phosphate group to ADP requires a large amount of energy, which is released when the bond is broken.

molecule of ATP—that is, the conversion of ATP to ADP (adenosine diphosphate) and the transfer of the phosphate group to glucose:

$$\text{glucose} + \text{ATP} \rightarrow \text{glucose-6-phosphate} + \text{ADP}$$

The 'activated' glucose molecule (glucose-6-phosphate) is now ready to participate in other cellular reactions.

The processes that occur in living cells can be likened to those in a complex piece of machinery. Fuel (in the form of nutrients) is broken down (oxidised, metabolised) to provide energy to carry out the functions of the machine. Manufactured engines, however, waste a lot of energy as heat, whereas living cells are highly efficient and use sophisticated energy-capturing systems such as ATP and other complex molecules to conserve energy.

Energy requirements of microbial cells

Microbial cells require energy to carry out a range of activities necessary for their growth and reproduction. These activities include:

- the synthesis of lipids, carbohydrates, enzymes and other types of proteins
- the formation of the various structural components of the cell
- the repair and maintenance of the cellular environment
- the accumulation and storage of nutrients and the disposal of waste products
- the active transport of substances into and out of the cell
- the movement of flagella and cilia.

STRUCTURE OF BIOLOGICAL MOLECULES

We now consider in more detail the structure of the biological molecules that make up living cells—carbohydrates, lipids, proteins and nucleic acids—and briefly describe the metabolic pathways involved in their synthesis and breakdown. Detailed discussion of all these pathways is beyond the scope of this text. However, a brief description of the major classes of molecules is given here, together with the most common pathways of energy production and biosynthesis.

Structure of carbohydrates

Carbohydrates (sugars) are a group of compounds composed primarily of carbon, hydrogen and oxygen, and their breakdown is the major source of energy in cells. The most common sugar unit is glucose. Glucose is a **hexose**, a 6-carbon compound containing six hydroxyl (OH) groups. Because of the stereoisomerism of the carbon atom, these hydroxyl groups can be arranged in different ways, giving rise to different **isomers** of glucose. In the cell, glucose exists in a ring form with a three-dimensional shape (see Figure 2.8).

The most abundant sugars are the 6-carbon sugars, which include glucose and fructose. Another important group—the **pentoses**—contain five carbon atoms. The most important sugars in this group are **ribose** and **deoxyribose**, which occur in nucleic acids.

Monosaccharides, disaccharides and polysaccharides

Sugars are found in nature as single units (**monosaccharides**), double units (**disaccharides**) or polymers (**polysaccharides**). The monosaccharides include the hexoses and pentoses described above. Disaccharides and polysaccharides are made up of basic hexose units joined together. The way in which they are linked (i.e. the direction of the bond) is referred to as an α -linkage or a β-linkage and determines the shape of the molecule (see Figure 2.9).

The most common disaccharide is sucrose, which consists of one molecule of glucose and one of fructose joined together. There are a number of different polysaccharides which have quite different characteristics that are determined by their structure (linkage). Cellulose is a straight chain polymer and a major component of plant cells—providing fibrous structural support. Starch is a storage polysaccharide found in potatoes and other root vegetables. It provides an easily digested source of energy. Animal cells contain glycogen as their storage polysaccharide. Dextran is a polymer of the glucose isomer dextrose and is used clinically as a blood plasma substitute.

Complex polysaccharide compounds located on the outer walls of some cells are responsible for conferring antigenic properties on the cell.

Structure of lipids

Fats, or triglycerides, are lipids consisting of glycerol and long-chain fatty acids. **Glycerol** is a 3-carbon compound containing three hydroxyl (OH) groups. **Fatty acids** are long chains of carbon and hydrogen (usually 16 or 18 carbon atoms in length) with a single carboxyl (COOH) group at one end. Triglycerides consist of a glycerol molecule combined with three long-chain fatty acids joined by ester linkages (see Figure 2.10).

Another important group of lipid molecules is the **phospholipids**. These consist of glycerol esterified with two long-chain fatty acids. The third position is occupied by a phosphate group, which can also be joined to another organic molecule such as choline. Phospholipids are an integral component of all cell membranes (see Chapter 3).

In phospholipids, one fatty acid chain is replaced by a phosphate group linked to an organic compound such as choline.

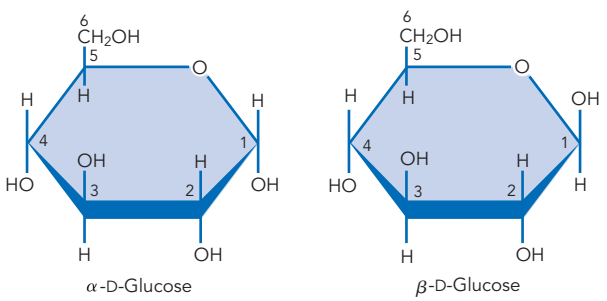
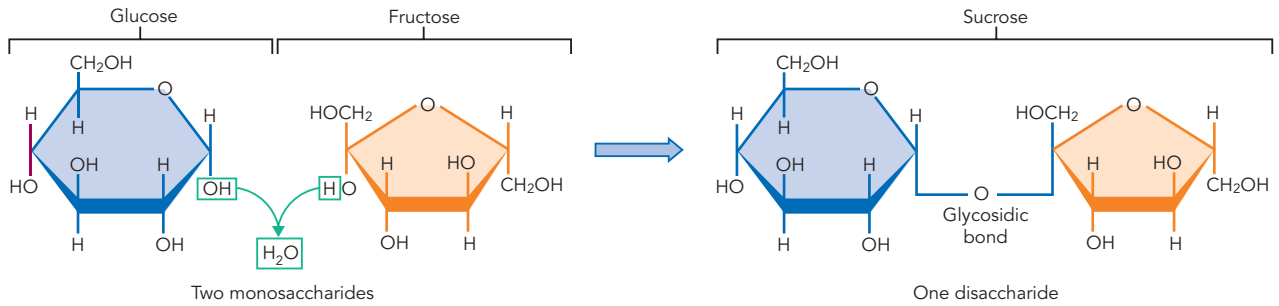


FIGURE 2.8

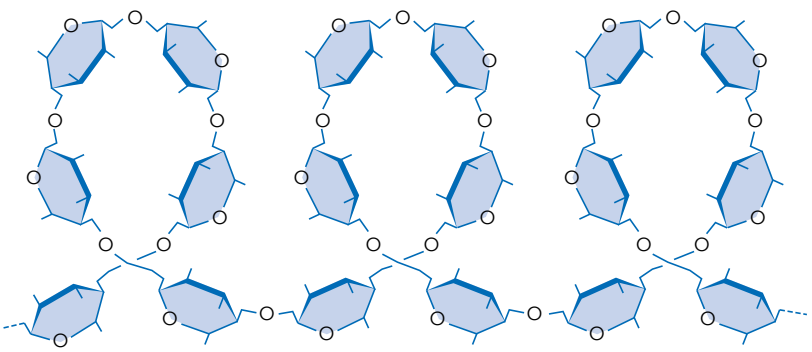
Ring structure of glucose

Sugars exist in biological molecules in a three-dimensional ring form.

(a) Formation of sucrose



(b) Structure of starch



(c) Structure of cellulose

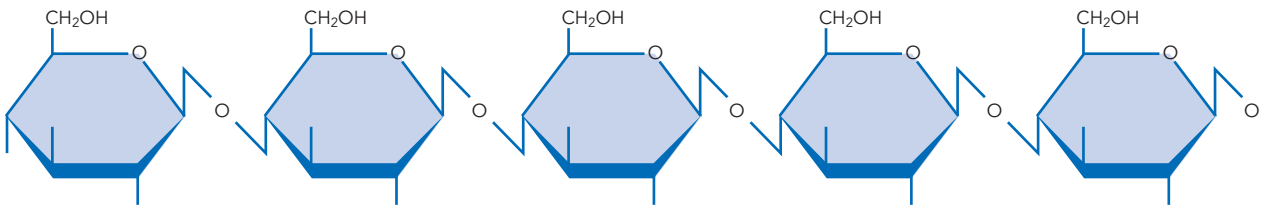


FIGURE 2.9

Structure of carbohydrates

(a) Two monosaccharides are joined to form a disaccharide by the removal of water and the formation of a glycosidic bond; (b) polysaccharides such as starch are formed by the linking of many monosaccharides in long chains; (c) structure of cellulose, β 1–4 linkage, gives rise to a straight-chain polymer which cannot be broken down by human enzymes.

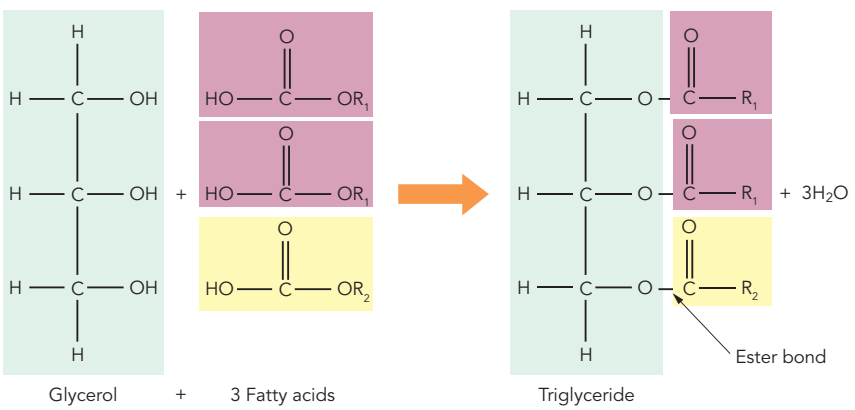


FIGURE 2.10

Structure of a triglyceride

Three long-chain fatty acids are combined with (esterified) a molecule of glycerol. The chain length and saturation of the fatty acids determine the properties of the lipid.

Structure of proteins

Proteins consist of chains of amino acids arranged in a specific sequence. There are 20 different naturally occurring amino acids that serve as the building blocks of proteins. Amino acids are a group of organic molecules that all contain an amino ($-NH_2$) group and a carboxyl ($-COOH$) group attached to various side-chain groups (usually designated R, R_1, R_2, etc.). The differences in the side-chain groups confer different properties on the amino acids. For example, glutamic acid has a carboxyl group in its side chain, so it is quite acidic; lysine has an amino group, so it is basic; cysteine has a sulfhydryl (SH) group, so it is capable of forming disulfide bridges (S–S) within the protein molecule. The structures of the 20 naturally occurring amino acids are shown in Figure 2.11.

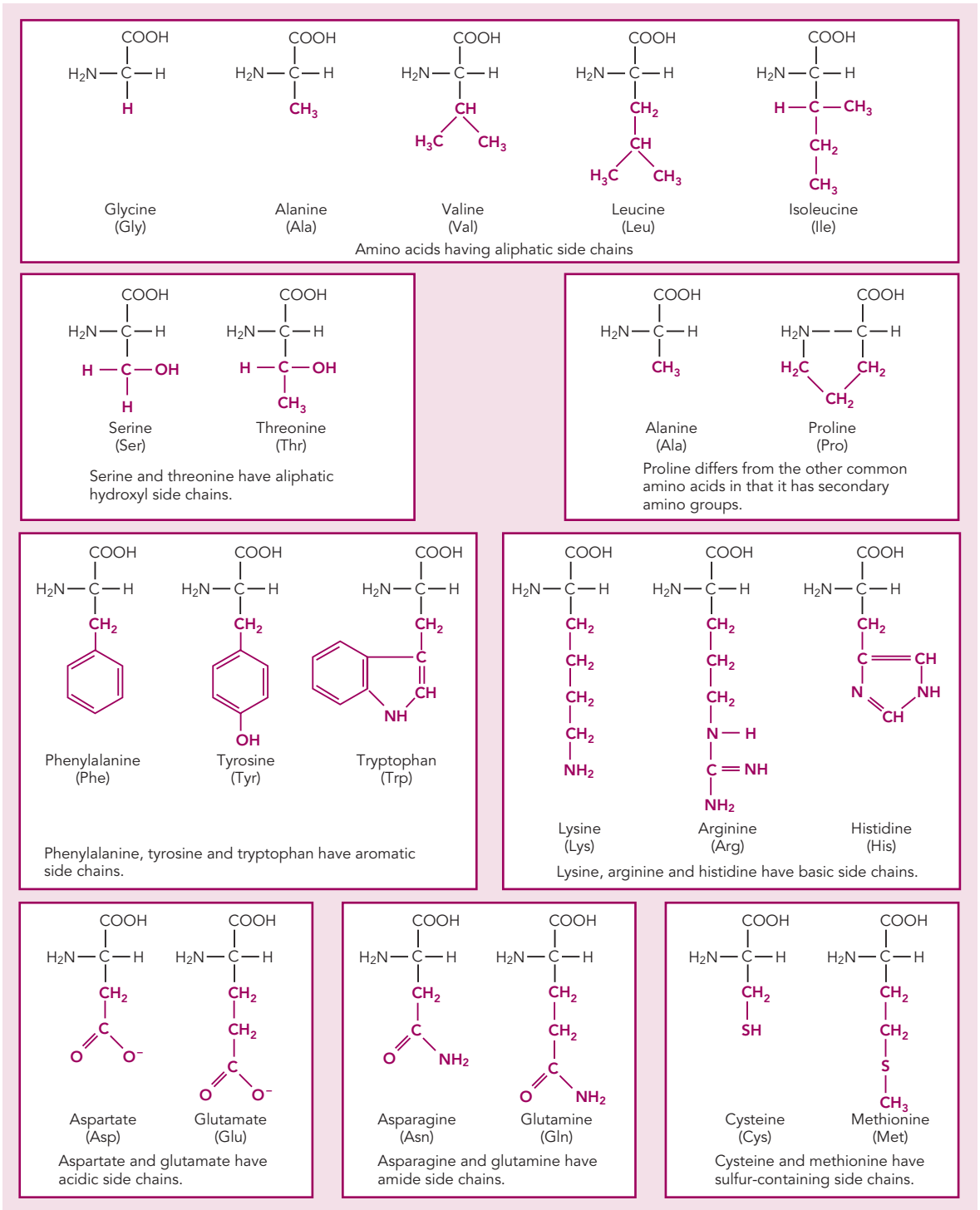


FIGURE 2.11

Structures of the 20 naturally occurring amino acids

The nature of the R side chain confers special properties on the amino acid molecule.

When two amino acids join together, they do so by the removal of a molecule of water from the amino and carboxyl groups of adjacent acids, forming a peptide bond (Figure 2.12). Short sequences of amino acids joined together are called **peptides**; longer ones are called **polypeptides**. Proteins are long polypeptide chains, folded and cross-linked to form compounds with a specific molecular structure.

The nature of the side chains of the amino acids affects the overall structure of the protein. The size, shape and charge of each amino acid determine the ability of the protein to fold and assume a particular conformation and activity (Figure 2.13). When this conformation is disrupted, the proteins are said to be 'denatured' and their function, or enzyme activity, may be lost.

BIOCHEMICAL PATHWAYS OF ENERGY PRODUCTION

Breakdown of polysaccharides

The ability of an organism to utilise different carbohydrates as an energy source depends on its ability to synthesise the appropriate enzymes. For example, human saliva contains the enzyme amylase, which can break the *a*-linkages found between the glucose units in starch (see Figure 2.9(b), above). However, humans cannot synthesise the enzyme which breaks the β 1–4 linkage that occurs between the glucose molecules in cellulose (Figure 2.9(c)), and so they are unable to metabolise cellulose. Cellulose therefore acts as fibre, or roughage, in the human diet and aids in digestion and formation of faeces without providing any energy.

Many microorganisms, however, produce an enzyme which can break the β-linkages and release single glucose units. Ruminant animals such as cows rely on the bacteria present in their stomachs to break down cellulose, which is the primary polysaccharide in grass and other fibrous plants. The ability of soil microorganisms to break down cellulose is important for the decomposition of plant organic matter.

Starch, with its branched structure, is mainly a storage carbohydrate. It can be utilised by many organisms, including humans, as a source of energy. Enzymes in the mouth

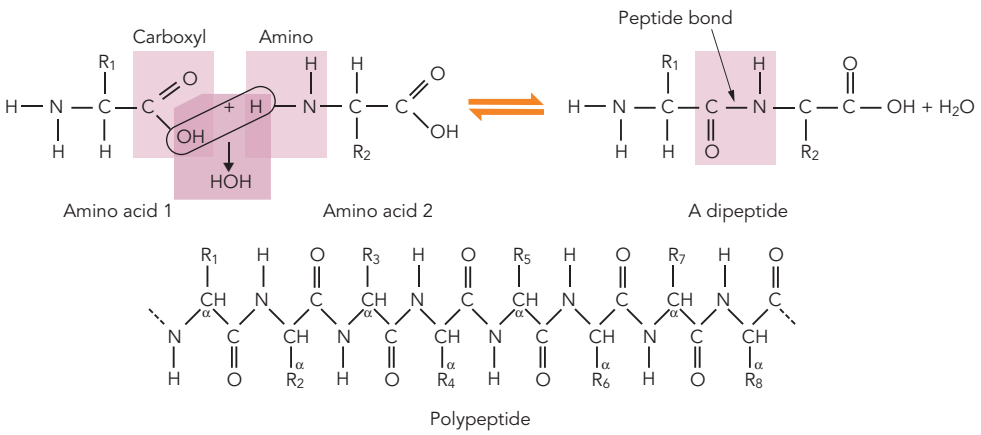
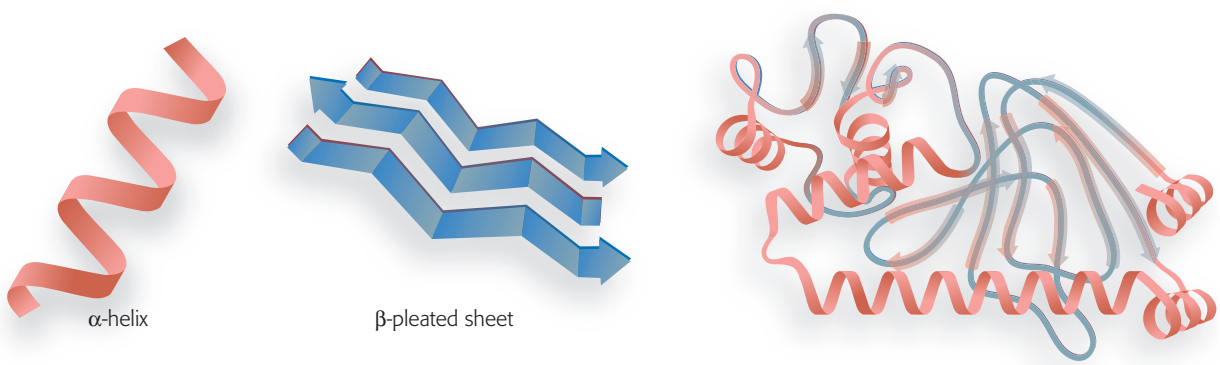


FIGURE 2.12

Synthesis of a peptide bond

Two amino acids join together to form a dipeptide. Water is eliminated from adjacent molecules to form the peptide bond.



(a) Secondary structure: α-helix or β-pleated sheets held together by hydrogen bonds.

(b) Tertiary structure: three-dimensional shape of proteins involves hydrogen bonds and covalent bonds.

FIGURE 2.13

Structure of proteins

(salivary amylase) and small intestine (pancreatic amylase) break it down to the disaccharide, maltose, for digestion and absorption. Most microorganisms use carbohydrates as their primary source of energy. They are broken down by various reactions to single glucose units, which then enter the pathways described below.

Breakdown of glucose

This is a brief overview of the principal pathways of glucose catabolism in microorganisms. The first stage is called **glycolysis**, or the **Embden-Meyerhof pathway** (see Figure 2.14(a)). It consists of ten reactions, each catalysed by a different enzyme, and is essentially the same in all living

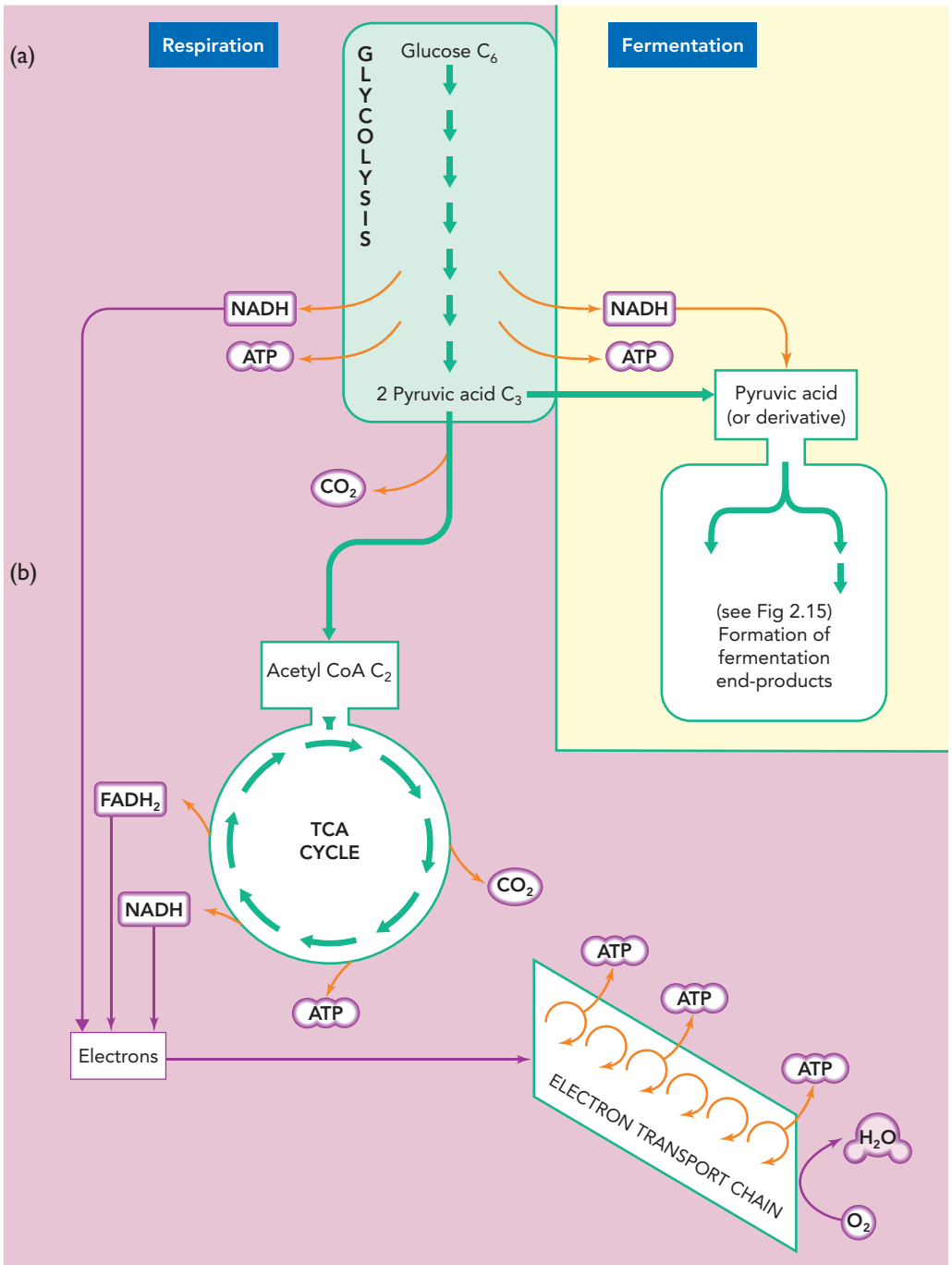


FIGURE 2.14

Overview of glucose metabolism

(a) Embden-Meyerhof pathway of glycolysis
One molecule of glucose gives rise to two molecules of pyruvic acid with the overall yield of two molecules of ATP.
(b) The reactions of the TCA cycle
Pyruvic acid from glycolysis is converted to the two-carbon intermediate acetyl CoA which enters the cycle. Each turn of the cycle results in the release of two molecules of CO_2. ATP is produced by substrate-level phosphorylation and by oxidative phosphorylation via the electron transport chain.

cells, plant, animal or microbial. It does not require the presence of oxygen. During this process, each molecule of glucose (six carbon atoms) is broken down to form two molecules of **pyruvic acid** (three carbon atoms), yielding only a small amount of energy. A number of other sugars, including fructose, can enter the glycolytic pathway and be converted to pyruvic acid, but the pathways involved are not described here.

Pyruvic acid is an important intermediate that can be further metabolised, either aerobically (respiration) or anaerobically (fermentation). Under aerobic conditions, **respiration** occurs, in which pyruvic acid is first converted to a two-carbon compound called **acetyl CoA**, which then undergoes a series of reactions known as the **Krebs cycle**, or **tricarboxylic acid (TCA) cycle**. This cycle leads to the formation of carbon dioxide and water, and large amounts of energy are released and stored as ATP. Acetyl CoA is a central intermediate that also occurs in the metabolism of lipids and proteins.

Respiration

Organisms that are capable of aerobic respiration oxidise pyruvic acid (three carbon atoms) to acetyl CoA (two carbon atoms), which then enters the Krebs, or tricarboxylic acid (TCA), cycle (Figure 2.14(b)). The TCA cycle provides a mechanism whereby molecules of acetyl CoA continuously enter the cycle and are broken down to carbon dioxide (CO_2) and water with the release of large amounts of energy in the form of ATP. A supply of inorganic phosphate (Pi) is necessary for these reactions to occur.

The intermediate steps require the transfer of electrons to the coenzyme, nicotinamide adenine dinucleotide (NAD), which is part of the electron transport chain used to convert the energy from these reactions into ATP. The TCA cycle also provides intermediate compounds that link into pathways involved in the synthesis of lipids, proteins and nucleic acids (see Figure 2.16, page 36).

Oxidative phosphorylation and the electron transport chain

In the presence of oxygen, pyruvic acid is oxidised via the TCA cycle with the release of large amounts of energy. The cell uses a process called **oxidative phosphorylation** to capture this energy in the storage molecule, ATP. The process involves the transfer of electrons from the reduced coenzyme, NADH, along a series of specialised carrier molecules located in the cell membranes to the final electron acceptor, molecular oxygen. This is known as the **electron transport chain**. It is a complex system comprising a series of steps in which each oxidation reaction is linked to a reduction reaction—that is, the energy released from one reaction is immediately used to carry out another reaction and to synthesise ATP.

Summary of glucose oxidation

The overall process of the aerobic oxidation of one molecule of glucose can be written:

$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O$$
glucose + oxygen → carbon dioxide + water

Each molecule of glucose produces 38 molecules of ATP.

The energy equation can be written:

38 ADP + 38 Pi → 38 ATP
adenosine diphosphate + inorganic phosphate → adenosine triphosphate phosphate

Fermentation of pyruvic acid

Under anaerobic conditions pyruvic acid undergoes **fermentation**, which is the term used to describe catabolic reactions that occur in the absence of oxygen. The energy yield is low compared to respiration. In microorganisms a number of different fermentative pathways may be followed, some of which give rise to useful products (see Figure 2.15).

No single organism has all the enzymes required for all these pathways. In fact, most organisms use only one pathway. Microbiologists can use this property to identify a particular organism by characterising the end products they release into the environment from fermentation reactions. Conversely, pure cultures of specific microorganisms can be added to a particular substrate to produce a desired product. Many of the products of microbial fermentation are of great use to humans. For example, yeast (*Saccharomyces*) is added to grape juice to produce alcohol in wine-making; yeast is also added to bread to produce carbon dioxide, which causes the bread to rise; and lactobacilli are added to various milk products to induce 'souring' during the production of cheese and yoghurt. These processes are described in more detail later in this chapter (see Table 2.1, page 39).

Anaerobic respiration

Some microorganisms use inorganic substances as the final electron acceptor instead of oxygen. For example, some species of *Pseudomonas* and *Bacillus* can use the nitrate ion (NO_3^-) as the acceptor, forming nitrite (NO_2), nitric oxide (NO) or nitrogen gas. Others use sulfate or carbonate. The amount of ATP formed is not usually as great as that produced when molecular oxygen is the final electron acceptor, but is quite significant.

The metabolic reactions described above are for glucose, which is the primary source of energy in most cells. However, lipids and proteins are also broken down with the release of energy and the formation of small molecules (building blocks) that can be used in other cellular processes.

Breakdown of lipids

Triglycerides are readily broken down by enzymes called **lipases**, which split the fatty acids from the glycerol molecule. The glycerol is then converted to dihydroxyacetone phosphate, an intermediate in glycolysis. The fatty acids are broken down by β-oxidation into two-carbon units of acetyl CoA which enter the TCA cycle. The metabolism of fats therefore yields large amounts of energy.

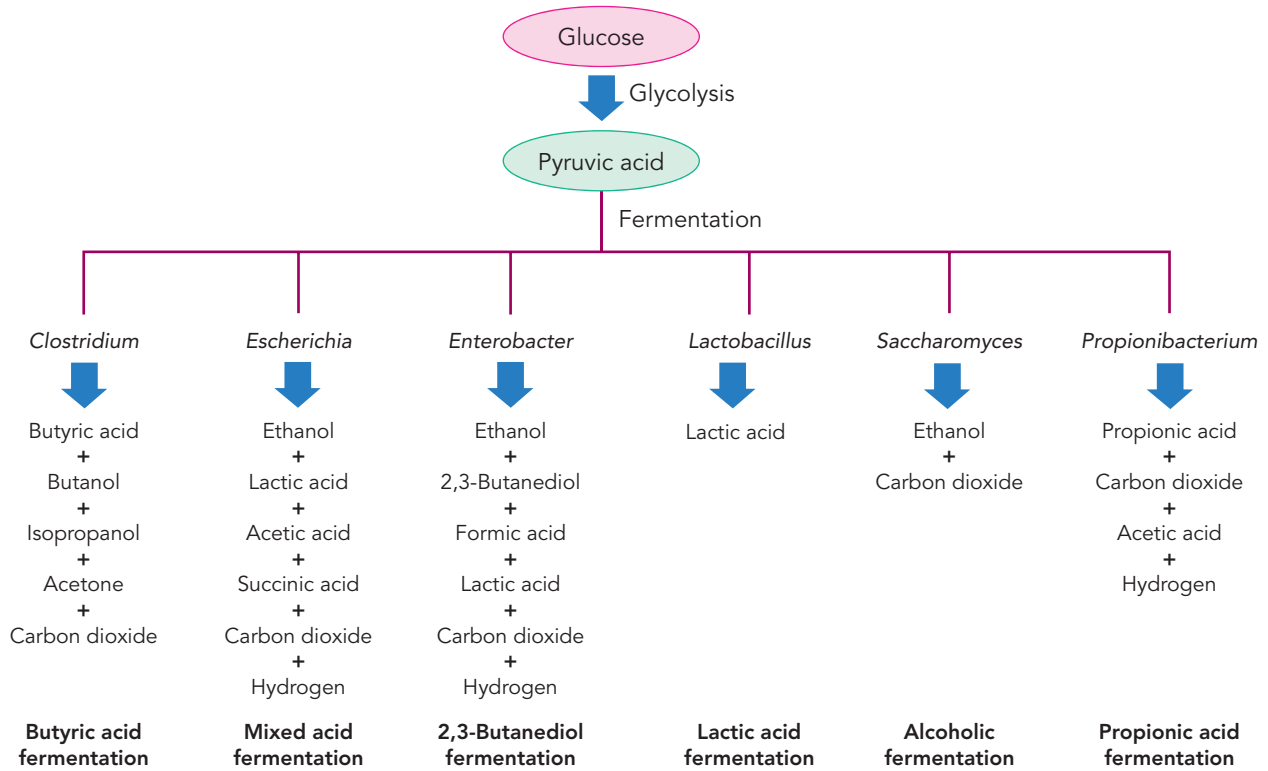


FIGURE 2.15

Major pathways of fermentation

Different microorganisms use characteristic fermentative pathways. The products of fermentation can be used to aid in identification of the microorganisms.

Breakdown of proteins

Proteins are broken down in most bacterial cells by proteolytic enzymes (called proteases or peptidases) to form smaller peptide fragments or single amino acids. The amino acids are reused for protein synthesis or are metabolised further. The removal of the amino group gives rise to compounds that can enter the TCA cycle and be broken down, producing energy in the form of ATP.

ANABOLISM—BIOSYNTHESIS OF CELLULAR COMPONENTS

The energy released in the reactions described above is used by the cell in a number of different ways. ATP is needed for the synthesis of the chemical components of the cell—that is, the carbohydrates, lipopolysaccharides, RNA and DNA, structural proteins and enzymes, the cell wall and the phospholipids of the cell membrane. The small building blocks of these complex macromolecules are activated by combining with ATP so that they have sufficient energy to enter their respective biosynthetic pathways.

Energy is also used for active transport of substances into and out of the cell and for the movement of flagella and cilia.

Biosynthesis of carbohydrates

Microorganisms utilise different pathways for the synthesis of carbohydrates, depending on the availability of nutrients and the particular needs of the organism.

Autotrophs are organisms that can use carbon dioxide from the air as their primary source of carbon; that is, they can live and reproduce without access to complex molecules. They are able to use energy from the sun during photosynthesis to synthesise glucose from carbon dioxide with the release of oxygen. These organisms include photosynthetic bacteria (cyanobacteria, green sulfur and purple sulfur bacteria), algae and green plants.

Heterotrophs, which include most bacteria, fungi and protozoa, must be provided with a source of organic carbon in their environment in order to synthesise glucose and larger polysaccharides. The intermediates in the TCA cycle, or other breakdown products from lipid or protein metabolism, can be a suitable source. Depending on the needs of the cell and the availability of energy in the form of ATP, various biosynthetic pathways are utilised which are the reverse of (or parallel to) those described for the breakdown of carbohydrates. However, some microorganisms have strict nutritional requirements; these are described in Chapter 3.

One important complex polysaccharide molecule that is synthesised only by procaryotic cells is **peptidoglycan**, a compound that provides strength and rigidity to bacterial cell walls. Peptidoglycan is a complex molecule composed of sugar molecules cross-linked by peptide bridges (see Chapter 3).

Biosynthesis of lipids

Bacterial lipids are usually formed by the condensation of long-chain fatty acids with a molecule of glycerol to form triglycerides. The fatty acids are synthesised from units of acetyl CoA, which occur as a breakdown product of carbohydrate metabolism.

Biosynthesis of amino acids

Amino acids are required for the synthesis of proteins and also serve as precursors for the purine and pyrimidine bases which are the building blocks of the nucleic acids RNA and DNA. Amino acids are synthesised in microbial cells by the addition of an amino group ($-NH_2$) to various intermediates in the TCA cycle. The nitrogen may be derived from ammonium salts (NH_4^+) or nitrates (NO_3^-), or from nitrogen in the atmosphere. Some bacteria are capable of using atmospheric nitrogen to form nitrogenous compounds in a process called **nitrogen fixation**.

Biosynthesis of proteins

The biosynthesis of proteins occurs under the direction of the genetic material of the cell, the DNA. It requires the participation of RNA and other nucleotides and is described in detail in Chapter 4.

INTERRELATIONSHIP OF METABOLIC PATHWAYS

Living cells are in a constant dynamic equilibrium of breakdown, synthesis and repair. The metabolic pathways are interrelated so that, for example, acetyl CoA molecules formed from breakdown of lipids can be used in the TCA cycle to produce energy; intermediates in the TCA cycle can be converted to amino acids and then incorporated into proteins. The integration of these processes allows for the most efficient use of nutrients and energy. Some microorganisms can synthesise all their cellular requirements from simple organic or inorganic compounds. However, microorganisms show great diversity in their nutritional requirements and some require more complex molecules such as sugars, amino acids, and vitamins or cofactors. Some mutant strains of bacteria may have an absolute requirement for a particular compound (e.g. a fatty acid or amino acid). Figure 2.16 illustrates the interrelationship between the pathways of bacterial metabolism. It is beyond the scope of this text to examine all these pathways in detail, but it is important to remember that most microorganisms of medical importance are **heterotrophic**—that is, they use complex carbon-containing molecules as their primary nutritional source. This property is useful for the identification and control of potential pathogens.

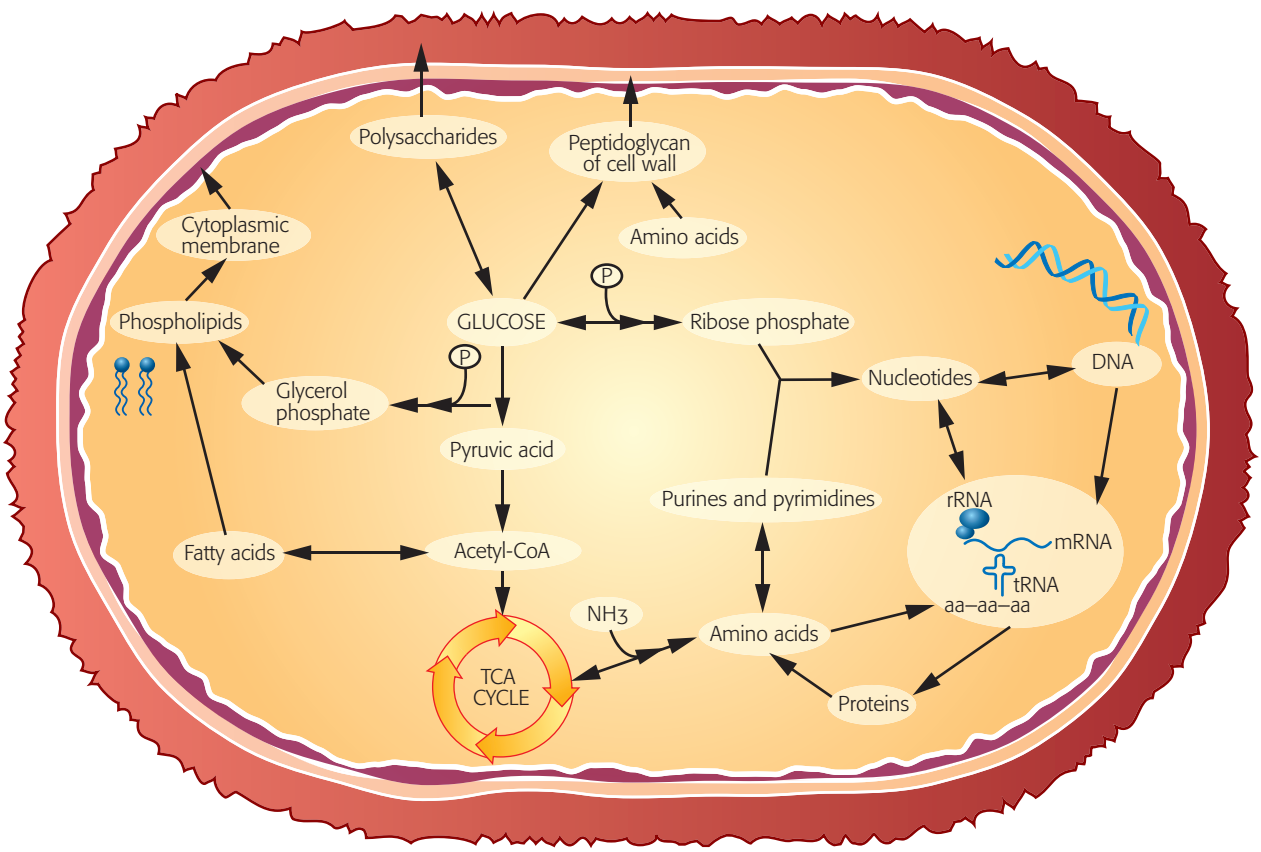


FIGURE 2.16

Interrelationship of metabolic pathways

Bacteria are able to synthesise all their cellular requirements when supplied with a simple carbon source, nitrogen (ammonia) and inorganic salts.

PRACTICAL APPLICATIONS OF MICROBIAL PROCESSES

The metabolic pathways described in this chapter represent only a small fraction of the reactions carried out by living cells. These reactions are controlled by specific enzymes produced by cells according to the information contained in their genes. In animal cells, the production of a set of enzymes by a cell is usually fixed, and reflects the function of that cell. If the required enzymes are not produced or are produced in the wrong amount, the workings of the cell are greatly affected. The measurement of the amount of enzyme produced by a cell can be used to diagnose whether a cell is functioning normally. For example, in humans, liver function tests measure the levels of different liver enzymes in blood to determine whether the liver is damaged by diseases such as hepatitis or cirrhosis.

In the microbial world, bacteria are able to adapt the metabolic pathways they use to the nutrients available. Because the bacterial cell is so small, it only synthesises enzymes for its immediate needs, even though it may carry the genetic information for other enzymes. When provided with alternative carbon sources it is often able to synthesise the enzymes necessary for metabolism of that compound. The metabolic pathways employed therefore reflect the environment in which the organism is generally found—for example, the sulfur bacteria occur in hot springs and synthesise enzymes that use sulfur compounds as electron acceptors.

The metabolic abilities of specific microorganisms are used by scientists in a number of areas including diagnostic microbiology, industry, scientific research and genetic engineering. The ability of microorganisms to break down large biological molecules is important for the decomposition of waste matter in the environment.

Diagnostic microbiology

In the laboratory most pathogens grow readily on plates made of a rich medium containing horse blood, 'blood agar', but in order to identify them they must be grown on additional media. The ability (or lack of it) to grow under different environmental conditions is used by microbiologists to differentiate between species of microorganisms that may appear morphologically identical. This is the principle behind the selective and differential media used in a diagnostic microbiology laboratory. A brief introduction is appropriate here to show how an understanding of metabolic pathways helps to identify different pathogens.

A **selective medium** is one to which one or more chemical compounds have been added to prevent the growth of certain microorganisms, but not others. The 'compound' is often something as simple as a high salt concentration. A **differential medium** is one to which some sort of indicator, usually a dye, has been added. This allows the clinical microbiologist to differentiate between various bacteria on the basis of chemical reactions that occur during growth.

Sometimes the medium is both differential and selective at the same time. For example, mannitol salt agar (MS agar) is used in the identification of a mixture of streptococci and different species of staphylococci. Staphylococci grow readily in the high salt concentration, whereas the growth of streptococci is inhibited. *Staphylococcus aureus* produces enzymes, which utilise mannitol (a sugar alcohol) and convert it to an acid that turns the pink indicator dye in the medium to yellow. *Staphylococcus epidermidis* appears similar under the microscope to *Staphylococcus aureus*, but does not metabolise mannitol, so there is no acid production and no colour change in the medium (see Figure 2.17).

A battery of different biochemical tests is used in the microbiology laboratory to assist in the identification of a particular microorganism, and many of these involve the measurement of an enzyme reaction. In effect, these tests detect the presence or absence of the enzyme required for a reaction similar to the ones described above, thus indicating the kind of organism that is present. Enzyme reactions that produce gases such as carbon dioxide or hydrogen can also be seen by the appearance of bubbles in the culture medium. Enzymic breakdown of sulfur compounds with the release of hydrogen sulfide is detected by reacting the gas with iron (ferric) compounds—as well as by its offensive smell!

Many commercial companies market diagnostic kits for the identification of microorganisms. These kits test for the presence of several different enzymes in a single specimen. Some of these reagents are very sensitive and can give a positive diagnosis when only a small amount of specimen is available. This topic is discussed further in Chapter 15.

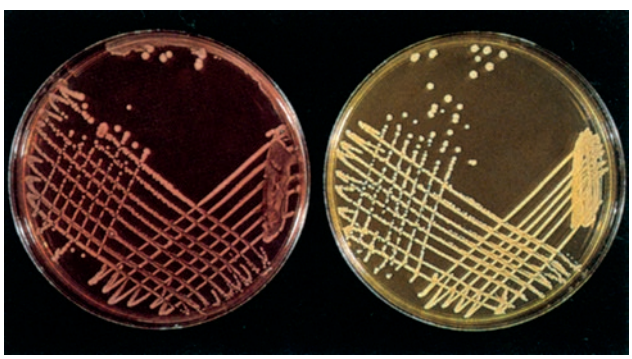


FIGURE 2.17

Mannitol-fermentation test

Growth on mannitol salt agar distinguishes different species of staphylococci. Staphylococcus aureus *ferments mannitol, producing acid, which turns the indicator in the medium to yellow.* Staphylococcus epidermidis *does not produce a colour change.*

Source: Dr Penny Bishop.

INDUSTRIAL APPLICATIONS OF MICROBIAL METABOLISM

Microorganisms are used in industry in a number of different ways—for example:

- as living cell cultures in the production of food
- in the preparation of purified enzymes
- as a source of primary or secondary metabolites.

Live microbial cells

Live cell preparations, such as yeasts, and lactic acid bacteria are widely used in the food industry. Fermentation of sugar by yeast cells under anaerobic conditions produces alcohol and carbon dioxide. For example:

- Wine is made from fermented grape juice. Wild yeast cells are found on grapes in nature. In practice, the wine they produce is of variable quality, so most winemakers add a pure culture of the yeast, *Saccharomyces ellipsoideus*, and control the fermentation by the amount of oxygen present. An understanding of metabolism can be used to achieve the desired product. When oxygen is low or absent, yeast fermentation produces mainly alcohol. When oxygen is available, carbon dioxide is also formed. When the alcohol concentration reaches 12–14 per cent the yeast cells die. Champagne is made by a secondary fermentation in which fresh yeast cells and sugar are added to still wine in a sealed bottle. The carbon dioxide produced cannot escape and remains as bubbles in the champagne.
- Beer is also a product of yeast fermentation. An extract of grains such as barley is used to make a sugar substrate, or 'malt'. Hops are added for flavour. Special strains of yeast, *Saccharomyces carlsbergensis* or *Saccharomyces cerevisiae*, are used.
- The leavening of bread also relies on the properties of the yeast cell. When added to the dough, yeast produces alcohol and carbon dioxide gas. The carbon dioxide causes the bread to rise. When the bread is baked, the alcohol and carbon dioxide evaporate, leaving the distinctive holes in the bread. Sourdough bread is made by adding cultures of lactic acid bacteria to the dough to produce the distinctive 'sour' taste.
- Lactic acid bacteria produce lactic acid by fermentation of glucose and are responsible for the 'souring' of milk to produce cheese, yoghurt, buttermilk and other products. Various other bacteria and some fungi are added to produce intermediate compounds which impart a distinctive appearance and flavour to cheeses (e.g. different strains of the mould *Penicillium* are used for blue cheeses and camembert).
- Cultures of the nitrogen-fixing bacterium *Rhizobium* are frequently inoculated on to leguminous plant seeds to encourage the formation of root nodules where nitrogen fixation can take place.
- Mushrooms are cultivated for food.

Microbial enzymes

Microorganisms can also provide a source of enzymes for use in industry. Some chemical reactions are difficult to carry out by non-enzymic means. As explained earlier in this chapter, enzymes are biological catalysts that allow chemical reactions to occur more efficiently by lowering the activation energy—often giving a much higher yield of product for a lower cost. Specific enzyme proteins can be extracted from large-scale preparations of microorganisms, purified and used in the commercial production of a

CASE HISTORY 2.1

Diagnosis of illness

A 10-year-old boy visited the GP's surgery complaining of a sore throat and looking flushed. On examination he was found to have a temperature of 38.4°C and his tonsils were red and swollen with areas of pus on them. The doctor also noticed that he had a number of infected sores on his arms and legs. His mother explained he had scratched some mosquito bites. The doctor took swabs from the boy's throat and also from the skin sores and sent them to the pathologist. He started the boy on some broad spectrum antibiotics.

In the microbiology laboratory, each specimen was cultured on blood agar and also on Mannitol salt agar. The skin specimen grew well on blood agar, and on mannitol salt it produced a yellowish colour. The throat specimen grew on blood agar, but there was no visible growth on mannitol salt agar.

Questions

1. What does this tell you about the bacteria present at each site?
2. What would be the preliminary identification of the organisms from each site?
3. Which infection do you think is most likely responsible for the boy's temperature (see Chapter 7)?
4. What further tests would be needed to decide whether the doctor had prescribed the best treatment (see Chapter 12)?

particular compound. Examples are protein-digesting enzymes (proteases in laundry detergents) and glucose isomerase, which converts glucose to fructose for use in the confectionery industry. Another useful enzyme is penicillin acylase, used in the manufacture of semi-synthetic antimicrobial drugs.

Primary metabolites

Many different organic compounds are produced by microorganisms in the metabolic reactions outlined in this chapter. **Primary metabolites** are products formed in the major pathways of fermentation. These compounds can often be obtained in sufficient yields to be of use commercially. Some of the more important metabolites are described below.

- Citric acid, an intermediate in the TCA cycle, is widely used as a flavouring in foods and beverages.
- Sorbose, produced when the bacterium *Acetobacter* oxidises sorbitol, is used to make ascorbic acid (vitamin C).
- Acetic acid (vinegar) is a product of the oxidation of alcohol (one of the products of fermentation) by members of the genera *Acetobacter* and *Gluconobacter*. Sometimes the *Acetobacter* are an accidental contaminant of food and cause acid production or 'souring'. Vinegar can be produced commercially from the alcohol present in wine or any other product of alcoholic fermentation. Vinegar is used to preserve (pickle) food because most harmful bacteria do not tolerate acid conditions.
- Vitamins and growth factors are used as food supplements for humans and in animal feeds. Most vitamins are made commercially by chemical synthesis, but some are too complex and so are produced by microbial fermentation. These include vitamin B12 and riboflavin.
- Some amino acids are produced by microbial means. Chemical synthesis of amino acids usually results in the formation of a mixture of the D- and L-stereoisomers. Naturally occurring amino acids exist only the L- form. Using microorganisms to produce the L-amino acid means that a pure product can be obtained. Some useful amino acids are:
  - the salt of glutamic acid, monosodium glutamate (MSG), used as a flavour enhancer
  - phenylalanine and aspartic acid, which are components of the sweetener, aspartame
  - lysine, an essential amino acid for humans, produced as a food supplement.

Some uses for products of microbial fermentation are listed in Table 2.1.

Secondary metabolites

Secondary metabolites are an interesting group of organic compounds, usually produced by microorganisms when nutrients have been depleted and the number of cells is no longer increasing rapidly. These compounds do not appear to be essential for growth or reproduction. The formation of these metabolites is limited to certain kinds of organisms and is very dependent on growth conditions. It is possible to select for specific strains of an organism in order to enhance the yield of the desired metabolite.

Antibiotics form one of the most important groups of secondary metabolites, and their discovery has had an enormous impact on the practice of medicine since the first commercial development of penicillin in the 1940s. More than 5000 antibiotic substances have been described, but most of them are too toxic for human use. Most commercially useful antibiotics are produced by filamentous fungi and bacteria of the actinomyces group (see Table 2.2). They are described more fully in Chapter 12.

TABLE 2.1 Some useful fermentation reactions

PRODUCT	USES	SUBSTRATE	MICROORGANISM
Lactic acid	Cheese, yoghurt Sauerkraut	Milk Cabbage	*Lactobacillus* spp.
Propionic acid and carbon dioxide	Swiss cheese	Milk	*Propionibacterium*
Ethanol	Beer Wine Fuel	Malt extract Grape juice Agricultural waste	*Saccharomyces cerevisiae* (yeast) *Saccharomyces ellipsoideus* (yeast) *Saccharomyces cerevisiae*
Acetic acid	Vinegar	Ethanol	*Acetobacter* (bacterium)
Glycerol	Industry/Food/Pharmaceutical	Molasses	*Saccharomyces cerevisiae*
Citric acid	Flavouring	Molasses	*Aspergillus* (fungus)
Sorbose	Vitamin C	Sorbitol	*Acetobacter*

TABLE 2.2 Commercial production of antibiotics

ANTIBIOTIC	MICROORGANISM	TYPE
Penicillin	*Penicillium chrysogenum*	Fungus
Cephalosporin	*Cephalosporium* spp.	Fungus
Bacitracin	*Bacillus subtilis*	Bacterium
Polymixin B	*Bacillus polymyxa*	Bacterium
Cycloheximide	*Streptomyces griseus*	Actinomycete
Streptomycin	*Streptomyces griseus*	Actinomycete
Erythromycin	*Streptomyces erythreus*	Actinomycete
Aminoglycosides	*Streptomyces* spp.	Actinomycete
Tetracycline	*Streptomyces rimosus*	Actinomycete

MICROORGANISMS AS TOOLS IN SCIENTIFIC RESEARCH

The metabolic processes that occur in microorganisms are very similar to those occurring in the cells of higher organisms. In fact, much of the scientific knowledge about the metabolism of animal cells was derived from laboratory research using bacteria. Bacteria are easy to grow in large numbers on a defined medium (i.e. controlled nutrients and growth conditions). They provide the research worker with a uniform population of cells, making it easier to interpret results. The simple genetic material (the DNA is all on one circular chromosome) provided the ideal system for early genetic mapping experiments. The techniques and knowledge obtained from this 'simple' system have now been adapted for use in more ambitious projects such as mapping of the DNA of the human genome. A knowledge of the processes of DNA replication in bacteria provided the scientist with a tool for manipulating the genetic information in cells—**genetic engineering**.

Genetic engineering

A very important use for microorganisms in recent years has been the production of specific compounds for medical use by the process of genetic engineering. The discovery and isolation of enzymes called restriction endonucleases—bacterial enzymes that were able to rupture strands of DNA at specific sites in the nucleic acid chain—allowed scientists to manipulate the structure of bacterial DNA. Scientists have developed methods of inserting genetic information into microbial cells in such a way as to direct the microbe to synthesise large amounts of a desired compound. Examples of genetic engineering include the production of human insulin, human growth hormone and some vaccines. Genetic engineering is described in more detail in Chapter 4.

ENVIRONMENTAL USES FOR MICROORGANISMS

Microorganisms play an essential role in the environment in decomposition and recycling of nutrients.

Decomposition

The catabolic reactions carried out by many microorganisms are essential for the decomposition of organic matter such as plant material and sewage. The enzymes produced by microorganisms break down complex carbohydrates and other biological compounds, releasing small organic molecules into the soil. These molecules are a source of nutrients for growing plants.

Hydrocarbon metabolism

Hydrocarbons are organic compounds containing only hydrogen and carbon, and are usually insoluble in water. Two examples are oil and petroleum. The chemical breakdown of hydrocarbons is a slow process, requiring oxygen. Very few microorganisms can utilise hydrocarbons for growth. The exceptions are some strains of *Pseudomonas*, *Nocardia* and *Mycobacterium*, and some yeasts and moulds. Cultures of *Pseudomonas* have been used to help disperse oil spills. Usually, the hydrocarbon metabolism would proceed too slowly to be of use, but it was found that the addition of other nutritional requirements, nitrogen and phosphate to speed up metabolism and growth of the bacteria enabled the oil to be broken down and dispersed.

Microorganisms have been used in bioremediation projects such as those undertaken at the Sydney Olympic site (see Spotlight box: Bioremediation—environmental uses for bacteria).

Bioremediation—environmental uses for bacteria

SPOTLIGHT ON

When the Homebush site in Sydney was chosen for the 2000 Olympic Games, one of the problems confronting the organisers was the fact that for many years the area had been used to dump the city's waste. Wilson Park is a 12-hectare portion of Crown Land adjacent to the Olympic site. It was used for the production of town gas for Sydney between 1953 and 1974, and approximately 230 000 tonnes of hydrocarbon (tar) waste remained buried on the site (see Figure 2.18(a)).

A feature of the Sydney 2000 bid had been the guarantee of a 'green games', so the main task was to clean up the site to a level where the risks to humans and the ecology of the area could be contained.

A number of toxic volatile organic compounds such as benzene, toluene, ethyl benzene and xylene were emanating from the buried tar and were found in the groundwater on site. The challenge was to remove these compounds and make the area safe.

Three Sydney scientists were commissioned by the Olympic Coordination Authority to undertake the project.

They found that there were high numbers of hydrocarbon-degrading bacteria present in the topsoil of the site. In the laboratory these bacteria were capable of completely metabolising benzene to carbon dioxide and water if simple plant fertilisers containing nitrogen and phosphorus were added to the cultures. The isolated bacteria included species of *Pseudomonas*, *Bacillus*, *Microbacterium*, *Actinomyces* and *Gordonia.* Some of these bacteria were similar to those used to clean up oil spills overseas.

The remediation project consisted of providing a healthy vegetated topsoil cover to act as a microbial biofilter. The enhanced bacterial activity in the soil was responsible for degrading the hydrocarbon gas pollutants and stopping their reaching the surface. In this way the park was made safe for human use once more (see Figure 2.18(b)).

FIGURE 2.18(a)

Aerial view of gasworks at Wilson Park, Silverwater, in 1962

FIGURE 2.18(b)

Wilson Park, 2000, after remediation and prior to Sydney Olympic Games

Source: Photos by D. Sheumack from D. Sheumack, M. Howe, B. Bicknell, M. Muir, J. Pym, E. Ling and K. Hughes 2000, Contamination assessment and bioremediation at Wilson Park. *Proceedings of the 15th International Clean Air and Environment Conference*, Sydney, Clean Air Society of Australia and New Zealand Inc., November 2000, Hanitro Pty Ltd, vol. 1: 191–98.

SUMMARY

- Metabolism is the overall term used to describe the chemical reactions of breakdown (catabolism), synthesis (anabolism) and repair that occur in living cells.

STRUCTURE OF BIOLOGICAL MOLECULES

- Living matter is made up of complex molecules containing the elements carbon, hydrogen, oxygen, nitrogen, phosphorus and sulfur, and other, trace elements.
- Organic compounds are compounds containing carbon; they exhibit stereoisomerism by forming four covalent bonds with other atoms.
- The arrangement of atoms within a molecule determines its shape and function.
- The particular structure of biological molecules enables them to combine with complementary molecules. This is termed 'specificity'.

ENZYMES AND CHEMICAL REACTIONS

- Enzymes are proteins that act as biological catalysts to lower the activation energy of a reaction.
- The production of enzymes is under the control of the DNA of the cell.
- Enzymes have a particular shape, containing an active site that is specific for the substrate; some require a cofactor for activity.

ENERGY PRODUCTION IN BIOLOGICAL SYSTEMS

- Photosynthesis uses energy from the sun to convert carbon dioxide and water to glucose.
- Cells break down glucose and release energy by the processes of glycolysis, fermentation and respiration.
- ATP is a small, biological, energy-rich storage molecule.
- Breaking the bond of the terminal phosphate group of ATP releases large amounts of energy, used by the cell for essential processes.

STRUCTURE OF BIOLOGICAL MOLECULES

Structure of carbohydrates

- Carbohydrates (sugars) are organic compounds made up of carbon, hydrogen and oxygen.
- The most common sugars are hexoses and pentoses; they may exist as monosaccharides, disaccharides or polysaccharides.

Structure of lipids

- Fats, or triglycerides, are composed of glycerol and long-chain fatty acids.

Structure of proteins

- There are 20 different naturally occurring amino acids.
- Proteins consist of chains of amino acids joined by peptide bonds in a specific sequence and folded into a particular shape.

BIOCHEMICAL PATHWAYS OF ENERGY PRODUCTION

- Energy is produced from the oxidation of carbohydrates by a process of glycolysis, followed by either fermentation or respiration.
- Fermentation occurs anaerobically and results in the formation of a number of useful products, depending on the microorganism.
- Respiration via the Krebs (TCA) cycle occurs in the presence of oxygen with the formation of carbon dioxide, water and large amounts of energy, which is stored as ATP.
- Proteins are broken down by proteolytic enzymes into amino acids, which can take part in other reactions or in the synthesis of new proteins.

ANABOLISM—BIOSYNTHESIS OF CELLULAR COMPONENTS

- Autotrophs are organisms that use carbon dioxide to synthesise glucose.
- Heterotrophs require a source of organic carbon to synthesise cellular components.
- Bacteria synthesise lipids from acetyl CoA and glycerol.
- Amino acids are synthesised by incorporation of nitrogen from nitrates or ammonia into the intermediates of the TCA cycle.
- Some bacteria can use atmospheric nitrogen to synthesise nitrates and ammonia.
- Proteins are formed in a complex series of reactions, directed by the nucleic acids, DNA and RNA.

INTERRELATIONSHIP OF METABOLIC PATHWAYS

- Microorganisms can use many different interrelated metabolic pathways to synthesise cellular requirements.

PRACTICAL APPLICATIONS OF MICROBIAL PROCESSES

- Many of the reactions carried out by microorganisms are of benefit to humans.
- Biochemical reactions are used to identify bacteria.
- Microorganisms and their products are used in the food industry.
- Genetic engineering involves the use of microorganisms to produce compounds for human use.

ENVIRONMENTAL USES FOR MICROORGANISMS

- Microbial metabolism is important for the decomposition of sewage and other organic matter.

STUDY QUESTIONS

1. What is meant by 'metabolism'?
2. Which elements make up biological molecules?
3. What name is given to compounds containing carbon?
4. (a) What name is given to the process in which carbon dioxide from the air is converted to glucose?
   (b) What type of microorganisms can carry out this process?
5. How do microbial cells obtain energy for their synthetic reactions?
6. Why are enzymes called biological catalysts?
7. What is meant by the 'active site' of an enzyme?
8. Give two examples of naturally occurring monosaccharides, disaccharides and polysaccharides.
9. Which metabolic pathway produces most energy?
10. Which compound is the key intermediate that links different metabolic pathways?
11. Give three examples of fermentative processes that are of use to humans.
12. How do microorganisms contribute to the recycling of nutrients to the environment?

TEST YOUR UNDERSTANDING

1. Give three examples of specificity in biological systems.
2. Explain how the properties of the carbon atom contribute to the shape and function of biological molecules.
3. Describe the difference between autotrophic and heterotrophic organisms.
4. How can the biochemical properties of microorganisms be used to aid in their identification?

CHAPTER

3

Bacteria

CHAPTER FOCUS

- What are the major properties of bacterial cells?
- What are the growth requirements of bacteria?
- How are bacteria identified?
- How are bacteria classified?
- What are the major groups of medically important bacteria?

INTRODUCTION

The largest group of microorganisms of medical significance are the **bacteria**. Most bacteria are able to survive and reproduce independently under a wide range of environmental conditions. There are thousands of different kinds of bacteria, which can be differentiated on the basis of size, shape, morphology, staining characteristics, nutritional requirements, biochemical activities, cell wall structure, and the composition of their ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). Most bacteria are harmless; in fact, many are beneficial to humans. Bacteria that cause disease are called **pathogens**. They are responsible for many skin and wound infections, and are the causative agents of various defined diseases. In this chapter we examine in detail the properties of bacteria, describe their classification and structure, together with their growth requirements and methods of identification, and give a brief overview of the different kinds of bacteria of medical importance.

CLASSIFICATION OF BACTERIA

Bacteria are classified as procaryotes, single-celled organisms characterised by a lack of a membrane-bound nucleus or other defined organelles. According to the classification developed by Carl Woese, which we examined in Chapter 1, they are grouped into two domains:

1. The domain **Bacteria** forms the largest group and includes the **eubacteria**, or true bacteria, and a small subgroup of bacteria capable of photosynthesis, the **cyanobacteria** (formerly called blue-green algae).
2. The domain **Archaea** contains the **archaebacteria**. These bacteria are thought to be of ancient origin and are able to live in extreme conditions of temperature and osmotic pressure. The features that distinguish the archaea from eubacteria include the lack of peptidoglycan in the cell wall, the presence of branched-chain fatty acids in their cell membranes and their unique ribosomal RNA. They are not known to cause disease in humans so are not discussed further in this text.

Nomenclature

All bacteria are given two names: the **genus**, which is usually signified by a capital letter, followed by the **species**. For example, *Staphylococcus aureus* and *Staphylococcus epidermidis* are the names of two different species within the same genus. A species may consist of a collection of similar **strains** that differ slightly from each other. The strain is usually designated by a letter or number after the species—for example, *Escherichia* (genus) *coli* (species) 0157 (strain). There are no rules about how bacteria get their names. The name may reflect the characteristic shape of the organism (Bacillus), where it is found (coli = colon), the name of the scientist who identified it (Escherich) or the disease it causes (tetani = tetanus). Names are always written in *italics.*

Size, shape and appearance of bacterial cells

Bacteria vary significantly in their **morphology**—that is, their size and shape. They range in size from 0.2 μm to 1 μm in diameter, and 1 μm to 10 μm in length. When stained and viewed under a light microscope with a magnification of 1000 times, bacteria appear as small spheres or rods see Figure 3.13, page 59. An electron microscope with a magnification of 50–100 000 times can be used to provide a 3-D image by scanning electron microscopy (SEM), or to examine the internal structure by transmission electron microscopy (TEM). There are three basic shapes of cells: spherical, rod-shaped and spiral (see Figure 3.1). Sometimes, groups of cells remain together after cell division to form clusters or chains. Some bacteria are motile; that is, they are capable of movement, possessing one or more extracellular appendages called **flagella** (singular: flagellum) which enable them to swim.

A spherical or round bacterium is called a **coccus** (plural: cocci). This is typically a small cell and may occur singly, in pairs (**diplococci**), in clusters (**staphylococci**) or in chains (**streptococci**). Frequently, the grouping of the cocci is reflected in the naming of the organism. For example, *Streptococcus pyogenes* is an organism with a cellular arrangement consisting of chains of cocci.

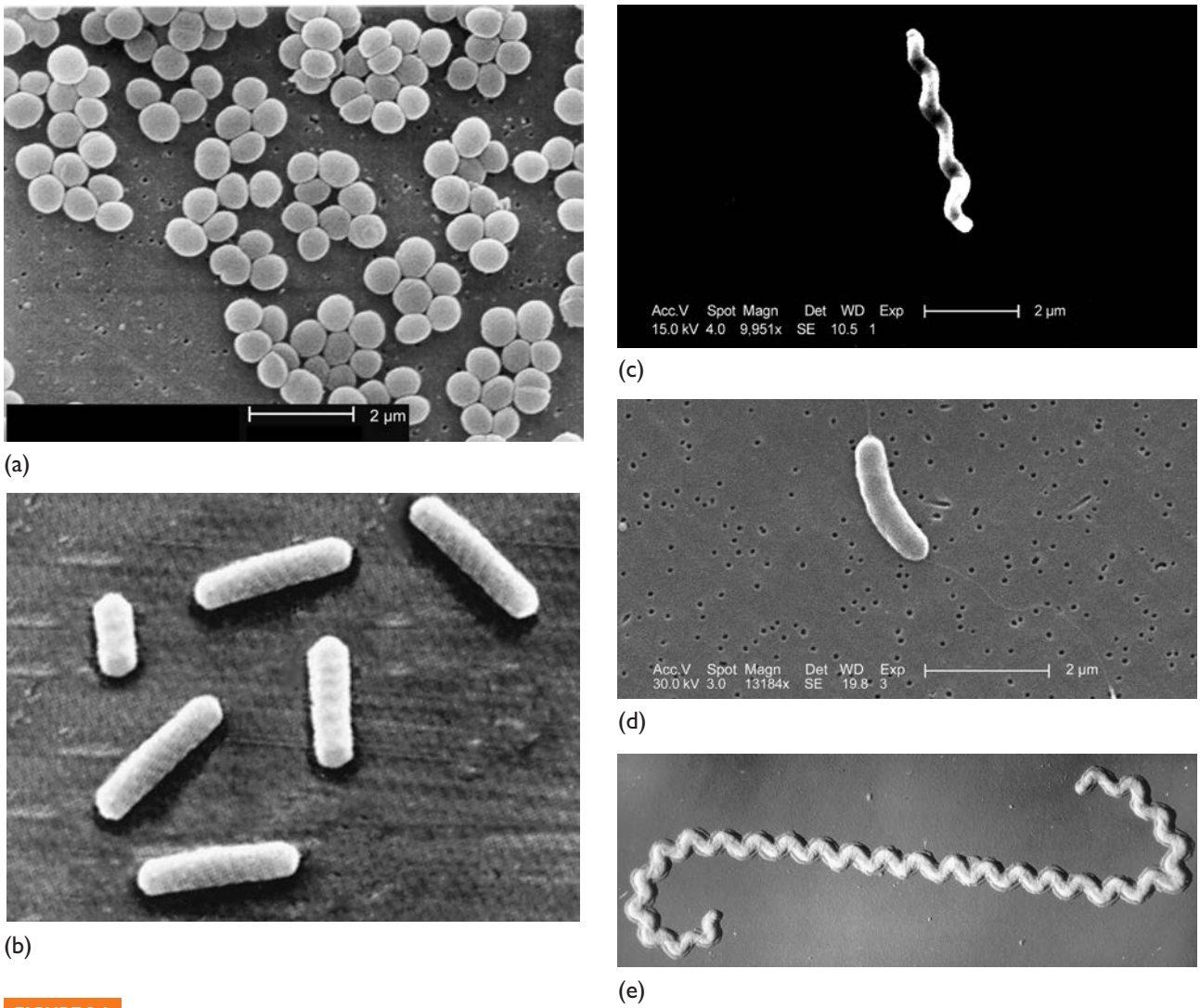
A rod-shaped or cylindrical bacterium is called a **bacillus** (plural: bacilli). Most bacilli appear as single cells, but a few are joined end to end to form **diplobacilli** or **streptobacilli**. Some form palisades where the bacteria are arranged in a row like a fence (e.g. species of *Corynebacterium*).

Spiral bacteria may have one of three shapes. The **spirochaetes** have a corkscrew-like appearance and a strong axial filament running the length of the cell. Rotation of this filament propels the cell along. **Spirilla** (singular: spirillum) are not as tightly coiled and move by means of a flagellum, a propeller-like tail. **Vibrios** are slightly curved rod-shaped cells, resembling a comma or incomplete spiral.

Most bacteria grow in a characteristic shape and this can be a useful means of identification. However, the actual size of the cell can be influenced by the availability of nutrients in the medium. A few bacteria occur in more than one shape (e.g. some species of *Rhizobium* and *Corynebacterium*), which makes identification more difficult. These bacteria are termed **pleomorphic** (*pleo* = many).

Cellular characteristics

One of the most useful ways to group bacteria is on the basis of their staining properties. Nearly all bacteria fall into one of two categories—Gram-positive or Gram-negative—depending on their cell wall structure (see below). Mycobacteria are identified by their reaction with the acid-fast stain.



(a)

(b)

(c)

(d)

(e)

FIGURE 3.1

Scanning electron micrographs of bacteria showing diversity of morphology

(a) Spherical clusters of staphylococci; (b) rod-shaped bacilli; (c) spiral Campylobacter*; (d) curved 'comma-shaped'* Vibrio cholera*; (e) spirochaete* Leptospira interrogans.

Sources: (a) and (b) Centers for Disease Control (CDC) & Dr Gary Lee; (c) Janice Carr/Centers for Disease Control (CDC); (d) Center sfor Disease Control (CD); (e) Dr Lee Smythe.

Strains of streptococci can be differentiated on the basis of the antigenic properties of carbohydrate molecules in their cell walls (see Lancefield's groupings on page 61, below).

Biochemical properties

Bacteria can also be identified and classified on the basis of their metabolic reactions. This is a useful procedure in diagnostic microbiology laboratories and is described more fully in Chapters 2 and 15.

In recent years molecular analysis of bacterial DNA and ribosomal RNA has replaced many of the older classification methods and led to the reclassification of some bacterial species.

STRUCTURE OF BACTERIA

Bacteria have a relatively simple structure compared to most other organisms. A typical procaryotic cell is shown diagrammatically in Figure 3.2. There are no defined structures within the cytoplasm, and the genetic material (DNA) is not enclosed in a nucleus. The cell wall provides structural support and the cell membrane carries out many of the functions that occur in sub-cellular structures of higher organisms. The following sections describe the structure of bacterial cells in detail.

Internal composition of bacterial cells

The **cytoplasm** of bacterial cells is about 80 per cent water and contains the proteins, carbohydrates, lipids and salts that are essential for the activities of the cell. Electron micrographs of bacterial cells usually have a granular appearance, with some areas appearing to be denser than others. The granules, or **inclusions**, can be identified by staining with simple dyes such as methylene blue or iodine. These inclusions are usually

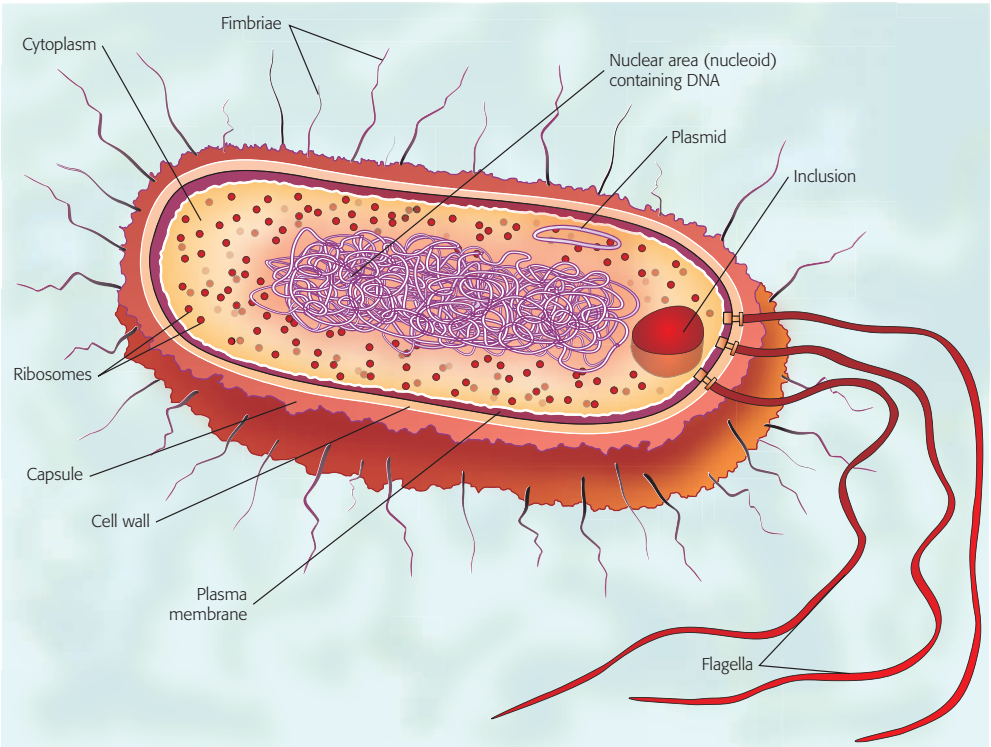


FIGURE 3.2

Schematic diagram of a typical procaryotic cell

polymers of cellular material and act as storage for the cell. For example, **volutin** is a polymer of phosphate molecules. Polymers of glucose, such as **glycogen** or **starch**, are also frequently present. Occasionally, cells contain **vesicles**, or **vacuoles**. These are usually filled with gas to give the cell buoyancy.

The nuclear area of the cell contains a single circular chromosome, consisting of a double strand of DNA (deoxyribonucleic acid) which carries the genetic information necessary for the structure and function of the cell. Bacteria often carry one or more extra pieces of DNA, small circular structures called **plasmids**. Plasmids vary in size and replicate independently of the main chromosomal DNA. They appear to carry information that is not essential for the normal metabolism or growth of the cell, such as genes for resistance to antibiotics or the production of toxins.

The gene for the production of the **sex pili**, which are involved in the transfer of genetic information on plasmids from one bacterium to another during conjugation, is also carried on a plasmid. The transfer of genetic information by plasmids is used by scientists in the process of genetic engineering, described in the next chapter.

Protein synthesis in the cell takes place on the **ribosomes**. They are small spherical particles consisting of two subunits, each containing protein and ribonucleic acid (RNA). Ribosomes of procaryotic cells are designated 70S, which refers to their rate of sedimentation when subjected to ultracentrifugation. They are slightly smaller and less dense than the ribosomes of eucaryotic organisms, which are designated 80S. Messenger RNA (mRNA) attaches to the ribosomes to provide a site for the incorporation of amino acids into the growing peptide chain. Groups of ribosomes attached to messenger RNA are called **polyribosomes**, and give the cytoplasm of the cell a granular appearance (see Figure 3.3).

External structure of bacterial cells

Glycocalyx

Some bacteria are surrounded by a **glycocalyx**, a sticky, polysaccharide-containing layer that is secreted on to the cell surface. Depending on its structure and function, it is referred to as a **capsule** or **slime layer**. These layers protect the cell from drying out.

Bacterial capsules are composed of polysaccharide and/or polypeptide molecules. They are usually synthesised inside the cell and excreted to the cell surface where they form an organised gelatinous layer firmly attached to the cell wall. An important function of the capsule is to help the bacterium adhere to host cell surfaces. The capsule possesses specific molecules that bind to complementary receptor molecules on the surface of the susceptible animal cell. The capsule also has a protective function, allowing a bacterial pathogen to avoid ingestion by the white blood cells of the immune system (**phagocytosis**). The presence of a capsule is therefore considered to contribute to the **virulence** (ability to cause disease) of the organism. For example, encapsulated strains of *Streptococcus pneumoniae* are highly virulent, whereas strains that lack a capsule are unable to produce disease symptoms.

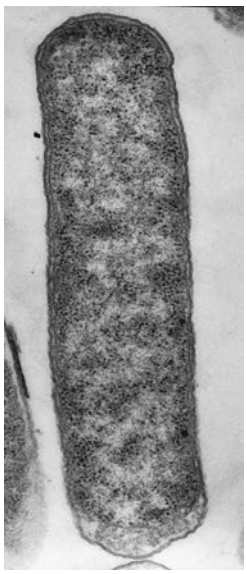


FIGURE 3.3

Transmission electron micrograph of *E. coli* (×60 000) showing cell wall membrane and granular cytoplasm

Source: Dr Penny Bishop.

The composition of the capsule is unique to the particular strain of bacteria in which it is found. The oral bacterium, *Streptococcus mutans*, forms a capsule containing **glucan**, a polymer of glucose, which is a major component of dental plaque. It helps the bacteria to adhere to the tooth surface and cause dental decay.

Slime layers are composed of glycoproteins and polysaccharides and usually exist as a loosely attached, less defined structure than capsules. The presence of a slime layer assists in the attachment of the bacteria to host cells and appears to contribute to the formation of biofilms on medical devices.

Flagella

Many bacteria are motile—that is, capable of movement in a water environment. The most common way in which procaryotic cells move is by means of **flagella** (singular: flagellum), which are thin, rigid filaments that are usually many times longer than the cell itself. Some bacteria have only one flagellum located at one end of the cell; others have two, one at each end of the cell. A few bacteria have numerous flagella, located all over the cell (see Figure 3.4). Flagella consist of protein subunits arranged in a complex structure. They are attached to the cell membrane and wall of the bacterium by a structure called a **basal body**, a series of concentric protein rings that allow the flagellum to rotate. Movement of the bacterium is achieved by rotation of the flagella with a propeller-like motion, a process requiring energy in the form of the high-energy storage molecule adenosine triphosphate (ATP). Motile bacteria appear to respond to a chemical stimulus in their environment and swim towards it. This phenomenon is called **chemotaxis**.

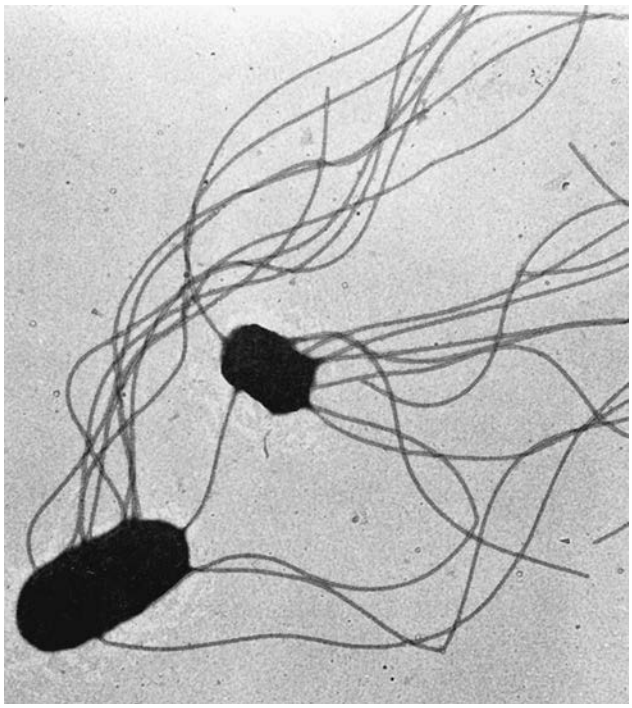


FIGURE 3.4

***Proteus vulgaris*, a bacterium with numerous flagellae**

Source: G. Jayachandran, Sydney Medical School, University of Sydney.

Pili and fimbriae

Pili (singular: pilus) and **fimbriae** (singular: fimbria) appear as hair-like appendages on the outside of some Gram-negative bacterial cells. Each has a distinct function. The thicker sex pili (1 to 10 per cell) are located on the surface of the cell; they are involved in the joining together of bacterial cells to allow transfer of the genetic material, DNA, from one cell to another during conjugation. There are several thousand fimbriae per cell (sometimes called attachment pili) (see Figure 3.19, page 63). They are thinner than pili and concerned mainly with the attachment of the bacteria to surfaces. The presence of fimbriae contributes to the ability of the organism to cause disease by enabling it to adhere to the host cell and colonise it. For example, strains of *Neisseria gonorrhoeae* that lack fimbriae are not as pathogenic as strains that have fimbriae to help attach the organism to the mucosal cells lining the host genital tract.

Cell wall

The most characteristic structure of bacteria is the cell wall (see Figure 3.5). It is a semi-rigid structure present in almost all bacteria and is responsible for determining the shape of the cell as well as some of its staining properties. It also provides mechanical support so that the cell does not burst when exposed to conditions of osmotic pressure that allow movement of water into the cell. Bacterial cell walls contain varying amounts of different macromolecules, which bestow certain characteristics on the cell. One of the major

components is a complex substance called **peptidoglycan**, which consists of repeating units of a disaccharide molecule containing two glucose derivatives, N-acetylglucosamine and N-acetylmuramic acid. The disaccharide molecules are cross-linked by an interbridge of four or five amino acids to form a complex rigid network (see Figure 3.5a). Based on the composition of the cell wall, nearly all bacteria can be classified into one of two groups, Gram-negative or Gram-positive.

Structure of Gram-positive cell walls

Cells classified as Gram-positive have a defined cell wall structure which determines their reaction in the staining procedure developed by the Danish microbiologist Hans Christian Gram (see Figure 3.12, page 58). The main component of Gram-positive cell walls is a thick layer of peptidoglycan. Attached to the peptidoglycan are other complex polysaccharides called **teichoic acids**. These teichoic acids have a strong negative charge, which appears to influence the passage of materials in and out of the cell and contribute to the antigenic specificity of the cell wall (see Figure 3.5b).

Structure of Gram-negative cell walls

The group of bacteria classified as Gram-negative have a more complex outer structure than the Gram-positives (see Figure 3.5c). The cell wall consists of a thin layer of peptidoglycan covered by an outer membrane containing **lipopolysaccharides**, **lipoproteins** and **phospholipids**. The outer membrane has several functions. It provides a barrier to the entry of various substances (e.g. some antibiotics) into the cell and also prevents the action of certain enzymes that break down the cell wall (e.g. **lysozyme**). The lipopolysaccharide component, called the O-polysaccharide or O-antigen, has a distinctive structure in each strain of bacteria. This is useful in serological tests to distinguish between different strains of bacteria (see Chapter 15). The lipid portion, known as **lipid A**, is an **endotoxin**. It is responsible for some of the toxic effects (such as fever and shock) that occur when certain Gram-negative bacteria containing lipid A infect the human body (see Chapter 10).

Structure of acid-fast bacteria (AFB)

Some bacteria (e.g. the mycobacteria) have an outer layer composed of peptidoglycan, similar to the Gram-positive wall, but covered by a thick waxy layer that interferes with the Gram-stain procedure. The presence of mycobacteria (e.g. the causative agent of tuberculosis, *Mycobacterium tuberculosis*) is usually detected in sputum or lung biopsies by using the **Ziehl-Neelsen acid-fast stain** (see Figure 3.14, page 59). Mycobacteria are often referred to as 'acid-fast bacilli' (AFB).

Damage to the cell wall

An intact cell wall is important to the structural integrity of the bacterial cell and its ability to survive in the environment. Thus the bacterial cell is particularly vulnerable to substances that destroy or damage the wall. Among these substances is the enzyme **lysozyme**, which occurs in egg white as well as in human tears and saliva. Lysozyme exerts an antibacterial effect, damaging the cell walls of Gram-positive bacteria by breaking the bonds in the peptidoglycan layer. When the wall is destroyed, the cell is left surrounded by only the plasma membrane. This structure is called a **protoplast** and it is very vulnerable to rupture by osmotic pressure. Lysozyme does not have such a dramatic effect on the cell walls of Gram-negative bacteria because it is unable to penetrate the outer layers of the cell wall and so cannot destroy the peptidoglycan layer.

Certain antibiotics specifically target the cell wall. **Penicillin** exerts its effect by inhibiting the formation of the cross-linking amino acid interbridges during the synthesis of peptidoglycan in rapidly growing cells (see Figure 3.5a). For this reason, rapidly multiplying Gram-positive bacteria are particularly susceptible to the action of the penicillin group of antibiotics. Gram-negative organisms are not as readily affected because their outer membrane interferes with the entry of penicillin to the cell and they contain only a thin layer of peptidoglycan. Some of the semi-synthetic penicillins, which are able to penetrate the outer membrane, are more effective against Gram-negative organisms (see Chapter 12).

Cell membrane

All bacterial cells have a **cell membrane**, or **plasma membrane**, which lies just underneath the cell wall. Bacterial membranes have a structure that is essentially similar to the membranes of all other living cells. It consists of a double layer of phospholipid molecules (a **phospholipid bilayer**) with an irregular arrangement of proteins embedded in the lipid layers. Bacterial cell membranes consist only of proteins and phospholipids, whereas eucaryotic cell membranes also contain sterols and carbohydrates that bestow additional properties on the eucaryotic membrane. The structure of the membrane is uniquely suited to its function in the cell. When examined under the electron microscope, it appears as a double layer consisting of phospholipid molecules arranged in a particular orientation (Figure 3.6b).

Phospholipids are compounds consisting of a molecule of glycerol esterified with two long-chain fatty acids (see Chapter 2). Fatty acids are long hydrocarbon chains that are **non-polar** (i.e. do not have any charged groups) and so are insoluble in water. They are therefore described as **hydrophobic**, or 'water-hating'. The phosphate group, which is attached to the third hydroxyl group of the glycerol molecule, is **polar** (i.e. charged) and is usually joined to another charged molecule such as ethanolamine or choline, or another molecule of **glycerol**. This part of the phospholipid molecule is termed **hydrophilic** ('water-loving'). Phospholipids therefore have two distinct regions—a polar, water-soluble, hydrophilic 'head' and a non-polar, hydrophobic 'tail' (see Figure 3.6c).

In the cell membrane, two layers of phospholipids are arranged so that the polar, hydrophilic 'heads' are oriented towards the 'outside' water environment, while the non-polar, fatty acid 'tails' are oriented towards the hydrophobic interior of the membrane. Embedded in the phospholipid bilayer are protein molecules which carry out various functions. This widely accepted model of membrane structure is usually called the *Singer-Nicholson model* or **fluid-mosaic**

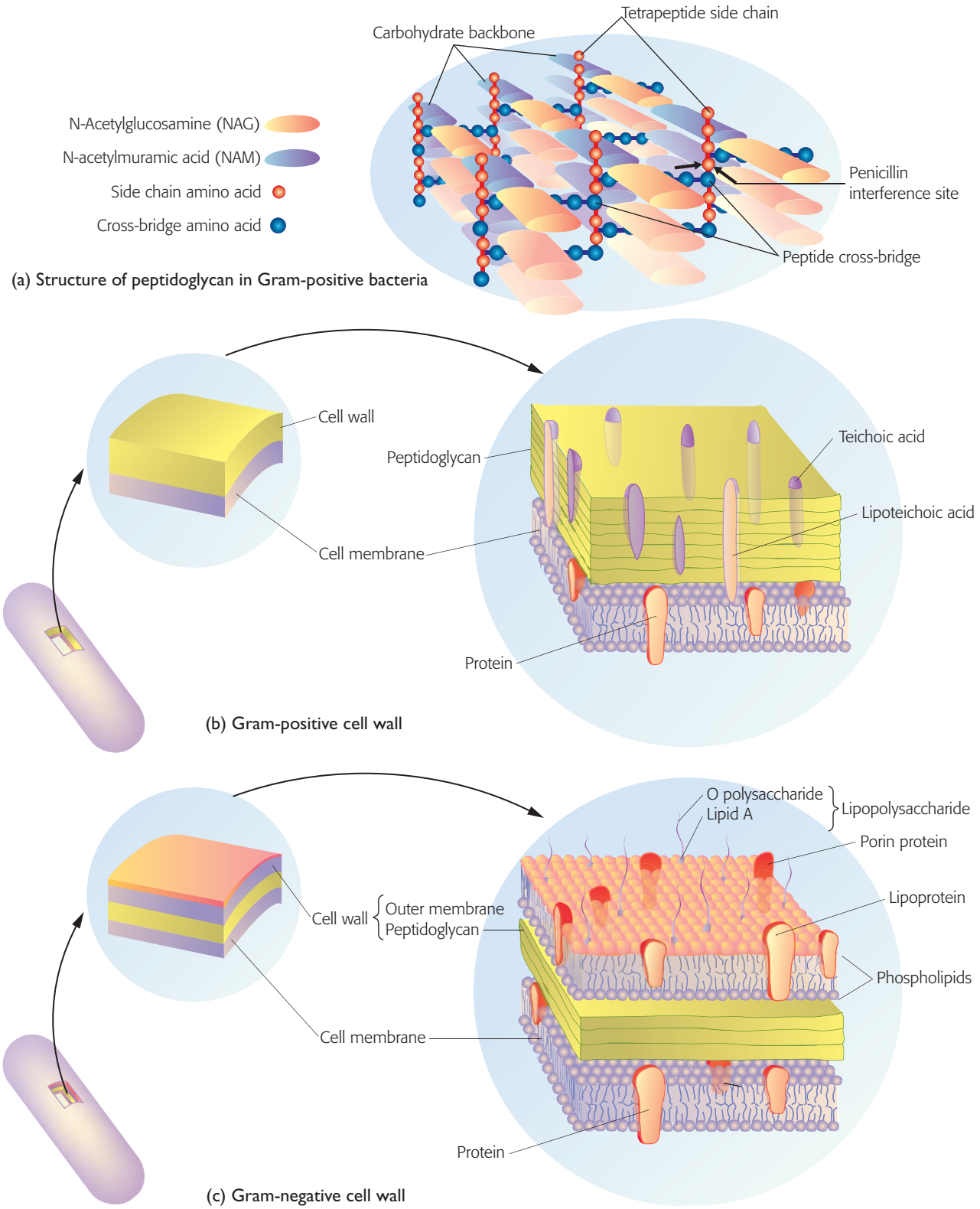


FIGURE 3.5

Bacterial cell walls

(a) Structure of peptidoglycan in Gram-positive bacteria. Together the carbohydrate backbone (glycan portion) and tetrapeptide side chains (peptide portion) make up peptidoglycan. The frequency of peptide cross-bridges and the number of amino acids in these bridges vary with the species of bacterium. The small arrows indicate where penicillin interferes with the linkage of peptidoglycan rows by peptide cross-bridges.
(b) Structure of the Gram-positive cell wall.
(c) Structure of the Gram-negative cell wall.

model. The term 'fluid-mosaic' describes a major characteristic of this membrane structure. The nature of the fatty acids allows the lipid and protein molecules to move sideways within the lipid matrix (see Figure 3.6b).

Functions of bacterial cell membranes

Movement of substances in and out of the cell: osmosis

Osmosis is the passage of a solvent (usually water) from one side of a membrane to another. Usually, the cell membrane allows unrestricted movement of small molecules such as water, oxygen and carbon dioxide. The water molecules move from an area of 'high' water concentration (i.e. a dilute solution) to an area of 'low' water concentration (e.g. a more concentrated solution inside the cell). **Osmotic pressure** is the pressure required to prevent the flow of water molecules across a selectively permeable membrane.

Unlike human cells, which use homeostatic mechanisms to maintain a constant environment, bacterial cells can be subjected to different kinds of solutions in their environment. This may create differences in the osmotic pressure exerted on the cell. If the overall concentration of salts and ions (solutes) in the solution outside the cell is the same as inside, the solution is said to be **isotonic** (and water enters and leaves the cell at the same rate). If the solute concentration is higher outside the cell than inside, the solution is said to be **hypertonic**. Water leaves the cell and the cell dehydrates. If the solute concentration is lower outside than inside (e.g. as in distilled water), the solution is said to be **hypotonic**. Water passes through the membrane into the cell and the cell may burst. The rigid cell wall plays an important part in protecting bacteria from the effect of variations in osmotic pressure.

Passive and active transport

The main function of the plasma membrane in procaryotic cells is to act as a selectively permeable barrier for the cell. Many water-soluble compounds cannot penetrate the nonpolar hydrophobic interior of the membrane. They must be assisted across the membrane by proteins acting as carriers and forming pores or channels that allow the passage of selected substances into and out of the cell. This is often termed **facilitated diffusion** or **passive transport**, as it does not require the expenditure of energy. It allows the movement of a substance across a membrane from an area of high concentration to an area of low concentration. This enables the cell to absorb the nutrients and substances it requires for growth and to rid itself of waste products.

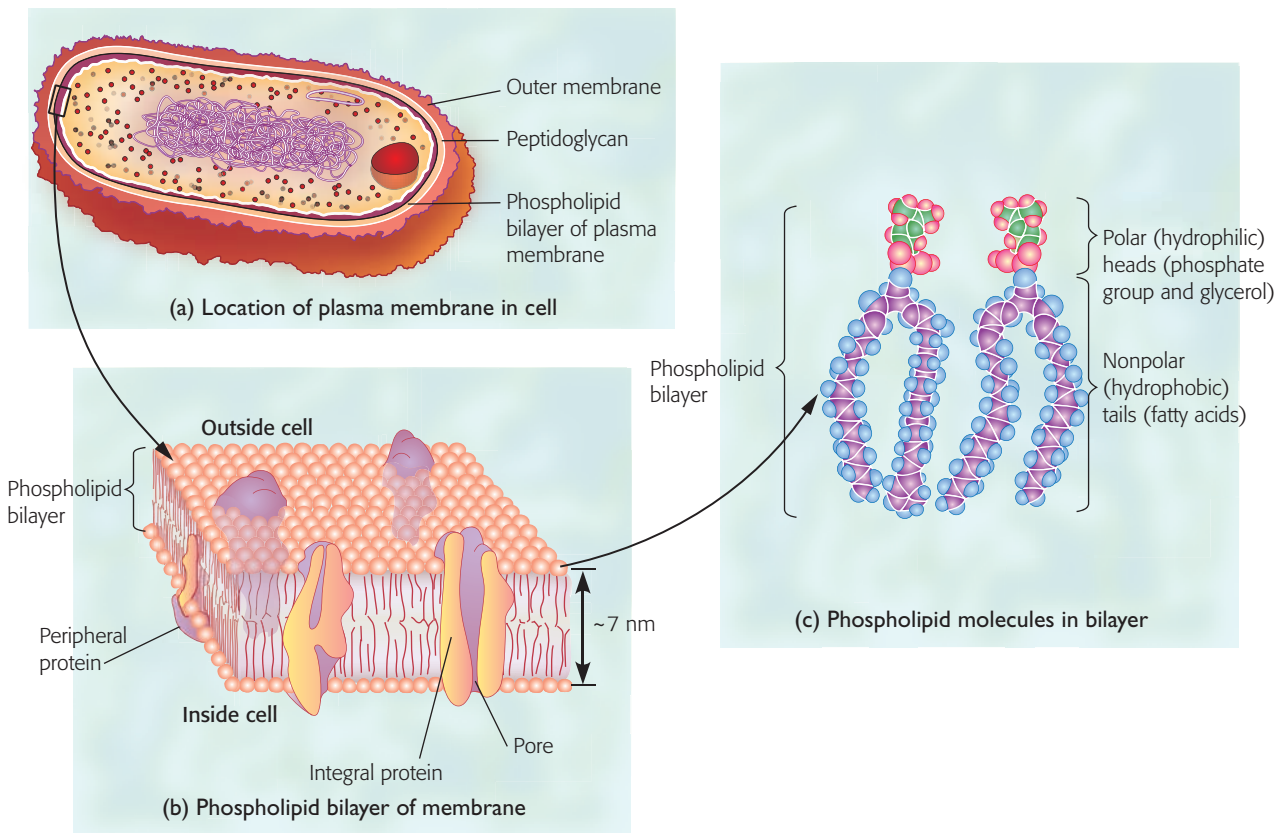


FIGURE 3.6

Plasma membrane

(a) A diagram showing the position of the phospholipid bilayer which forms the inner plasma membrane of Gram-negative bacteria.
(b) A portion of the inner membrane showing the phospholipid bilayer and proteins. The outer membrane of Gram-negative bacteria is also a phospholipid bilayer.
(c) Space-filling models of several molecules as they are arranged in the phospholipid bilayer.

Active transport occurs when a substance is transported into or out of a cell *against* a concentration gradient; that is, there is a higher concentration of the substance on one side of the membrane. This usually requires the expenditure of energy. Specialised protein molecules called **transport proteins** carry the substance through the membrane.

Specialised functions of bacterial cell membranes

Procaryotic cells lack other cellular membranes, so the plasma membrane in bacteria is the site of proteins that carry out additional functions normally performed by the organelles in eucaryotic cells. These include:

- synthesis of cell wall components
- cellular respiration and synthesis of ATP
- secretion of proteins and enzymes, which are released from the cell as extracellular enzymes or toxins.

The membrane is a vital part of the cell and as such is very susceptible to damage. Some antimicrobial compounds exert their effect by disrupting the structure of the membrane. These include disinfectants, such as alcohol and the quaternary ammonium compounds, and the polymyxin group of antibiotics.

Endospores

When environmental conditions are unfavourable, certain Gram-positive bacilli are able to form endospores. Bacterial sporulation does not occur when cells are growing rapidly, but only when growth ceases due to the exhaustion of essential nutrients. An **endospore** is a specialised type of resting cell which is formed inside the bacterial cell membrane. It is surrounded by a **spore coat**, consisting of thick layers of peptidoglycan and protein, to provide protection. Bacterial endospores can survive conditions that destroy normal **vegetative** (growing) **cells**, such as boiling for up to five hours, freezing, desiccation (dehydration), and exposure to chemicals and radiation. The endospore develops inside the bacterial cell membrane and can be located by special staining procedures (see Figure 3.7).

The spore has a lower water content than vegetative cells and does not carry out metabolic reactions. It contains nucleic acid and the various cellular enzymes and substances that are essential for spore germination to occur when conditions become favourable. When this happens, water enters the spore, germination occurs, and the vegetative cell that is produced resumes normal growth and metabolism. Endospore formation is *not* a method of reproduction—one bacterial cell gives rise to only one endospore, which then germinates into a single cell again. It is, however, a very important method of survival for certain species of bacteria and has important clinical significance.

Bacterial endospores have been shown to survive in the soil for many years and there are even reports of their being found in fossils. Spores of *Bacillus anthracis*—the organism responsible for anthrax, a disease that affects humans as well as cattle—were found more than 20 years later at a site where diseased animals had been buried.

Many of the Gram-positive bacteria that form endospores are important pathogens. These include members of the genus *Clostridium*—*C. perfringens* (food poisoning and gas gangrene), *C. tetani* (tetanus) and *C. botulinum* (botulism).

In the food industry, spores may survive the normal cooking process and cause food poisoning. An example is *C. perfringens*, which is common in the environment. Spores of *Clostridium* may be ingested in food, germinate in the intestine and produce symptoms of gastroenteritis (stomach cramps and diarrhoea) 12–24 hours later. This organism is also the cause of gas gangrene. If wounds containing dead tissue are contaminated with *C. perfringens*, either from the environment or from non-sterile medical equipment, gangrene may develop.

C. botulinum spores are widespread in nature and may sometimes be found in preserved or canned foods that have not been adequately sterilised. The organism grows in the food under anaerobic conditions, producing a powerful neurotoxin (poison).

C. tetani is a common inhabitant of the large intestine of humans and animals. Tetanus spores are frequently found in faeces or in soil where manure is spread. When introduced into deep puncture wounds, the spores germinate, grow anaerobically and produce the tetanus toxin responsible for spasms, paralysis and death. A vaccine is available against tetanus.

Knowledge of the existence of highly resistant forms of bacteria is important for the development of adequate methods of sterilisation and disinfection in hospitals, and methods of preservation in the food industry. Because they survive in soil or in dust, these resistant endospores can contaminate hospital areas such as operating theatres unless strict attention is paid to hygiene. Contamination of deep surgical wounds can give rise to serious infections, as the organisms can grow anaerobically. As discussed above, bacterial endospores are resistant to heat because of their comparatively low water content, and require treatment at 121°C for 15 minutes in an autoclave to be killed. The complex nature of the spore coat also makes them highly resistant to most germicides.

REPRODUCTION IN BACTERIAL CELLS

The rate at which bacteria reproduce is determined by both physical and chemical factors in their environment. Physical factors include the availability of water and the correct osmotic pressure, temperature, and pH, and the presence or absence of oxygen. Chemical, or nutritional factors, include a suitable carbon source, nitrogen, phosphorus, sulfur, trace elements and minerals. Some bacteria have an absolute requirement for a particular compound or growth factor. Overall, bacteria can grow in a wide range of environments, but each species has its own set of optimal conditions that allow it to achieve its maximum growth rate. For most human pathogenic bacteria, these conditions are very similar to those found in the human body.

Most bacteria are free-living and can reproduce without using another cell. The exceptions, which replicate inside a host cell, are species of chlamydia and rickettsia, which are bacterial obligate intracellular parasites, and the

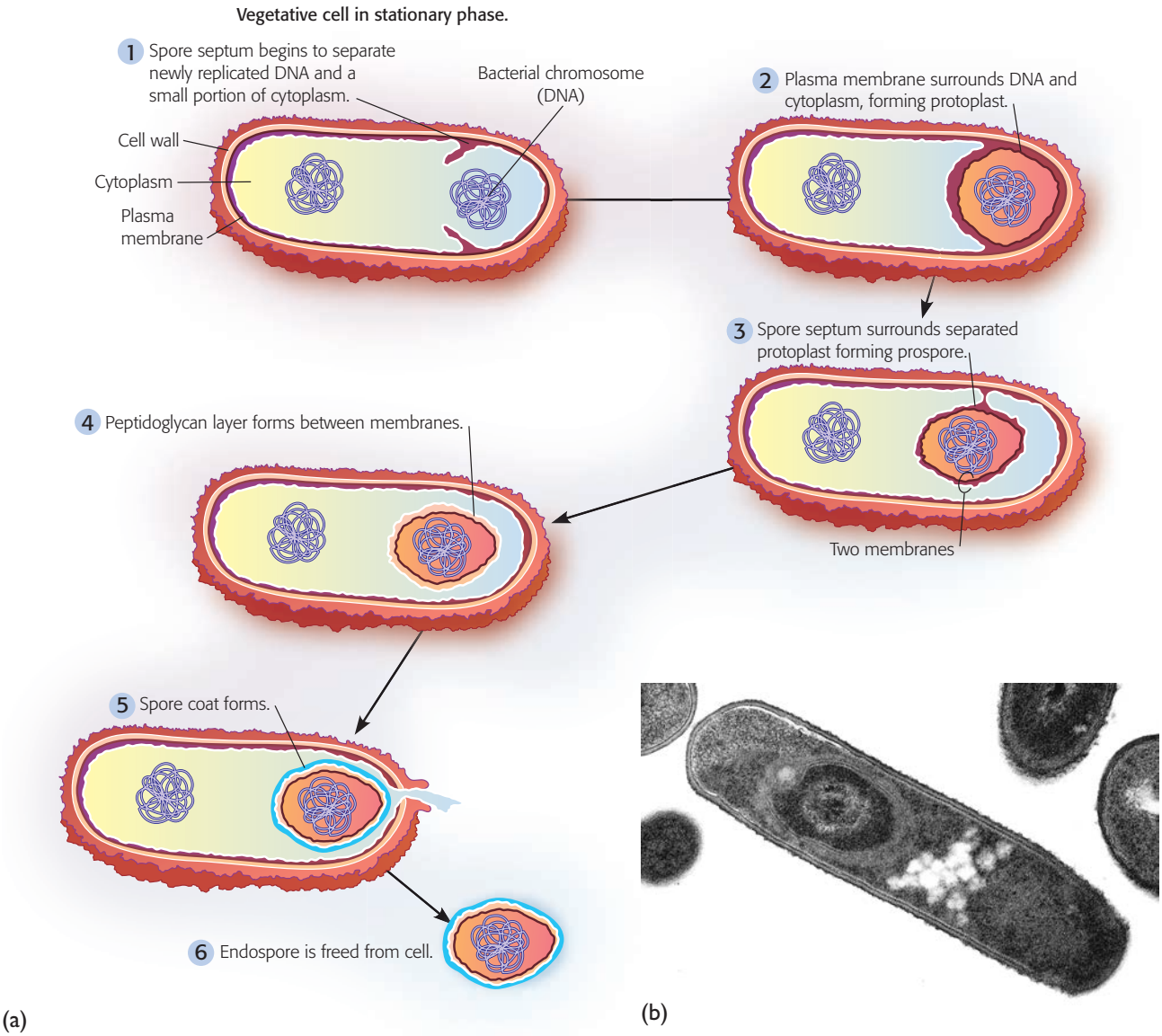


FIGURE 3.7

Formation of endospores by sporulation

(a) Diagrammatic representation of endospore formation; (b) TEM of Bacillus subtilis *showing spore formation.*

Source: (b) Dr Penny Bishop.

mycoplasmas, which do not have a cell wall and so are vulnerable to changes in osmotic pressure.

Physical factors

Water and osmotic pressure

The most important requirement for bacterial reproduction is the presence of water. Bacteria grow best in an environment that is saturated with water. Although many bacteria can survive dry conditions, they require water to reproduce. They obtain all their nutrients from their surroundings, so the composition of substances in the water environment will affect their growth. The dissolved substances exert osmotic pressure on the bacterial cell, which will be able to reproduce optimally if the growth medium is isotonic. This means that, if a bacterial cell is placed in a hypotonic solution such as distilled water, water moves into the cell, which swells up and becomes turgid. If the pressure becomes too great to be contained by the rigid structure of the bacterial cell wall, the cell bursts and is destroyed (cell **lysis**).

If the cell is placed in a hypertonic solution—that is, a solution with a higher salt concentration (osmotic pressure) than the bacterial cytoplasm—water tends to move out of the bacterial cell into the surrounding medium, causing the cell membrane to shrink away from the wall. This greatly slows down or inhibits growth. This phenomenon is useful in food preservation. High concentrations of salt or sugar (hypertonicity) in foodstuffs prevent the growth of bacteria

CASE HISTORY 3.1

Food poisoning due to *Clostridium perfringens*

An outbreak of gastroenteritis occurred at a Melbourne nursing home. One elderly woman died, and 25 of the 80 residents became ill with stomach cramps, nausea and diarrhoea. The outbreak prompted investigations to determine the cause and source of the illness. Food and faecal samples were tested, environmental swabs taken and food-handling procedures reviewed.

At the nursing home, the cause of the infection was shown to be *Clostridium perfringens*, which had contaminated the pureed food prepared for the residents. The temperature of the cool-room at the nursing home was found to be 8–12°C, well above the maximum recommended temperature of 5°C. The prepared food was usually pureed and then kept warm until eaten. It was not reheated thoroughly before serving.

Comment

C. perfringens is an endospore-forming Gram-positive bacterium which occurs widely in the environment. It is a common cause of gastroenteritis, especially in institutions or at large gatherings. Bacterial endospores survive the cooking process and germinate in warm food, multiplying rapidly at temperatures up to 45°C. Unless the food is reheated to over 70°C to kill the bacteria, after ingestion they continue to multiply in the intestine, producing enterotoxins which cause the typical disease symptoms.

Source: Tallis, G., Ng, S., Ferreira, C., Tan, A. and Griffith, J. (1999), A nursing home outbreak of *Clostridium perfringens* associated with pureed food. *Australian and New Zealand Journal of Public Health* 23(4): 421–23. doi 10.1111/j.1467-842X.1999.tb01287.x.

Questions

1. What food-handling procedures contributed to this outbreak?
2. Why are elderly people more susceptible to infection?
3. Suggest ways in which this type of occurrence could be avoided in the future.

and subsequent food spoilage. Some examples are salted or cured meat and fish, jams and fruit syrups.

Oxygen

Bacteria can be divided into groups according to their requirement for molecular oxygen for growth. Organisms that use oxygen are called **aerobes**. Those that *require* oxygen for growth are called **obligate aerobes**. Obligate aerobes such as *Mycobacterium tuberculosis* are often found as pathogens in areas of high oxygen tension, such as the lungs. Bacteria that reproduce in the absence of oxygen are called **anaerobes**. Some of them are termed **obligate anaerobes** because they are not only inhibited, but destroyed, in the presence of oxygen. These obligate anaerobes are killed by a special form of oxygen called a **free radical**, or **superoxide**, which is a compound formed in most cells during aerobic oxidation, but usually destroyed by two enzymes—**superoxide dismutase** and **catalase**. Obligate anaerobes lack these enzymes and the cells are killed by the toxic effect of the free radicals.

Two pathogenic organisms that are sensitive to oxygen are *Clostridium tetani* (tetanus) and *Clostridium perfringens* (gas gangrene). Tetanus and gangrene are infections that occur in deep puncture wounds or necrotic tissue where the supply of oxygen is depleted. Both infections are sometimes treated by placing the patient in a hyperbaric oxygen chamber, where tissues are saturated with oxygen in an attempt to kill the causative organisms.

Some bacteria, called **facultative anaerobes**, have developed the ability to grow in either the presence or absence of oxygen. They carry on aerobic metabolism when oxygen is present, but change to anaerobic pathways when oxygen is limited. Many of these organisms are found in the human intestine.

The oxygen requirement of pathogenic organisms is an important factor to consider when collecting specimens for laboratory diagnosis. Even a short exposure to oxygen (ten minutes) is sufficient to kill some anaerobes. Special techniques have been developed to collect and transport sensitive organisms (see Chapter 15).

Temperature

Most bacteria will grow at the environmental temperatures usually encountered in temperate zones, 10–39°C, but each will have a minimum, maximum and optimum temperature. Organisms that grow at moderate temperatures (25–35°C) are called **mesophiles**. Those that thrive in low temperatures (optimum 10°C) are called **psychrophiles** (cold-loving), and those that like high temperatures (optimum 60°C) are termed **thermophiles** (heat-loving) (see Figure 3.8). Optimum growth (fastest reproduction) is represented by the peak of the curve. Notice that the reproductive rate drops off very quickly at temperatures only a little above the optimum. At either extreme of the temperature range, the reproductive rate is much lower than the rate at the optimum temperature.

The rate of growth of most bacteria is greatly reduced at temperatures below 10°C, such as occur in a domestic refrigerator (4°C), a common method of preserving food. However, some bacteria will survive long periods of storage in the cold and even withstand freezing. Although food spoilage is greatly reduced at low temperatures, the bacteria are not killed and it is important to remember that food may still be contaminated by pathogens that were present before refrigeration.

Pathogenic bacteria usually have an optimum growth temperature similar to the human body (i.e. 37°C).

pH

The level of acidity or alkalinity of the culture medium (pH) is very important for bacterial growth. For most bacteria, the pH optimum, at which maximum growth occurs, is close to neutral—that is, pH 7. Most bacteria do not grow at all above pH 8 or below pH 6. Some bacteria produce organic acids during metabolism, causing a drop in pH that inhibits further growth. An important group of bacteria that can tolerate acid conditions belongs to the genus *Lactobacillus*. They produce lactic acid by fermentation of lactose and are responsible for the souring of milk to make products such as yoghurt. These bacteria can only tolerate moderate levels of acid (i.e. pH 4), so when the pH drops below 4 the culture stops growing. This is an excellent method of preserving milk products—spoilage is inhibited because most other bacteria cannot tolerate even mildly acid conditions.

Nutritional requirements

The essential element in all living cells is carbon. Most bacteria are **heterotrophic**—that is, they need to be supplied with a suitable simple organic carbon compound for growth. The bacteria break down carbon compounds into smaller molecules, which they use to synthesise their own cellular requirements. A few bacteria are **autotrophic** and can carry out photosynthesis using carbon dioxide from the air as their sole carbon source.

Bacteria also need a supply of other elements to synthesise the organic molecules (proteins, carbohydrates, nucleic acids) that make up their cellular components. These include nitrogen, phosphorous, sulfur, and various minerals and trace elements. In the environment, bacteria derive all their nutrients from their surroundings. When grown in the laboratory, bacteria are cultured on a specially prepared medium (see 'Growth media', page 60).

Organic growth factors

A few bacteria have requirements for specific organic compounds that must be available for growth to occur. These may be vitamins, which act as coenzymes. Often the requirement will be for a specific amino acid that the bacterium is unable to synthesise for itself, but which is essential for the formation of protein. This inability may be inherent in the species, or it may be the result of a mutation that has given rise to a particular strain of bacteria that has lost the ability to produce the enzymes required to make this compound for itself.

PATTERN OF BACTERIAL REPRODUCTION

Growth is usually defined as an increase in size. However, when microbiologists speak about bacterial growth, they are usually referring to an increase in numbers of bacterial cells. This may appear confusing until it is realised that bacteria do not increase in size very much during their lifespan. As soon as a bacterial cell has approximately doubled in size, it divides to form two identical daughter cells. Bacterial growth is therefore defined as *an increase in cell numbers*.

Cells use the energy derived from the metabolic breakdown of nutrients to produce new cellular components. In most bacteria, as the cell elongates, the genetic information contained in the chromosome is duplicated and one new

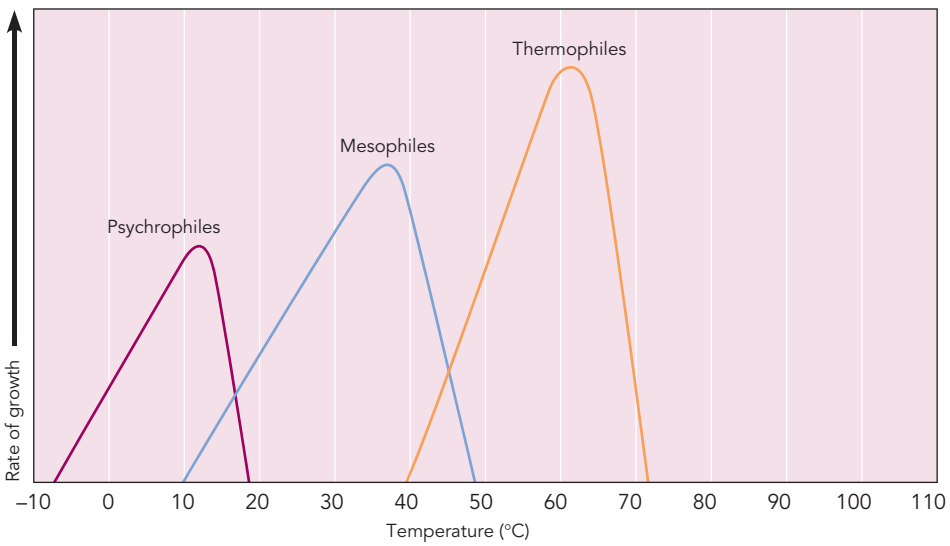


FIGURE 3.8

Typical growth rates of different types of microorganisms in response to temperature

chromosome moves to each end of the cell. The membrane and cell wall begin to grow inwards at a point about halfway along the cell, until a septum forms. The cell then splits, forming two new cells that contain identical components to the parent cell. This whole process is called **binary fission** (Figure 3.9).

The two daughter cells are identical; they are both completely new cells—not one old cell and one new cell. The time taken for the cell to reproduce itself is called the **generation time**; this can vary enormously, depending on the type of organism and the environmental conditions. Under favourable conditions, some bacteria can reproduce in 20–30 minutes. Important clinical species, such as *Escherichia coli*, can reproduce at this rate. This means that a single cell can give rise to over 1 million cells in 8–10 hours (see Table 3.1, below). This is why an apparently slight infection in a patient can rapidly become serious.

Other species (e.g. *Mycobacteria* and some anaerobic organisms) are very slow-growing and may take up to 12 hours to reproduce even when conditions are optimal.

The time required to culture and identify an organism from a clinical specimen depends very much on the growth rate. Many bacteria produce visible growth in an overnight culture (18 hours), but some bacteria take days or even weeks. It is important to be aware of differences in growth rate when trying to identify a specimen in the laboratory. Cultures must be incubated for long enough to enable cells to multiply and be identified, otherwise the presence of a pathogen may be missed.

Phases of growth

When bacteria are grown in the laboratory in liquid medium, the medium becomes cloudy or turbid. It is possible to measure the increase in numbers of bacteria at various time intervals and plot the values on a graph representing a growth curve. When cells are first inoculated into fresh medium containing all the nutrients they require, they take a short time to adapt before they start to divide. This is termed the **lag phase**. The cells then begin to duplicate their contents and undergo binary fission, doubling the cell numbers with each generation.

Thus, for each cell present initially, there are two cells after one generation, four (or 2^2) cells after two generations, eight cells (2^3) after three generations, and so on. In other words, the number of cells increases by a power of 2 each generation time. Mathematically, this is described as a **logarithmic**, or **exponential**, **increase**. It can be seen from the calculation in Table 3.1 and the graph in Figure 3.10 that a huge number of bacterial cells can be produced in a short time. This phase of growth is termed the **logarithmic**, or **log**, **phase** when the cells are generally reproducing at a maximum rate. This type of growth rate is very sensitive to environmental conditions.

The logarithmic rate of bacterial growth continues only while conditions are optimal. As the number of bacteria increases, nutrients are used up and there is an accumulation of waste products. These may inhibit essential enzyme reactions in the cell, or cause a change in the pH of the medium, both of which will slow growth. As the density of cells in the culture increases, the availability of oxygen may decrease. The rate of cell division therefore slows down and eventually cell division ceases, or some cells may die instead of dividing.

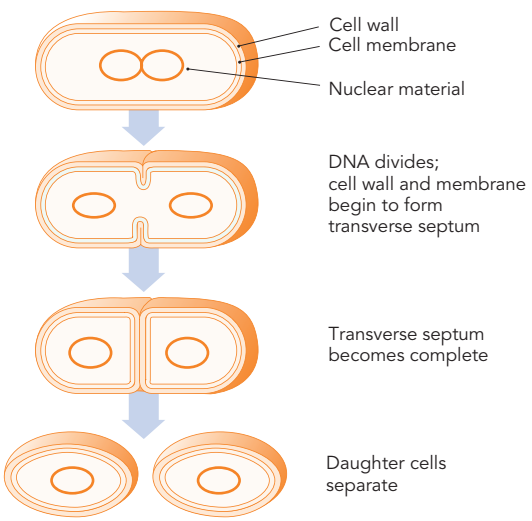


FIGURE 3.9
Binary fission in a bacterium

TABLE 3.1 **Bacterial growth**

TIME (HOURS)	NUMBER OF CELLS
0	1
0.5	2
1	4
1.5	8
2	16
2.5	32
3	64
3.5	128
4	256
4.5	512
5	1 024
5.5	2 048
6	4 096
6.5	8 192
7	16 384
7.5	32 768
8	65 536
8.5	131 072
9	262 144
9.5	524 288
10	1 048 576

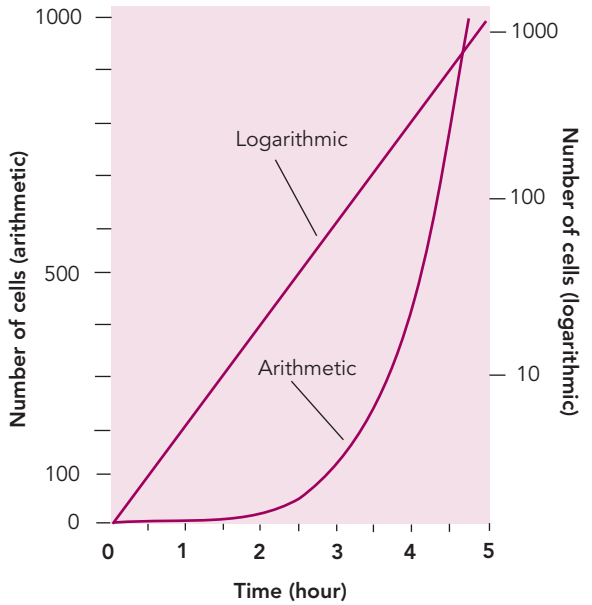


FIGURE 3.10

Rate of growth of bacterial culture

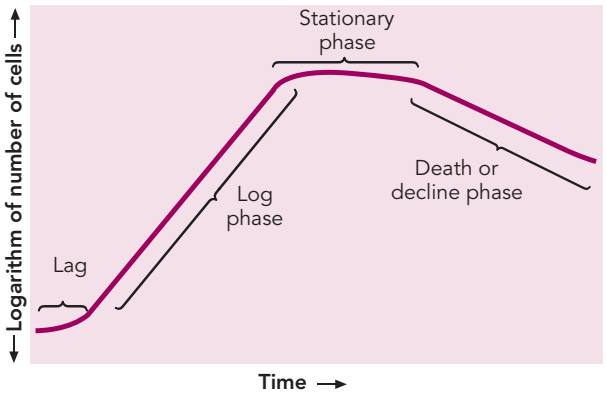


FIGURE 3.11

Typical growth curve for a bacterial culture

When the rate of cell division equals the rate at which cells are dying, the culture is said to be in the **stationary phase** of growth. Many important secondary metabolites are produced under the limited nutritional conditions encountered in the stationary phase (see Chapter 2, page 39).

Gradually, the rate of cell division stops completely and some of the cells die, so the number of viable cells is reduced and the culture enters the **decline** or **death phase**. Figure 3.11 illustrates the four phases of growth that are usually observed in cultures grown in the laboratory in liquid medium.

When cells are grown on solid medium, the dynamics of growth are similar. Single cells divide rapidly until a colony becomes visible to the naked eye. (A colony about 0.1 mm in diameter usually contains 10^4 to 10^5 cells.) Cells at the edge of the colony have a source of nutrients, so continue to divide, but cells in the centre of the colony pile up on top of each other and, without space or access to nutrients, gradually die off.

Pattern of growth in the human host

Our description of bacterial growth has been confined to laboratory cultures in closed or limited environments; under these conditions, growth is limited by the volume of culture medium contained in the tube or flask. The composition of this medium is continually being altered by the depletion of nutrients and accumulation of wastes. The rate of growth therefore slows down.

In the human body the bacteria causing an infection may be in an environment where there is a continuous supply of nutrients and oxygen via the bloodstream. Under these conditions there is no limit to growth and the infection develops unchecked. In the laboratory it is possible to devise a vessel called a **chemostat**, which allows continuous additions of fresh medium and removal of spent medium and cells. Cells can also be harvested so that bacterial growth can be maintained in the logarithmic phase. Large quantities of bacteria can be produced by this method. A chemostat is used for experiments to determine the effect of limiting the amount of various nutrients on the growth of a culture, or to prepare large quantities of bacterial cells for research or commercial use, or to encourage the formation of secondary metabolites.

IDENTIFICATION OF BACTERIA IN CLINICAL SAMPLES

A knowledge of the properties and growth requirements of bacteria is used in microbiology laboratories to isolate and identify bacteria from clinical or environmental specimens. Specimens from infected patients are sent to the microbiology laboratory in order to identify the organism responsible for the infection and determine the appropriate treatment.

The first step is usually to prepare a slide of the specimen that is then stained and examined under the microscope. In some cases this can provide a preliminary diagnosis, which is confirmed by growth of the specimen on a suitable medium, and further biochemical tests. Tests are then carried out to determine the most appropriate antimicrobial drug to prescribe.

Microscopic examination—Gram stain

Generally, bacteria can be better visualised and examined in a light microscope when they are stained. Staining can also help to visualise some internal structures (e.g. spores) and to identify and separate different bacteria. Before staining, the bacteria must be fixed (or attached) to a microscope slide to prevent them being washed away by the liquid stains. The stain(s) are then applied and after an appropriate time are washed off with water (see Figure 3.12).

Various stains are used in the microbiology laboratory to examine bacteria, or certain structures within them, but the **Gram stain** is the most useful and important staining procedure used. Developed in 1884 by the

Danish bacteriologist Hans Christian Gram, it has become the standard procedure for staining bacteria because it divides almost all bacteria into two groups, and serves as the first step in classifying and identifying bacteria. As we have already seen, bacteria stain as Gram-positive or Gram-negative, depending on the composition of their cell wall (see page 50). The Gram staining reaction is characteristic for each species.

After Gram staining, bacterial cells are either a dark violet or purple colour (Gram-positive) or a pink/red or orange colour (Gram-negative) and can be readily seen in the light microscope (Figure 3.13). The Gram stain is thus termed a *differential stain*. In Gram-stained smears of clinical specimens (e.g. swabs, cerebrospinal fluid), bacteria as well as host cells, especially polymorphonuclear leucocytes (white cells or pus cells), can be observed if present. A direct Gram stain of a clinical specimen can be performed within minutes of receiving the specimen in the laboratory. After microscopic examination of the stained smear, a presumptive diagnosis can be made in some circumstances. For example, in bacterial meningitis, the observation of bacterial cells and pus cells in a Gram stain of cerebrospinal fluid can sometimes give a good indication of the possible causative agent.

Several other stains are used by microbiologists for specific purposes. The **Ziehl-Neelsen**, or **acid-fast stain**, is important because it is used to identify bacteria belonging to the genus *Mycobacterium*, such as *M. tuberculosis* and *M. leprae*, the causative agents of tuberculosis and leprosy, respectively.

Mycobacteria have a waxy substance in their cell wall which prevents them from being stained using Gram's method. The acid-fast stain is a multi-step procedure that stains the mycobacteria a red colour, and all other bacteria and material another colour, usually blue (see Figure 3.14). It is useful for the detection of mycobacteria in sputum specimens from patients with pulmonary tuberculosis. Other bacterial stains include the spore stain (see Figure 3.15) and flagella stain, used to highlight spores and flagella for easier recognition. Some bacteria can be identified by treating the specimen with a specific fluorescent antibody. This is a rapid test and is useful for bacteria that are difficult to grow or grow only slowly in the laboratory, as the presence of the bacterium can be detected in a tissue sample (see Figure 3.16).

Stains are one of the more important techniques used by microbiologists to aid in the visualisation, classification and identification of bacteria. The Gram stain is usually the first step in the identification of bacteria. Although other more intricate and sensitive techniques have been developed for the detection of microorganisms in clinical specimens (e.g. the polymerase chain reaction and DNA probes—see Chapter 15), the ability to visualise the Gram reaction, shape and arrangement of bacterial cells is still very important in diagnostic medical microbiology.

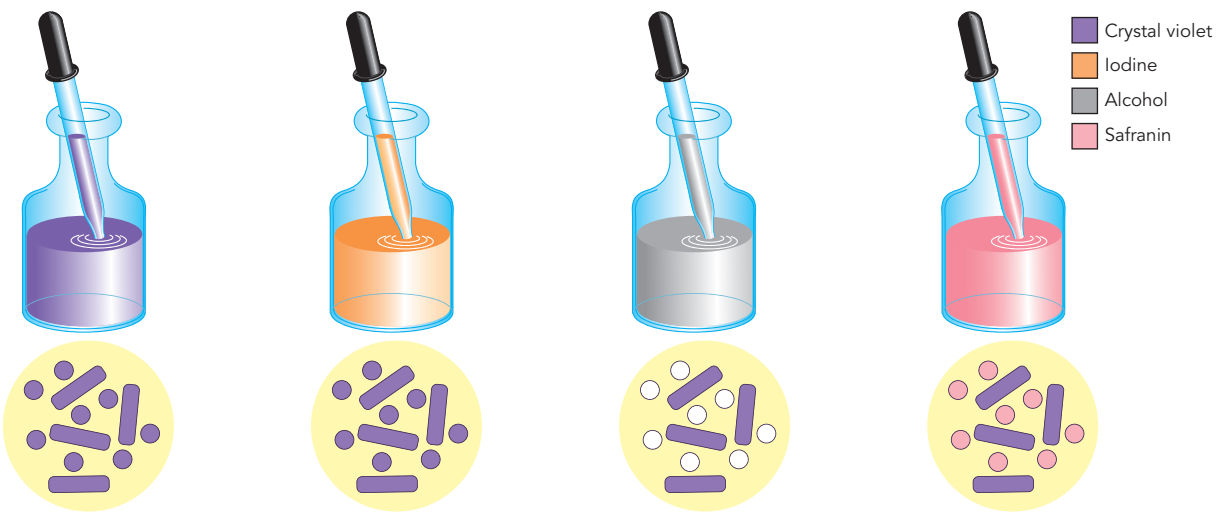
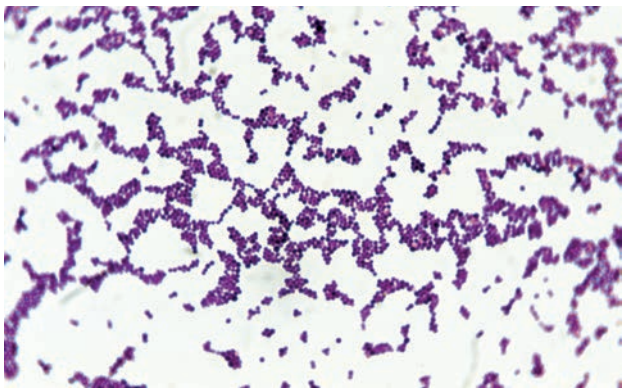


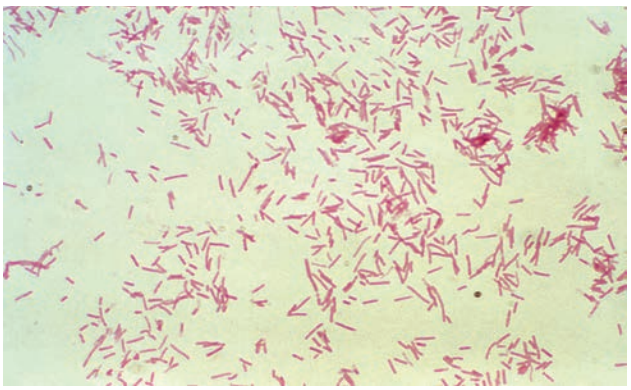
FIGURE 3.12

The Gram stain procedure

1. The slide is first covered with a solution of a purple dye, usually crystal violet. After a short time (approximately 30 seconds) the dye is washed off with water, leaving all the bacterial cells purple-coloured. 2. The smear is next treated with an iodine solution, which fixes the purple dye firmly in the cells. After the iodine is washed off all cells are still coloured a dark purple. 3. The smear is then treated briefly (a few seconds) with an alcohol or alcohol-acetone solution, followed quickly by a water rinse. This decolourises some cells by removing the purple dye from them. In this critical stage, Gram-positive bacteria retain the purple dye, but Gram-negative bacteria lose it, becoming colourless. 4. The final treatment (or counterstain) is with a red/pink dye, usually safranin or neutral red. This is necessary because the Gram-negative bacteria, which have lost their purple colour, would not be visible under the microscope. The cells that retained the purple dye in step 3 remain purple because it is a darker colour. The safranin is washed off after a short time (approximately 30 seconds) and the slide is then gently blotted dry and is ready for microscopic examination.



(a)



(b)

FIGURE 3.13

Gram stains

(a) Gram-positive clusters of Staphylococcus aureus*; (b) Gram-negative rods of* Bacteroides fragilis.

Sources: (a) G. Jayachandran, Sydney Medical School, University of Sydney; (b) CDC/Don Stalons.

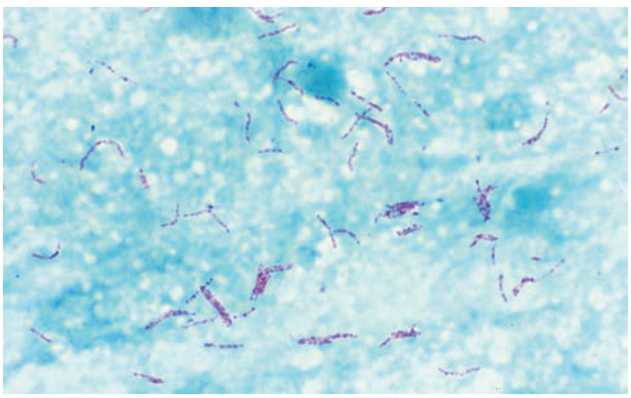


FIGURE 3.14

A Ziehl-Neelsen stain of sputum sample containing *Mycobacterium tuberculosis*

Source: Professor Richard Lumb.

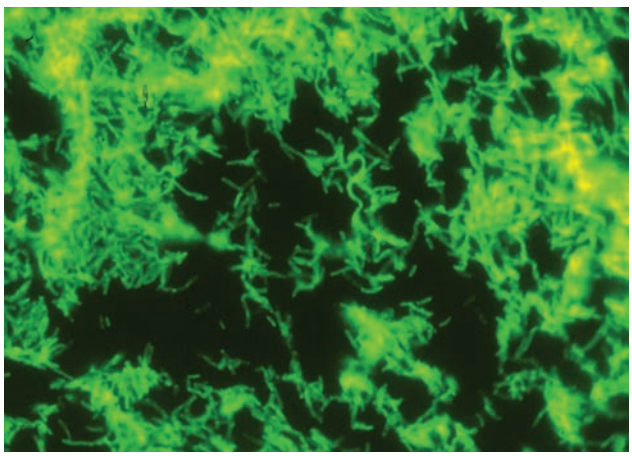


FIGURE 3.16

Fluorescent antibody stain of cells of *Legionella pneumophila*

Source: Courtesy of Dr A Smithyman, Cellabs, Sydney.

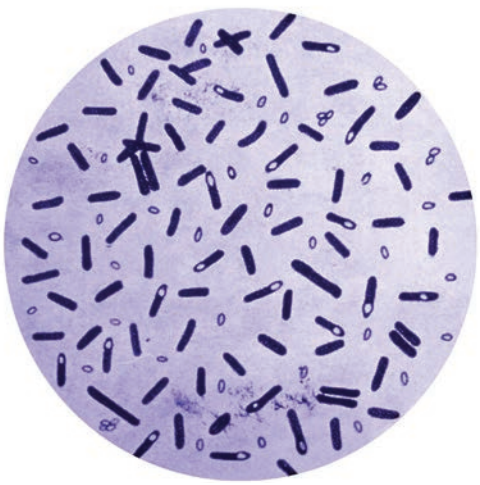


FIGURE 3.15

Spore stain of *Clostridium botulinum*

Source: Centers for Disease Control (CDC).

GROWTH OF CLINICAL SPECIMENS

The Gram stain gives the microbiologist an indication of the likely organism(s) that are present. In order to positively identify the particular pathogen that is present in a clinical sample, it is first necessary to isolate it as a pure culture—that is, a culture containing only one type of bacterium. Microorganisms that occur in clinical samples are almost invariably a mixture of normal flora and the invading pathogen. Specialised techniques have been developed to isolate and identify bacteria in the laboratory by growing them on different media. A wide variety of media are used, differing in composition according to the nature of the specimen being examined. Most media consist of a solution of salts and nutrients in water, adjusted to the correct pH. The solution is prepared and sterilised in order to kill any unwanted bacteria. If a solid medium is required, a solidifying agent is added; this is usually **agar**, a complex polysaccharide extracted from

seaweed. Agar is a useful gelling agent as it has the unusual property of melting at a much higher temperature (90°C) than it sets (45°C). The medium is poured into a petri dish, or 'plate', and allowed to set before being inoculated with a clinical specimen.

To isolate individual bacterial types, clinical specimens are usually grown on the agar plate and 'streaked out' to obtain single colonies for identification. Various methods are used to quantitate and identify the bacterial cells present (see Chapter 15).

Growth media

The media that are used may be:

- **Chemically defined**—one whose exact chemical composition is known.
- **Complex**—a medium in which most of the organic nutrients consist of small peptide or peptone fragments produced by partial digestion of large protein molecules, vitamins and growth factors. A commonly used medium is called nutrient broth. When it is required in solid form, agar is added and it is called nutrient agar.
- **Blood agar**—a medium used for the initial isolation of a clinical specimen. It consists of nutrient agar to which defibrinated horse or sheep blood has been added. It is especially useful in identifying organisms that damage red blood cells, such as the haemolytic streptococci.
- **Selective**—a medium containing substances that suppress the growth of unwanted bacteria while permitting the growth of the desired organisms. For example, MacConkey medium contains crystal violet and bile salts which inhibit the growth of Gram-positive organisms, but allow the growth of most Gram-negative bacteria.
- **Differential**—a medium used to distinguish between organisms growing on the same plate. An indicator, usually a dye, is added to the medium and it undergoes a colour change if certain organisms are present and able to metabolise the nutrients supplied.

Some media are both selective and differential. For example, mannitol salt (MS) agar contains the sugar alcohol, mannitol, as the carbon source, together with a high concentration of salt. When a clinical specimen is grown on MS agar, the salt-tolerant staphylococci will survive and grow, but other organisms will be inhibited. The strains of staphylococci present can be further differentiated because *Staphylococcus aureus* metabolises mannitol, producing an acid that turns the pH indicator dye in the medium from pink to yellow. *Staphylococcus epidermidis* does not produce acid, so there is no colour change (see Figure 2.17, Chapter 2, page 37).

Nucleic acid typing

Different strains of bacteria usually have slight differences in their DNA profile which can be detected using the techniques of molecular biology. Techniques such as the polymerase chain reaction (PCR) and pulse field gel electrophoresis (PFGE) are now being used in many clinical laboratories, especially for hard-to-grow microorganisms or when the specimen size is small (see Chapter 15).

Phage typing

A useful method of differentiating between strains of bacteria should be mentioned here. Some bacteria are attacked by viruses that are specific for a particular strain of bacteria within a species. A bacterial virus is called a **bacteriophage**, or **phage**. Bacteria isolated from different sources can be tested for their susceptibility to a particular phage. This helps to identify the strain of bacteria and can assist in epidemiological studies when clinicians are trying to identify the source of an outbreak of infection. For example, *Staphylococcus aureus* is frequently isolated from the noses of health personnel. Phage typing of the strain of *Staphylococcus* and comparison with the phage type of the outbreak can help to identify the source or carrier of the infection (see Figure 3.17).

DIVERSITY OF BACTERIA

It is beyond the scope of this text to discuss the details of bacterial classification, but it is important for students to be aware of the enormous diversity of microorganisms. Some bacteria of medical importance are described in the following sections.

A number of different criteria can be used to classify bacteria into groups. The formidable task of classifying bacteria was undertaken by a team of microbiologists and published under the title of *Bergey's Manual of Systematic Bacteriology*. The original basis for the classification included properties such as spore formation, cell wall structure, Gram stain, shape, motility, oxygen requirements and biochemical properties. This classification has now been modified to

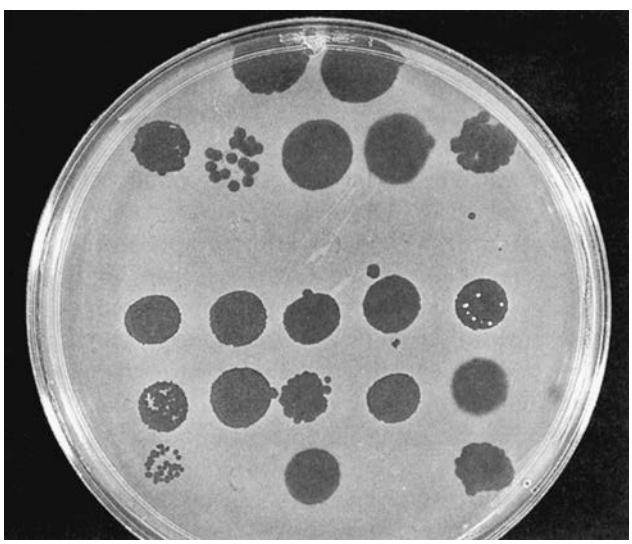


FIGURE 3.17

Phage typing of *Staphylococcus aureus*

A culture of the strain to be tested is spread over the whole plate and different phages inoculated in rows on the plate. The development of areas of lysis, or 'plaques', indicates that the strain of Staphylococcus *is sensitive to infection by some of the phages and allows identification of the strain.*

Source: G. Jayachandran, Sydney Medical School, University of Sydney.

include information obtained from analysis of the composition of the DNA and ribosomal RNA. Techniques such as cross-hybridisation and DNA sequencing can be used to determine how close the relationship is between different strains of bacteria. This information will no doubt lead to some revision of bacterial classifications in the future.

BACTERIA OF MEDICAL IMPORTANCE

There are literally thousands of different bacteria, but relatively few are pathogenic. Some of the more common bacteria are described here, grouped for convenience by their spore-forming ability, Gram-staining characteristics, shape and oxygen requirements. In the future these groupings may be changed to reflect new information about similarities in ribosomal RNA or base composition of DNA.

Gram-positive cocci (non-spore-forming)

Many of the medically important bacteria are Gram-positive cocci. The **staphylococci** and **streptococci** which are responsible for many skin and wound infections belong to this group.

Staphylococci appear as grape-like clusters when viewed under the microscope (Figure 3.1a, page 46). They are very tolerant in their growth requirements, surviving in conditions of relatively low moisture and high osmotic pressure (e.g. high salt concentration). *Staphylococcus aureus* and *S. epidermidis* are two common inhabitants of the human body, and are found in areas of low moisture such as the nose or skin. *S. aureus* is a common cause of wound infections in hospitals, where it poses a serious threat because of its ability to quickly develop resistance to antibiotics. It has also been identified as the agent responsible for toxic shock syndrome and for scalded skin syndrome.

Because of their tolerance of salt and dry conditions, staphylococci are often present in salted or preserved meats, such as bacon. Some strains of *S. aureus* produce toxins which contribute to their pathogenicity. They are a common cause of food poisoning, producing an enterotoxin that causes diarrhoea and vomiting. *S. aureus* can be distinguished from *S. epidermidis* (also a major cause of wound infections) by its ability to produce the enzyme, coagulase, and also by growth on the differential medium, mannitol salt, in which it produces a yellow colour due to a pH change in the medium as described above (see Figure 2.17).

Streptococci are Gram-positive cocci that usually occur in chains of varying length (although *Streptococcus pneumoniae* typically occurs in pairs). They are responsible for a wide variety of diseases: wound and throat infections, scarlet fever, glomerulonephritis, pneumonia etc. (described in Unit Four). They are often classified by their growth on blood agar. Beta (β)-haemolytic streptococci produce an enzyme that lyses red blood cells, creating clear areas of haemolysis on blood agar plates. Alpha (α)-haemolytic species reduce haemoglobin to methaemoglobin, causing a greenish colour change on blood plates. Gamma (γ) or non-haemolytic streptococci have no effect on red blood cells.

In the 1930s, Rebecca Lancefield discovered that the carbohydrates (antigens) in the cell walls of streptococci stimulated the formation of different antibodies. This method of identification is the basis of a system of classification whereby streptococci are placed in one of 14 groups using an alphabetical system (A, B, C, etc.). Human disease is often associated with the Group A streptococci (GAS), of which *Streptococcus pyogenes* is the most common (see Figure 3.18). Various strains produce different enzymes and toxins that have a number of pathogenic effects, causing sore throat, septicaemia and diseases such as scarlet fever and necrotising fasciitis ('flesh-eating' bacteria). The most pathogenic strains of streptococci contain a protein in their cell walls known as M-protein, which appears to be responsible for their virulence.

Group B *Streptococcus agalactiae* (GBS) is the organism most commonly associated with **puerperal fever** and neonatal meningitis. *Streptococcus pneumoniae* (pneumococcus) is a common inhabitant of the upper respiratory tract and can cause pneumonia, bronchitis, otitis media and meningitis.

Enterococci are common inhabitants of the large intestine. They are becoming increasingly important as hospital pathogens now that strains of enterococci resistant to vancomycin have been isolated (vancomycin-resistant enterococci, VRE). *Enterococcus faecalis* and *Enterococcus faecium* are the most common strains and are mainly a problem for susceptible, high-risk patients.

Gram-positive rods (non-spore-forming)

This group includes the genus *Lactobacillus*, whose members are able to produce lactic acid during carbohydrate fermentation. In humans they are found in the intestinal tract and oral cavity; they also occur in the vagina where they maintain a protective, slightly acid environment. They are used extensively in the food industry in the production of yoghurt, cheese, soured milk products and pickles, the lactic acid acting as a food preservative.

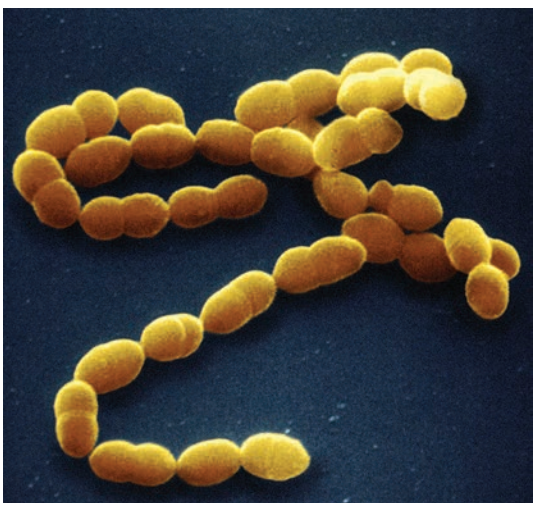


FIGURE 3.18

Coloured scanning electron micrograph showing chains of *Streptococcus*

Source: Morgan/Science. Hank/Getty Images.

Listeria monocytogenes is a Gram-positive rod that sometimes contaminates dairy products. It poses a serious threat to pregnant women, causing damage to the foetus and sometimes resulting in stillbirth (see Case History 8.2: Listeria infection, Chapter 8, page 167).

Some organisms are **pleomorphic** (i.e. their shape varies, often with the age of the cells). Best known are the **corynebacteria**, which tend to be mainly club-shaped. *Corynebacterium diphtheriae* is the causative agent of diphtheria. *Propionibacterium acnes* is found on the skin and is thought to be implicated in the skin infection, acne. Members of the genus *Actinomyces* are commonly found in the mouth and throat of humans and animals. They often form branched filaments. *A. viscosus* is involved in the colonisation of tooth surfaces, as a prelude to plaque formation by *Streptococcus mutans* and subsequent tooth decay. *Nocardia asteroides* causes skin abscesses and lung infections.

Endospore-forming Gram-positive rods

Some organisms form endospores as a means of survival under adverse environmental conditions. Some of these organisms are important pathogens. The two most important genera are *Bacillus* and *Clostridium*. *Bacillus anthracis* is the agent responsible for anthrax, a serious disease of cattle which can also affect humans. *Bacillus thuringiensis* is an insect pathogen.

Members of the genus *Clostridium* produce endospores that are highly resistant to heat. They are obligate anaerobes and produce toxins that are responsible for some serious human diseases. These include tetanus (*Clostridium tetani*), botulism (*C. botulinum*), and gangrene and food poisoning (*C. perfringens*). *C. difficile* is an opportunistic pathogen responsible for diarrhoea and pseudomembranous colitis, which can occur in patients on certain types of long-term antibiotic therapy (see Chapter 18, page 451).

Gram-negative aerobic rods and cocci

This is a very large group of organisms, some of which are important human pathogens.

The genus *Neisseria* comprises non-endospore-forming diplococci which only grow well at body temperatures. It includes two important pathogens: *Neisseria gonorrhoeae* (see Chapter 21), the causative agent of gonorrhoea, and *Neisseria meningitidis* (meningococcal disease) (see Chapter 20).

The genus *Pseudomonas* consists of highly motile flagellated aerobic rods that exist mainly in water and soil. Many of them synthesise distinctive pigments ranging in colour from blue through greenish-yellow to brown. *Pseudomonas aeruginosa* is an important opportunistic pathogen. It is often responsible for serious infections in immunocompromised patients, in patients suffering from cystic fibrosis and in burns units. Its ability to grow in water with only minimal nutrients, and its resistance to many disinfectants, antiseptics and antibiotics mean that it poses a particular threat in the hospital environment. *Burkholderia pseudomallei*, a small bacillus found in watery environments in Northern Australia, causes **melioidosis** which may start as a suppurative skin infection and develop into bacteraemia and pneumonia.

Legionella is a genus discovered in 1976 after an outbreak of atypical pneumonia in the United States. It is the cause of **Legionnaires' disease** and is spread mainly in water as aerosol droplets dispersed by wind from reservoirs, such as the water-cooling towers of air-conditioning plants, where the bacteria are growing.

Moraxella, which causes conjunctivitis, and *Acinetobacter* are both aerobic coccobacilli. *Acinetobacter baumannii* can occur as a hospital pathogen and exhibits resistance to many antibiotics.

Bordetella pertussis, the causative organism of whooping cough, is a Gram-negative rod. Despite the availability of a vaccine, there are still hundreds of cases of whooping cough in Australia each year.

Other important pathogenic organisms in this group include *Brucella* (brucellosis) and *Francisella tularensis*, a fastidious organism that requires media enriched with blood or tissue extracts for growth, and causes tularaemia.

Pasteurellaceae are very small Gram-negative bacilli and coccobacilli. They are non-motile and are very fastidious in their nutritional requirements. *Pasteurella* species infect mainly animals but can be transmitted to humans (often by animal bites).

Haemophilus influenzae is a small coccoid bacillus and an important human pathogen. Although it is often present as part of the normal flora in the upper respiratory tract, it is a common cause of middle ear infections and is responsible for potentially fatal meningitis and epiglottitis in young children (see Chapter 17, page 421). A vaccine for *H. influenzae* type b (Hib) is recommended in the childhood immunisation schedule in Australia.

The Vibrionaceae are a family of curved Gram-negative rods. The most important members are *Vibrio cholerae*, which causes cholera, and *Vibrio parahaemolyticus*, which is often found in shellfish and causes a form of gastroenteritis.

Gram-negative aerobic helical bacteria

These are slender helical Gram-negative rods that do not have an axial filament but use flagella for motility. *Campylobacter jejuni* is an important pathogen responsible for foodborne gastrointestinal infections. *Helicobacter pylori* has been shown to be associated with gastric ulcers in humans (see Chapter 18, page 458).

Facultatively anaerobic Gram-negative rods

The **Enterobacteriaceae** (**enterics**) form a large group of very important organisms, many of which inhabit the human intestine as part of the normal flora. The most important genera are *Escherichia, Salmonella, Shigella, Klebsiella, Serratia, Proteus, Yersinia* and *Enterobacter*.

Escherichia coli (*E. coli*) is one of the predominant inhabitants of the human intestine (Figure 3.19). Its presence in water is often used as an indication of faecal contamination ('coliform' count). It is generally considered to be part of the normal flora and relatively non-pathogenic, unless it is transferred to body sites other than the colon. It is one of the most common causes of urinary tract infections. Some

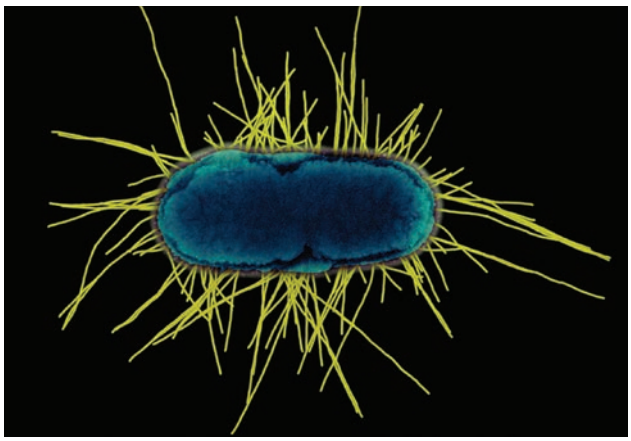


FIGURE 3.19

Coloured SEM of *Escherichia coli* showing fimbriae

Source: Getty Images.

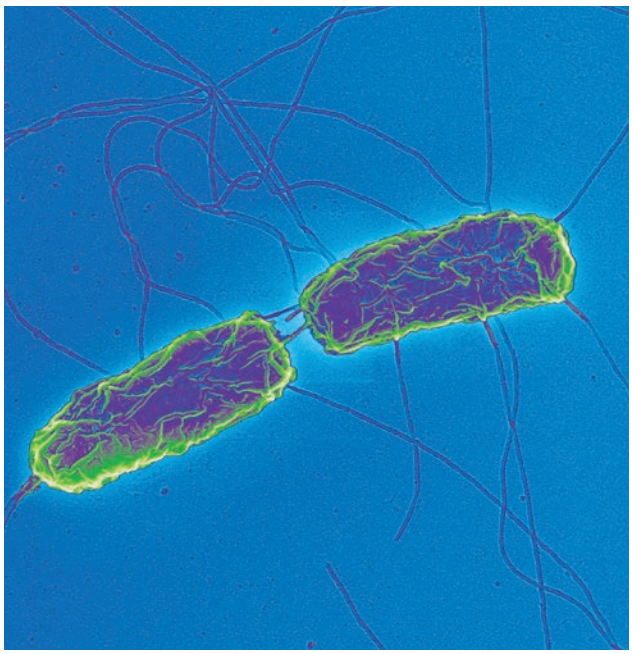


FIGURE 3.20

Coloured SEM of *Salmonella typhimurium after cell division*

Source: Science Photo Library.

strains produce enterotoxins that can cause diarrhoea or haemolytic uraemic syndrome (see Chapter 18, page 448). Species of *Shigella* are responsible for a severe type of diarrhoea called bacillary dysentery, or shigellosis, caused by the ingestion of contaminated food.

Almost all the members of the genus *Salmonella* are pathogenic (Figure 3.20). *Salmonella typhi* is responsible for typhoid fever. Strains of *Salmonella typhimurium* are often associated with chickens and are responsible for various gastrointestinal diseases and food poisoning. The different strains are identified on the basis of their surface antigens, which are referred to as 'serovars' or 'serotypes'.

Among other enterics, *Klebsiella pneumoniae* is a major cause of pneumonia, especially in children and susceptible patients. *Proteus* is an inhabitant of the large intestine and a common cause of urinary tract and wound infections; it has a distinctive 'swarming' appearance when grown on plates in the laboratory and an unpleasant odour. *Enterobacter cloacae* and *E. aerogenes* are opportunistic pathogens which may cause urinary tract infections. *Serratia marcescens* grows at room temperature with the production of a distinctive red pigment. In recent years it has been recognised as a significant cause of opportunistic health-care-associated infections.

The most pathogenic of all the enterics is *Yersinia pestis*, the cause of bubonic and pneumonic plague (see Chapter 19).

Anaerobic Gram-negative rods

Bacteroides, which live in the human gastrointestinal tract, are anaerobic Gram-negative rods that are non-motile, do not form endospores and are often responsible for infections due to puncture wounds or surgery, and for peritonitis.

Spirochaetes

The **spirochaetes** are long (10 μm) Gram-negative helical bacteria with two or more axial filaments for motility, giving them a distinctive corkscrew-like appearance (see Figure 3.1e, page 46). Some are free-living, occurring in sewage, mud and decaying organic matter. Others inhabit humans and animals as part of the normal flora. Among the spirochaetes is the causative organism of the sexually transmitted disease, syphilis—*Treponema pallidum* (see Chapter 21). Members of the genus *Borrelia*, which are usually transmitted by ticks, are responsible for relapsing fever and Lyme disease (see Chapter 19). *Leptospira* species are often acquired from water contaminated with animal faeces or urine and cause Weils disease characterised by kidney and liver damage.

Intracellular bacteria

Rickettsias are very small, obligate intracellular parasites and were not at first classified as bacteria because they can only reproduce inside a living cell. They are rod-shaped bacteria or coccobacilli that are **pleomorphic** (have many forms). They are Gram-negative and non-motile and most of them are transmitted to humans by the bite of insects or ticks. Some of the diseases caused by rickettsias include epidemic and endemic typhus, Rocky Mountain spotted fever in the United States, and scrub typhus in Australia. **Q fever**, caused by the rickettsia *Coxiella burnetii*, is harboured by ticks but appears to be mainly transmitted person to person in aerosols and from contaminated cattle.

Chlamydiae are also Gram-negative, non-motile intracellular parasites and are transmitted by close person-to-person contact. They have a complex developmental cycle. In the host cell they develop inside membrane-bound cytoplasmic vacuoles, forming large metabolically active reticulate

bodies. These divide many times by binary fission, rupture and release the smaller infectious particle, or elementary body, which can attack other cells. *Chlamydia trachomatis* is the agent responsible for trachoma. The organism can also be sexually transmitted and is a primary cause of non-gonococcal urethritis and pelvic inflammatory disease.

C. pneumoniae is now considered to be the most prevalent of the chlamydiae and is a significant cause of pneumonia, especially in elderly patients. It is transmitted person to person and is thought to be responsible for some infections that were previously identified as *C. psittaci*, the agent responsible for psittacosis in birds.

Mycoplasmas

Mycoplasmas are very small bacteria that do not form cell walls. They are mainly aerobic or facultatively anaerobic. Since they are not bound by a rigid cell wall, they are capable of assuming many shapes (**pleomorphic**), often producing fungus-like filaments. *Mycoplasma pneumoniae* is the cause of atypical pneumonia. The mycoplasmas and the **ureaplasmas** are often inhabitants of the vagina and are thought to be involved in intrauterine infections in pregnancy, which may result in abortion.

Mycobacteria

Mycobacteria are aerobic rod-shaped organisms that occasionally form filaments. They do not stain well with the Gram stain as they are surrounded by a waxy outer layer. An acid-fast (Ziehl-Neelsen) stain is used instead (see Figure 3.14, page 59), so they are also known as **acid-fast bacilli** (**AFBs**). *Mycobacterium tuberculosis* causes tuberculosis, and *M. leprae* is the causative agent of leprosy. *M. avium* and *M. intracellulare* are important opportunistic pathogens found in immunosuppressed patients. *M. ulcerans* is responsible for Bairnsdale ulcers, which occur in certain regions of Queensland and Victoria.

SUMMARY

CLASSIFICATION OF BACTERIA

- Bacteria are the largest group of medically important microorganisms.
- Bacteria can be differentiated on the basis of their cell wall structure, Gram stain, shape, motility, oxygen requirements, biochemical properties and analysis of DNA or ribosomal RNA.
- Bacterial names contain the Genus and species and are written in italics.
- Bacteria are classified as procaryotic cells. They are grouped into two domains, the Archaea and Bacteria.
- Bacteria occur in three main shapes—spherical (cocci), rod-shaped (bacilli) and spiral (spirilla, spirochaete or vibrio).
- Bacteria are also identified and classified on the basis of their staining properties, metabolic reactions, and by nucleic acid typing.

STRUCTURE OF BACTERIA

- Bacteria are simple single-celled organisms surrounded by a rigid cell wall, with no sub-cellular organelles.
- The cytoplasm contains water, enzymes, ribosomes, inclusion granules and a single, circular chromosome of DNA.
- In some bacteria, additional genetic information is carried on a plasmid.
- Ribosomes are the site of protein synthesis.
- Some bacteria are surrounded by a polysaccharide-containing capsule, the glycocalyx, which allows the bacteria to bind to surfaces and evade phagocytosis.
- Flagella are thin, rigid filaments which enable the bacteria to swim.
- Pili are short, hair-like appendages which aid in the attachment of bacteria to mucosal cells. Some are involved in bacterial conjugation.
- Bacterial cell walls are complex, semi-rigid structures.
- Depending on the structure of the wall, bacteria are classified as Gram-positive or Gram-negative.
- Mycobacteria have a waxy outer layer that necessitates their identification by the acid-fast stain.
- The cell wall can be damaged by certain chemicals or enzymes such as lysozyme. The synthesis of peptidoglycan is inhibited by penicillin.
- Bacterial cells have a plasma membrane, consisting of a phospholipid bilayer with proteins embedded in it.
- The plasma membrane is a semi-permeable barrier, controlling the passage of substances into and out of the cell.
- Bacterial cell membranes are the site of metabolic reactions which occur in the organelles of eucaryotic cells.
- Some Gram-positive bacilli form resistant endospores when environmental conditions are unfavourable.

REPRODUCTION IN BACTERIAL CELLS

- Bacteria require various physical factors for growth, including water, the correct osmotic pressure, the correct amount of oxygen, and the correct temperature and pH.
- Nutritional requirements include water, the elements carbon, nitrogen, sulphur and phosphorous, and various trace elements. Autotrophic bacteria use carbon dioxide from the atmosphere. Some bacteria require specific organic compounds.

PATTERN OF BACTERIAL REPRODUCTION

- Bacterial growth is defined as an increase in cell numbers.
- Bacteria reproduce by binary fission.
- The time taken for a cell to reproduce itself is called the generation time.

- When grown in the laboratory under controlled conditions, bacteria have four phases of growth: lag, logarithmic, stationary and death.

IDENTIFICATION OF BACTERIA IN CLINICAL SAMPLES

- The Gram stain is the most useful staining procedure used in the microbiology laboratory because it divides almost all bacteria into two groups: Gram-positive (purple stain) or Gram-negative (pink/red or orange stain), depending on the composition of their cell wall.
- Spore stains and Ziehl-Neelsen stains are also used.

GROWTH OF CLINICAL SPECIMENS

- Bacteria can be isolated from clinical specimens and identified in the laboratory by growing them on defined media.
- Nucleic acid typing and Phage typing are used to differentiate bacterial strains.

DIVERSITY OF BACTERIA

- *Bergey's Manual of Systematic Bacteriology* classifies bacteria on the basis of morphology and biochemical properties.
- They are grouped according to their shape, growth patterns and the diseases they cause.
- Newer methods include nucleic acid typing.

BACTERIA OF MEDICAL IMPORTANCE

- Many bacteria are of medical importance.

STUDY QUESTIONS

1. Describe the major shapes of bacterial cells.
2. What is meant by 'morphology'?
3. How does the structure of Gram-positive cell walls differ from the structure of Gram-negative cell walls?
4. What is meant by an 'acid-fast bacillus'?
5. Describe the structures that carry genetic information in bacterial cells.
6. What is the function of these extracellular bacterial structures—glycocalyx, flagella and pili?
7. What are the main requirements for bacterial growth?
8. Describe the four phases of bacterial growth.
9. Why are preparations of microorganisms usually stained before they are examined with a light microscope?
10. Why is the Gram stain so important in microbiology?
11. How are mycobacteria identified in clinical specimens?
12. What techniques are now used in addition to those used to classify bacteria by Bergey's method of classification?

TEST YOUR UNDERSTANDING

1. How does the structure of the plasma membrane control the passage of substances into and out of the cell?
2. Why don't bacteria grow in honey or jam?
3. Why is it important to use methods of sterilisation that destroy endospores?
4. Why is tetanus and/or gangrene sometimes treated by placing the patient in a hyperbaric oxygen chamber?
5. Explain how botulism occurs in preserved food.
6. How can phage typing help to identify the source of an infection?

FURTHER READING

Holt, J.G. (ed.) 1994, *Bergey's Manual of Systematic Bacteriology*, 9th ed. (Baltimore, MD: Williams & Wilkins). (A standard reference for identification and classification of bacteria.)

CHAPTER

4

Genes and biotechnology

CHAPTER FOCUS

- How does the unique structure of DNA ensure the exact replication of genetic information?
- What is the role of nucleic acids in cell replication and protein synthesis?
- What are genes, and how do they control cellular structure and function?
- What is the effect on a cell when its DNA is altered by mutation or recombination?
- How can the analysis of DNA composition be used to broaden our understanding of the nature and spread of disease?
- What is genetic engineering, and how is it used to benefit humans?

INTRODUCTION

Every living organism has distinctive characteristics that are determined by the composition of its genome or 'genes'. The genome consists of chromosomes that are contained in the nuclear region of the cell. They are made up of genes that carry the genetic information that is specific for each cell. Over the last 50 years, research into the nature of genes has revealed the unique structure of the genetic material, how characteristics are passed from one generation to the next, and how genes direct cellular structure and function. The culmination of this research was the completion of the Human Genome Project in 2000—a project that involved mapping all the genes that make up the human chromosomes.

Research using bacteria contributed to this project by providing much of our early understanding of the nature of the genetic material, and resulted in the development of methods for nucleotide analysis. The study of bacterial metabolism provided information about the mechanisms of gene expression and protein synthesis. A full description is beyond the scope of this book, but some aspects of this topic have special significance for the health professional. For example, nucleic acid analysis of clinical isolates of bacteria can be used to track the source and kind of infection. The ability of bacteria to mutate, and in some cases to transfer genetic material (resistance factors) between related cells, is of importance in the development of resistance to antimicrobial drugs.

This chapter examines the structure of the genetic material (genes) and its method of replication; the different kinds of nucleic acids, the way in which they are synthesised and their involvement in protein synthesis; and the way genes direct and control cellular function. The effect on the cell when the composition of the genetic material is altered by **mutation** or by **genetic engineering** is discussed. The material presented here is an overview of the topic and is an attempt to introduce the student to some of the terms used, and to show how an understanding of genetic processes can assist in the control and treatment of disease. We will focus on microbial genetics, but it is important to remember that many of these same processes occur in higher organisms.

GENES

What are genes, and how do they control cellular activity?

A gene is a piece of deoxyribonucleic acid (DNA), a compound that is made up of a linear sequence of **nucleotides**. A nucleotide consists of a nitrogen-containing compound, or 'base', joined to a pentose sugar molecule, deoxyribose, and to phosphate. There are four different bases in DNA: the purines, **adenine** (**A**) and **guanine** (**G**), and the pyrimidines, **thymine** (**T**) and **cytosine** (**C**) (see Figure 4.1). Each gene contains a specific sequence of nucleotides and is responsible for determining a particular characteristic of the cell.

A number of genes are joined together to form a structure called a **chromosome**.

In bacteria, all the genetic material is contained in a single circular chromosome containing many thousands of genes. It is not enclosed by a membrane, but is tightly coiled inside the cell (see Figure 4.2). Some bacteria contain one or more smaller pieces of DNA, called **plasmids**, which carry additional information and replicate independently of the chromosome. Eucaryotic cells usually have more than one chromosome and the DNA exists in a linear structure in association with proteins called **histones**. The chromosomes are contained within the nucleus and surrounded by the nuclear membrane. In addition, eucaryotic cells carry small amounts of DNA in their mitochondria. Viruses do not contain chromosomes, and their genetic information is contained in either single- or double-stranded DNA or single- or double-stranded ribonucleic acid (RNA) (see Chapter 5). In ribonucleic acid the pentose sugar is ribose instead of deoxyribose and the base thymine is replaced by **uracil** (**U**). The nucleic acids store information and direct the synthesis of proteins that are needed to maintain the structure and functions of the cell.

STRUCTURE OF DNA

The name 'nucleic acid' is derived from early studies which showed that the substance carrying hereditary traits was located in the nucleus of the cell. When DNA was first isolated in the 1940s and the bases were chemically analysed, it was found that the amount of thymine present

The Human Genome Project

SPOTLIGHT ON

The Human Genome Project was a worldwide cooperative study by scientists to map the complete sequence of the 100 000 genes that make up the human chromosomes. It required the determination of the sequence of about 3 billion nucleotide pairs. It is now theoretically possible to screen individuals and determine their exact genetic makeup. DNA analysis also allows scientists to test for defective genes that may be responsible for a particular disease. There are many ethical issues about how this information should be used. Attempts have been made to introduce selected pieces of DNA into embryonic cells in order to correct genetic defects, but these experiments are in the early stages and are also the subject of much discussion about the ethics of such procedures.

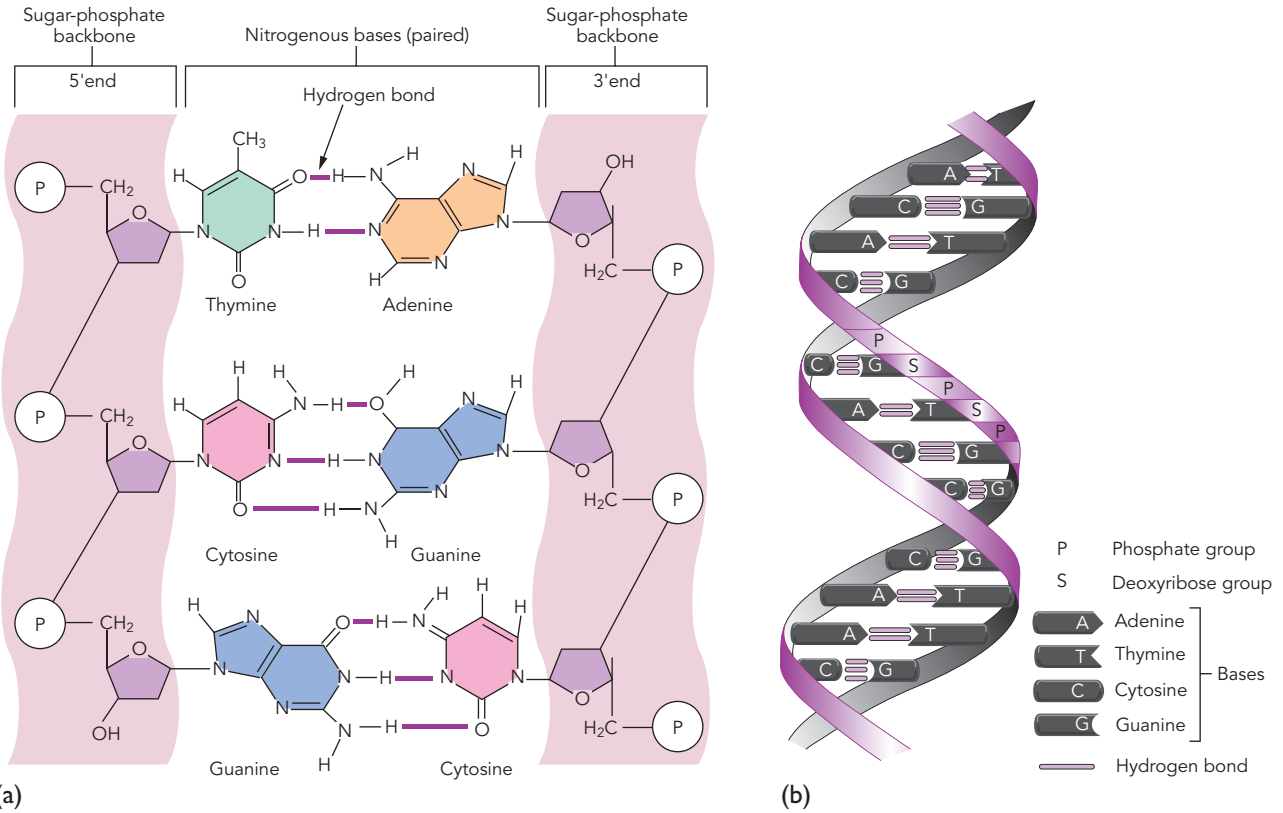


FIGURE 4.1

Structure of DNA

(a) Pairing of nitrogenous bases in sugar-phosphate backbone; (b) helical structure of DNA.

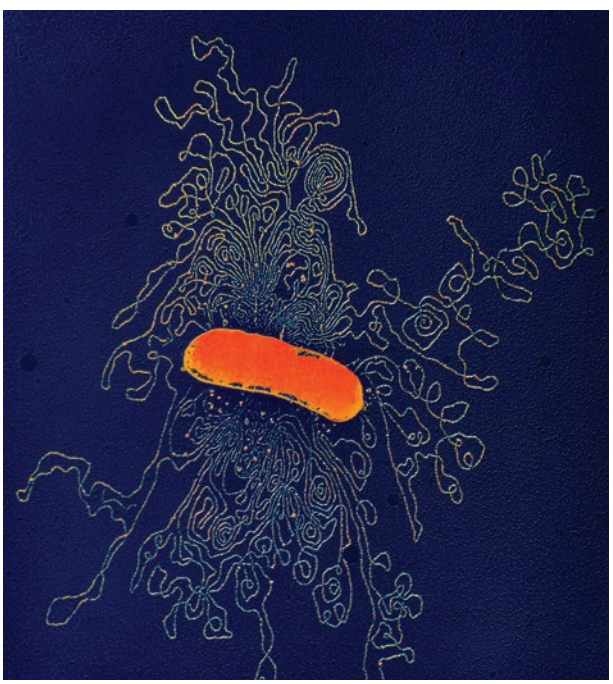


FIGURE 4.2

Electron micrograph of DNA of *Escherichia coli*, spilling out of a damaged cell

The DNA is normally tightly coiled and packed into the cell.

Source: Dr Gopal Murti/Science Photo Library.

was approximately the same as the amount of adenine and the amount of cytosine was approximately equal to guanine. However, the significance of this finding was not readily apparent and it was necessary to further elucidate the unique structure of DNA in order to understand that the pairing of these bases was crucial to the structure, and provided a mechanism for genetic information to be preserved and transferred accurately during cell division.

The results of careful research by English scientists Max Wilkins and Rosalind Franklin, on the X-ray crystallography of DNA, led James Watson and Francis Crick in 1952 to propose a structure for DNA that has laid the basis for our understanding of genetic inheritance.

Their breakthrough concept of a double helical structure for DNA satisfied the requirements for a structure that could be replicated exactly and thus carry the genetic information from one generation to the next.

The double helix

DNA consists of two strands of nucleotides twisted around each other into a double helical structure. The nucleotide bases are arranged in a specific sequence in each strand. The strand has a backbone of sugar and phosphate from which the bases protrude (see Figure 4.1a).

To enable the two strands to fit together into a double helix, the bases have to be held together by hydrogen bonds between the opposing strands, with adenine opposite

thymine and guanine opposite cytosine. This explains the equal ratios of adenine to thymine and guanine to cytosine.

Figure 4.1(b) shows the simple, elegant structure proposed for DNA. It explains the unique properties of the genetic material and, in particular, allows for the exact replication of the nucleotide sequence. The sequence of nucleotides in one strand exactly complements the sequence in the other strand, but runs in the opposite direction.

NUCLEIC ACID SYNTHESIS

The genetic information contained in the DNA molecule directs cell division and also the synthesis of proteins in the cell. During cell division the chromosomal DNA is replicated to produce two identical DNA molecules that are then transferred to the new cells. In the growing cell, DNA directs the synthesis of functional proteins using a complex system involving different types of ribonucleic acid.

REPLICATION OF CHROMOSOMAL DNA

The discovery that DNA existed as a double helix showed how genetic properties could be preserved during cell division. When a cell divides to form two new cells, a copy of its DNA must be transferred to each new daughter cell. Replication of DNA involves a number of steps. First, a helicase enzyme breaks the hydrogen bonds between the two strands of DNA, allowing them to separate (see Figures 4.3 and 4.4). A new strand of DNA is then built up on each of the original strands. Individual nucleotides are incorporated on to the complementary sites on the exposed single strands of DNA and the nucleotides are then joined together ('zipped up') by an enzyme called DNA polymerase. The sequence of nucleotides in each of the original strands acts as a pattern, or template, for the synthesis of the new strand. Base pairing ensures that two new identical double helices are formed. In this way the genetic properties of the cell are preserved from one generation to the next. The process occurs the same way in all living cells. A number of enzymes were discovered that could break DNA molecules at different points in the DNA chain, depending on the sequence of bases. These enzymes have been used successfully in the laboratory to recombine DNA molecules in various kinds of genetic engineering (see below).

PROTEIN SYNTHESIS

Most activities of the cell are carried out by proteins. Proteins are large organic molecules consisting of long chains of amino acids arranged in a specific sequence. The amino acids are held together by peptide bonds and folded in various ways in order to carry out their function in the cell (see Chapter 2, Figure 2.13, page 32). Enzymes are proteins, as are many of the structural components of the cell. Cells have to continually synthesise the proteins needed for metabolism, growth and repair.

DNA contains the genetic information required for the synthesis of these cellular components, so the cell needs a way of using this information to direct the synthesis of the correct protein structures. In order for this to occur, the

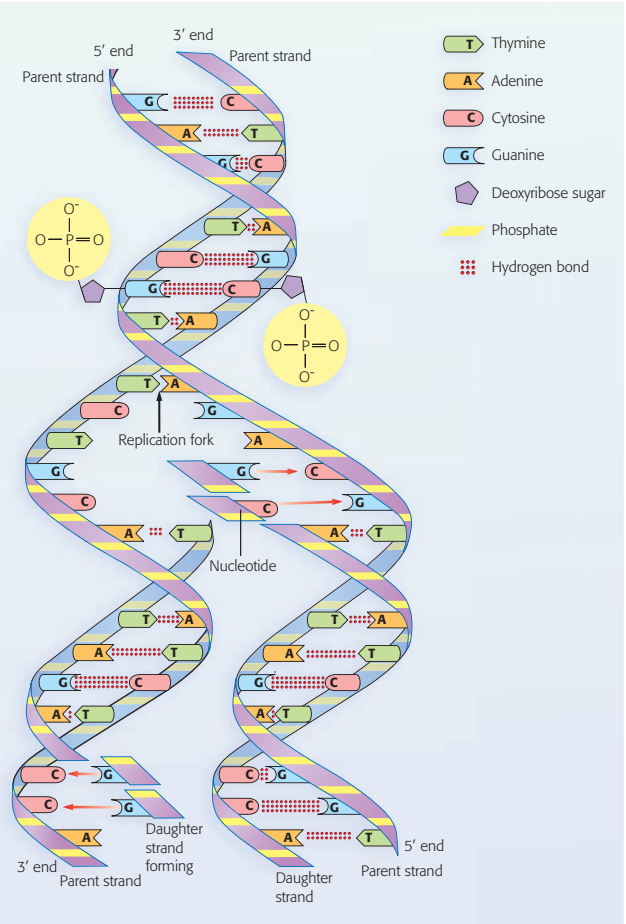


FIGURE 4.3

Two new identical double helices being formed

information in the DNA is first copied (transcribed) into another type of nucleic acid, ribonucleic acid (RNA), which then directs the synthesis of proteins in the cell.

Ribonucleic acid differs from DNA in that the sugar molecule in the nucleotides is ribose instead of deoxyribose and one of the bases, thymine, is replaced by the closely related purine molecule, uracil (U). In addition, RNA does not occur as a double helix, but usually in single strands.

Three distinct types of RNA take part in protein synthesis. They vary in molecular size and function.

1. **Ribosomal RNA** (**rRNA**) is a large molecule that combines with protein to form particles called ribosomes, which act as a support framework or site of protein synthesis in all cells.
2. **Messenger RNA** (**mRNA**) is a single strand of RNA that is synthesised on the chromosomal DNA template and carries the information or 'message' from the gene to the site of protein synthesis. Messenger RNA is bound to the ribosomes and provides a template for the correct sequence of amino acids to be joined together to form the protein.

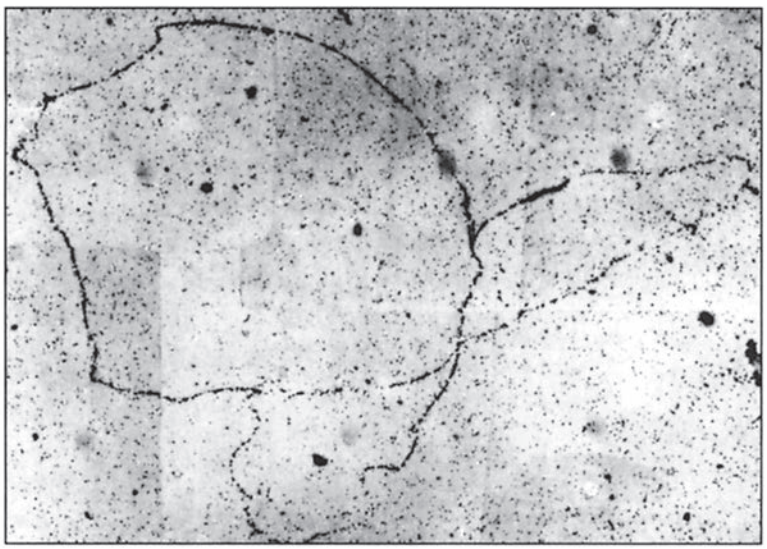
3. **Transfer RNA** (**tRNA**) (Figure 4.5) is a relatively small molecule, the function of which is to transfer amino acids to the mRNA strand on the ribosomes, in order to ensure that the correct amino acid is added to the growing peptide chain.

Mechanism of protein synthesis

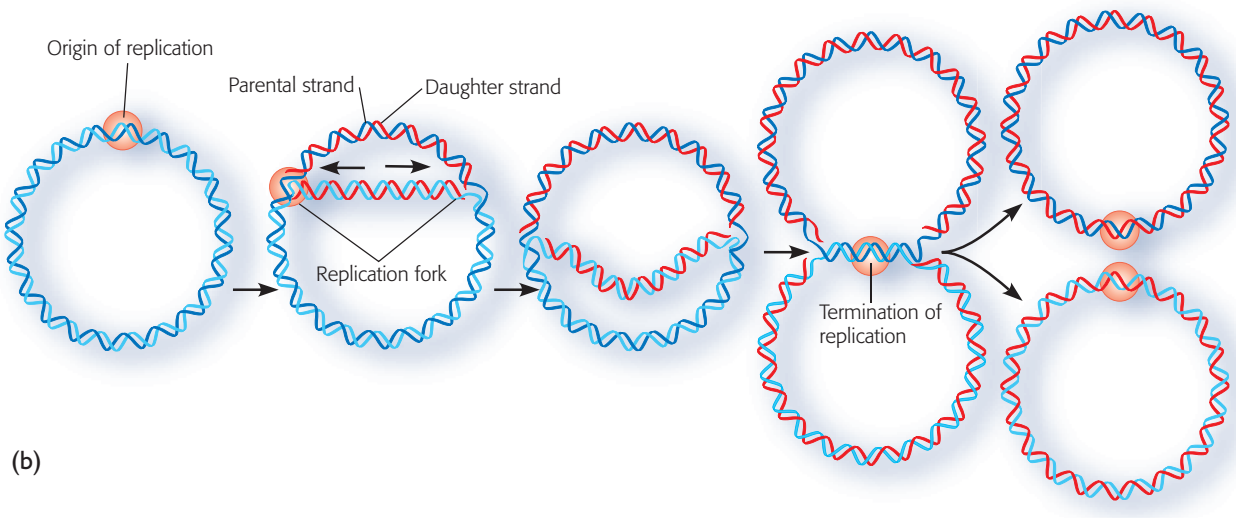
The synthesis of a functional protein with the correct amino acid sequence is dependent on the information in the chromosomal DNA being correctly transferred to the finished protein. Special terms are used to describe this process—transcription and translation.

Transcription is the term applied to the synthesis of mRNA on the DNA template—that is, the formation of a single strand of ribonucleic acid with a base sequence complementary to the base sequence of the DNA. The DNA is *transcribed* into a different form. The process could be likened to changing handwritten information into a typewritten form, but keeping the same language.

During transcription, the double strands of DNA are pulled apart and an enzyme called RNA polymerase binds to one of the strands of DNA at a site called the promoter. RNA nucleotides are then paired with the complementary DNA bases—adenine with thymine, uracil with adenine, and guanine with cytosine. As the messenger RNA is synthesised, the section of DNA that has been transcribed rejoins. When a terminator codon (see below) is reached, the RNA polymerase and the new mRNA are released. The mRNA that is produced may be a sequence containing the information from only one gene or from several closely related



(a)



(b)

FIGURE 4.4

Replication of bacterial DNA

(a) A radioactively labelled E. coli *chromosome in the process of replicating; (b) a diagram of the bidirectional replication of a circular bacterial DNA molecule. The new strands are shown in red.*

genes. The newly formed mRNA moves from the DNA to the ribosomes, where it is attached and acts as a template or pattern for the formation of protein molecules.

Translation refers to the formation of a protein, or polypeptide, composed of a chain of amino acids, using the mRNA as a template or pattern to ensure that the correct amino acid is inserted into the correct place. The product here is a long chain composed of amino acids in place of the nucleotides of the RNA and DNA. This process, therefore, can be likened to translating a message from one language to another (English to French, for example). In this case, the information in the nucleic acid is *translated* into protein.

The processes that are involved can be summarised as follows:

$$\text{DNA} \xrightarrow{\text{transcription}} \text{mRNA} \xrightarrow{\text{translation}} \text{protein}$$

During translation, the mRNA has to be able to direct the incorporation of amino acids into polypeptide chains in a specific sequence.

How is this achieved?

In the 1960s, several scientists working in the United States realised that the sequence of the nucleotide bases in the nucleic acid molecules could determine which amino acid was incorporated into the protein. They were able to show that a specific sequence of three nucleotide bases, called a **codon**, was responsible for the binding of each particular amino acid.

There are four different nucleotides. It can be calculated that, if there are three nucleotides in each codon, they can be arranged in $4 \times 4 \times 4 = 64$ different ways or codons—more than enough for the 20 naturally occurring amino acids found in proteins. We now know that some amino acids have more than one codon and some codons contain other messages, such as 'stop' and 'start'. Table 4.1 shows the nucleotide codon for each amino acid.

Transfer RNA

One further discovery was necessary to complete the picture. It was already known that small molecules of RNA, called **transfer RNA** (**tRNA**), could bind to amino acids. It was found that there was a specific tRNA for each amino acid and that on this tRNA there was a sequence of three nucleotides, or anticodon, that could recognise the matching codon on the messenger RNA (see Figure 4.5). It was now possible to visualise a scheme whereby the genetic information in the DNA could be translated into functional proteins (see Figure 4.6). Enzymes in the cytoplasm attach the appropriate amino acid to the molecule of tRNA with an anticodon corresponding to its binding site on the mRNA template. This ensures that the amino acid will be inserted into the

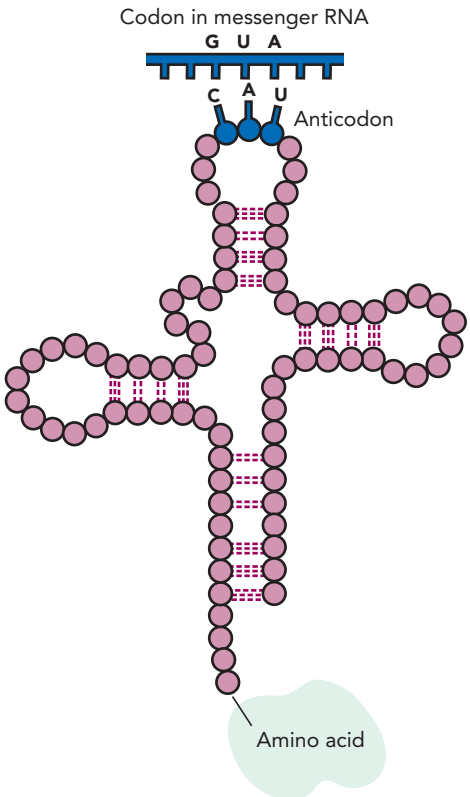


FIGURE 4.5

Structure of transfer RNA

The two-dimensional structure of a molecule of transfer RNA. The anticodon end will pair up with a codon on a strand of messenger RNA and deliver the desired amino acid, which is bonded to its other end. The molecule is maintained in its cloverleaf pattern by hydrogen bonding between strands that form the arms (dotted lines).

TABLE 4.1 **The genetic code**

Codons are written with the 5'-terminal nucleotide on the left. Note that most amino acids are represented by more than one codon and that variation at the third nucleotide in a codon is common.

Amino acids are written as follows:

Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ileu	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	Stop
GCU	CGU	GAU	AAU	UGU	GAG	CAG	GGU	CAU	AUU	CUU	AAG	AUG	UUU	CCU	UCU	ACU	UGG	UAU	GUU	UGA
GCG	CGG	GAC	AAC	UGC	GAA	CAA	GGG	CAC	AUC	CUG	AAA		UCC	CCG	UCG	ACG		UAC	GUG	UAG
GCC	CGC						GGC		AUA	CUC				CCC	UCC	ACC			GUC	UAA
GCA	CGA						GGA			CUA				CCA	UCA	ACA			GUA	
AGG									UUG					AGU						

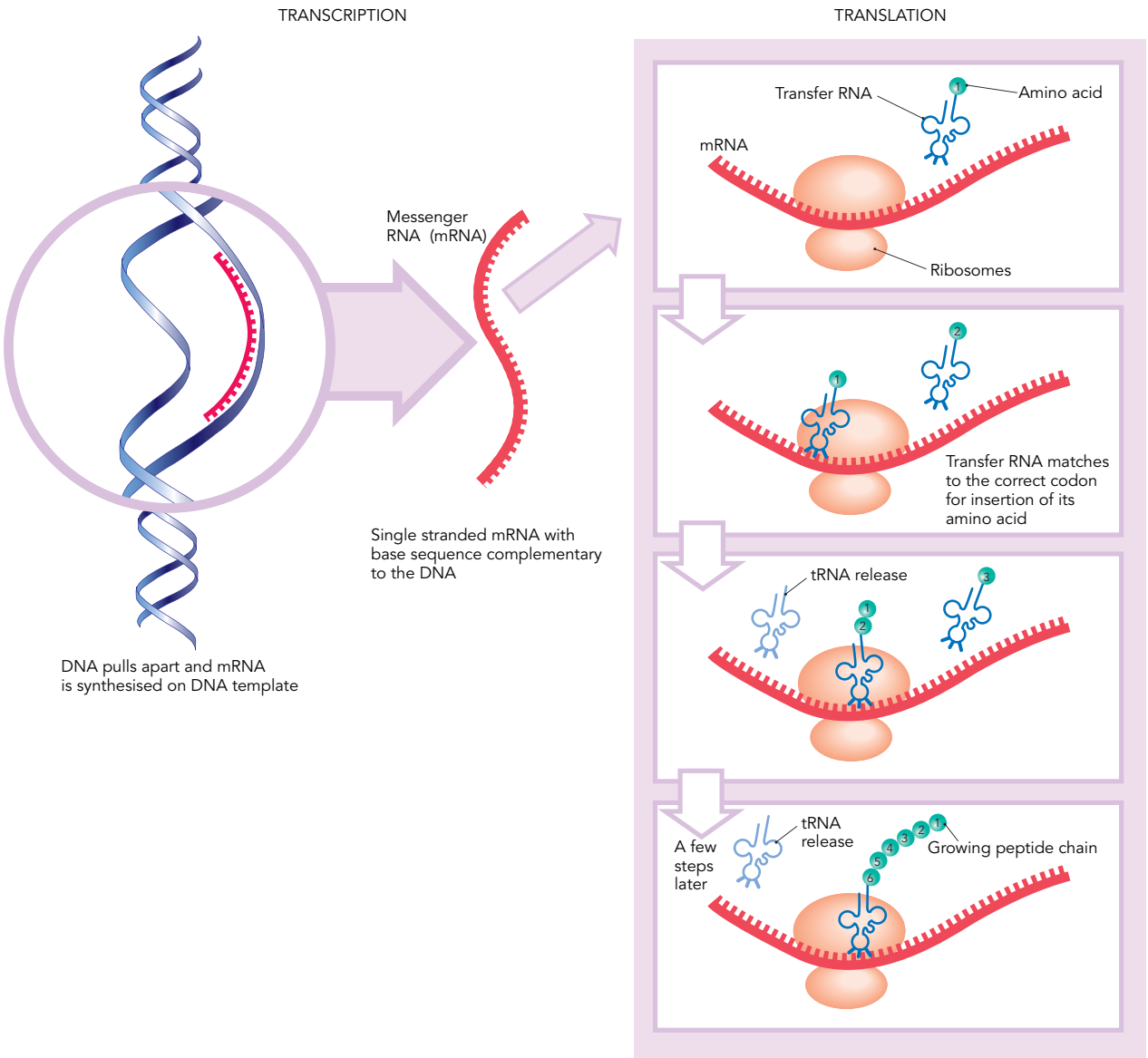


FIGURE 4.6

Main steps in protein synthesis

Diagrammatic representation of the events in protein synthesis. Genetic information encoded in DNA is transcribed into messenger RNA. The mRNA attaches to ribosomes in the cytoplasm and acts as a template for the insertion of the correct sequence of amino acids into the growing peptide chain by matching the codons on the transfer RNA.

correct place in the growing peptide chain. The aa-tRNA is attached to the mRNA template. The tRNA molecule is released and a ribosomal enzyme joins the amino acids together by peptide bonds.

In short: DNA makes RNA, and RNA makes protein.

When the ribosome reaches the 'stop' codon on mRNA, the polypeptide is released.

Gene expression and repression

The accurate transfer of the genetic information contained in the DNA of the cell into functional proteins is of primary importance in all living cells. It is sometimes said that a gene is *expressed* or *not expressed*. This refers to whether or not the cell is actually producing a particular protein for which it has the gene. Most cells carry information for the synthesis of enzymes (proteins) that they do not need to use all the time. Bacteria, especially, are very small and there is no space in the cell for proteins that are not actually carrying out a function. Thus, although the bacteria may carry the gene, it may not necessarily be expressed. It is rather like having a book full of recipes (the DNA) that are used only when required. This characteristic enables bacteria to make best use of their nutritional environment. This property of bacteria was discovered when it was found that *E. coli* only synthesised the enzymes necessary for the metabolism of lactose when it was grown in a medium containing lactose.

The mechanism that hinders the expression of a gene is called **repression** and is usually mediated by special proteins

called **repressors**, which interfere with the transcription of the gene to mRNA. On the other hand, substances such as lactose act as **inducers** to induce the transcription of the genes responsible for coding of enzymes necessary for lactose metabolism. Such enzymes are termed **inducible enzymes** because they are not part of the normal cellular makeup and are synthesised only when required.

Genotype and phenotype

It is important to understand the difference between the genotype and phenotype of an organism. Genotype refers to the genetic information, which is contained in the DNA. Phenotype describes the 'expression' of the genetic information—in other words, the characteristics of the organism that can be observed or measured, such as appearance and metabolism.

MUTATION

A **mutation** is defined as a permanent change in the sequence of the nucleotide bases in the DNA. From the foregoing discussion it can be seen that a change of even one base is likely to result in the formation of a protein with an altered amino acid sequence. Some mutations involve the change of only one base, a **point mutation**. Others involve a more dramatic change; for example, a **frame-shift mutation** occurs when the reading of the code misses a base or bases and gets out of phase. The protein formed as a result of a mutation may be non-functional or, on the other hand, it may have useful properties. Another outcome of mutation could be the formation of a '**nonsense**' **codon**, which would give a 'stop' signal, resulting in the synthesis of an incomplete peptide chain. The mutation may result in a different protein being formed or the cell acquiring different properties. In these cases the effect of a mutation is a change in the 'phenotype' of the organism. In fact, many mutations do not have any observable effect, as there is no significant alteration to the cellular proteins. However, some are lethal, resulting in the death of the organism. One interesting example of the effect of a single base substitution is the occurrence of the hereditary disease sickle cell anaemia, illustrated in Figure 4.8.

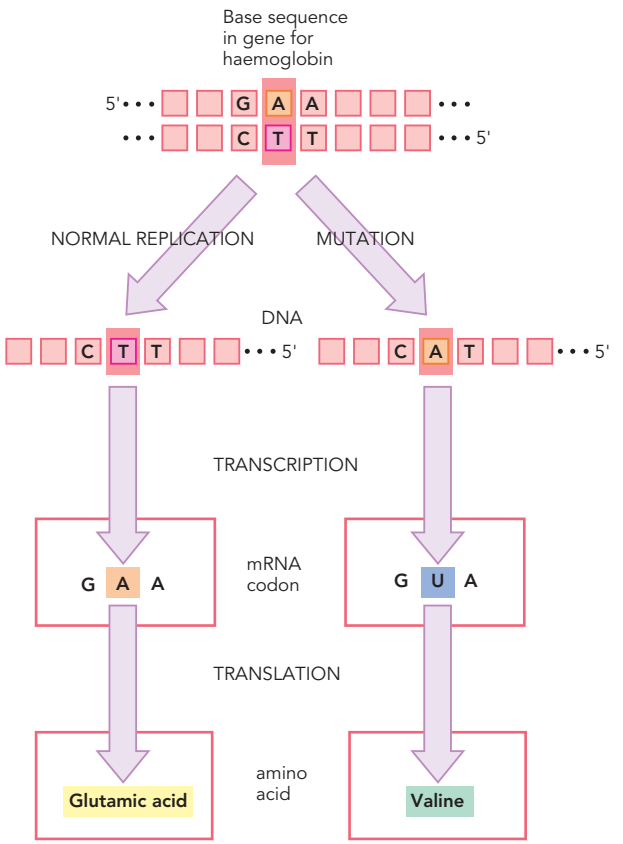


FIGURE 4.8

Possible effect of base substitution in a gene coding for a protein

The substitution of one base in the DNA sequence of the gene for haemoglobin gives rise to a different mRNA codon and leads to the insertion of the non-polar amino acid, valine, instead of the charged amino acid, glutamic acid.

Sickle cell anaemia

SPOTLIGHT ON

The inherited genetic disorder known as sickle cell anaemia is due to a single-base substitution in the DNA which gives rise to a haemoglobin molecule with one altered amino acid. The non-polar amino acid, valine, is substituted for glutamic acid. This change in the amino acid composition gives the molecule a different electric charge and so the haemoglobin molecules stick together under conditions of low oxygen tension, causing the red blood cell to change shape, or sickle, and become stuck in the capillaries see Figure 4.7.

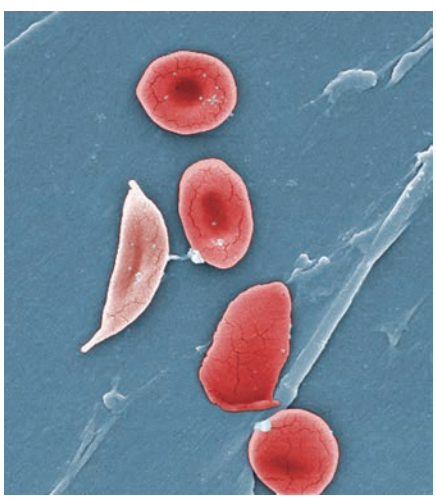


FIGURE 4.7

Scanning EM of cells of patient with hereditary sickle cell anaemia showing deformed shape of red blood cells

Source: Janice Haney Carr/Centers for Disease Control (CDC).

How do mutations occur?

Many mutations occur spontaneously when the cell makes a mistake in the replication of the DNA during cell division. In bacteria the rate of spontaneous mutation is considered to be about 1 in every 10^9 cell divisions. This may not seem very great but, considering the enormous rate at which bacterial cells divide, mutation is a very common event. Many of these mutations are not noticed because the change produces a cell that is not viable. Sometimes, the mutation results in the appearance of a cell with a characteristic that allows it to survive better, and so these cells flourish and multiply. A particularly important example is the development of resistance to antimicrobial drugs or antibiotics. Mutants that have resistance to a particular drug will survive and multiply in the presence of the antibiotic. Therapeutic or prophylactic use of the antibiotic then, has the potential to select for antibiotic-resistant mutants. Continued exposure to antibiotics, especially in the hospital environment, has led to the emergence of a population of resistant microorganisms (see Chapter 12).

Chemical mutagens

Some chemicals damage DNA and cause an alteration to the nucleotide sequence. These chemicals are called **mutagens**. The chemical may act by altering the structure of the base so that it is incapable of pairing with its correct partner. This results in an abnormal DNA being formed with different properties to the DNA of the parent cell. Chemicals that cause damage to human cells so that they mutate and become cancerous are called **carcinogens**.

Much of our current knowledge about mutation has come from research with bacteria. A useful test for determining the potential carcinogenic properties of a chemical substance is the **Ames test**. A test strain of bacteria is exposed to the chemical. If it causes an observable change to the properties of the bacterial strain, then it is considered to have properties that are potentially mutagenic or carcinogenic for humans and requires further testing.

Some viral infections can cause changes to the cellular DNA resulting in the cell becoming cancerous. These are called oncogenic viruses and are described in Chapter 5.

Radiation

Ionising radiation, such as X-rays and gamma rays, causes damage to the DNA by producing errors in replication. These rays penetrate tissue easily and can break the hydrogen bonds in the DNA chain, destroying chromosomes and leading to the death of the cell. Non-ionising radiation, such as UV light, also causes significant damage to cellular DNA by the production of **thymine dimers**. These distorted molecules are incapable of correct replication, and this may lead to the death of the cell or an alteration in its properties. UV light is used to destroy bacteria in clinical areas. The UV rays do not have great penetrating power but are useful for disinfecting air spaces and surface areas (see Chapter 11). Bacteria and some other organisms have enzymes that can repair radiation damage. These useful enzymes have been isolated and are used by scientists in the process of genetic engineering (see below). Sometimes, an error occurs during the process of DNA repair and gives rise to a mutant cell. Damage or alteration to DNA has a profound effect in all types of cells. UV radiation damage to the DNA in human skin cells may produce mutations that cause the cell to become cancerous, leading to the development of skin cancer, or melanoma.

Viral mutation

Viruses can also undergo mutation—usually as a result of a mistake in the replication of their nucleic acid during viral replication. This is especially common in retroviruses (see Chapter 5) and usually gives rise to viruses with different chemical structures (antigens) on their outer layer. This has the effect of changing the specificity of the virus so that it may be able to infect another type of cell—that is, cross the species barrier. Another effect of viral mutation is that existing antibodies to the virus will no longer be effective so a person will no longer be immune to that particular type of virus. This occurs regularly with the influenza virus.

TRANSFER OF GENETIC INFORMATION

The simplest kind of transfer of genetic information occurs during asexual cell division. In bacteria, cell division usually occurs by a simple process of binary fission (see Chapter 3, Figure 3.9, page 56)—each cell splits into two daughter cells. The DNA in the single circular chromosome replicates itself exactly and one of each of the new chromosomes moves to each new cell. In eucaryotic organisms the process of cell multiplication or mitosis is more complicated but also involves duplication of the genetic information with the transfer of identical DNA to the daughter cells.

Sexual reproduction mainly occurs in eucaryotic cells. DNA from each of the parent cells is combined and rearranged to produce offspring with different characteristics to the parents.

In nature, there are many variations on these processes, some of which are described below. DNA replication may involve any of the following: genetic recombination, 'crossing over', transformation, transduction or conjugation.

Recombinant DNA

Genetic recombination is the term used to describe the transfer of genes from one DNA molecule to another, thus forming a new pattern of genes on a chromosome. In eucaryotic cells, recombination usually takes place during sexual reproduction (**meiosis**) when chromosomes containing DNA molecules from each parent are lined up beside each other. If these chromosomes break and then rejoin, DNA from one chromosome can be transferred to the other in a process called '**crossing over**'. This is an ordered process that occurs regularly during sexual reproduction in eucaryotes and gives rise to the necessary genetic diversity observed in higher organisms (see Figure 4.9).

In bacteria, reproduction is usually asexual, so genetic recombination occurs relatively infrequently and requires

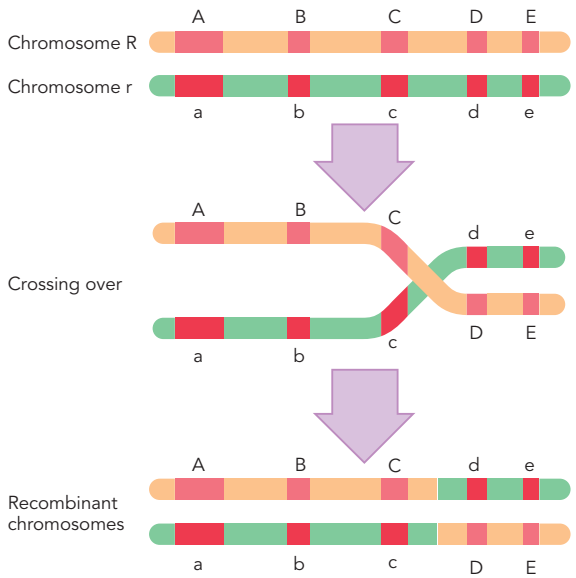


FIGURE 4.9

Genetic recombination between two adjacent chromosomes

The two homologous chromosomes R *and* r *carry a copy of the genes* A *to* E *and* a *to* e*, respectively. When the DNA strands break, cross over and rejoin, two recombinant chromosomes are formed carrying information from each parent.*

special conditions. However, research into the mechanisms of DNA replication and transfer in bacteria has contributed greatly to our knowledge of the whole genetic process. Horizontal DNA transfer, requiring a donor cell and a recipient cell, can occur between bacteria under certain circumstances. The new DNA formed in the recipient cell is referred to as **recombinant DNA** (see below).

Transformation in bacteria

In 1928, while attempting to find a vaccine for pneumonia, Frederick Griffith carried out experiments with two strains of the bacterium *Streptococcus pneumoniae*: a pathogenic strain with a polysaccharide capsule and a non-pathogenic strain that did not possess a capsule. At that time it was not known whether it was the protein or the DNA in the nucleus of the cell that carried the genetic information. However, it was known that heating the bacteria destroys the protein but does not damage the DNA.

Griffith mixed heat-killed bacteria from the pathogenic encapsulated strain of *Streptococcus pneumoniae* and live bacteria from the non-pathogenic strain that did not form a capsule, and injected them into a mouse. The mouse became sick and died (see Figure 4.10). It was found that a live, encapsulated pathogenic strain had been produced.

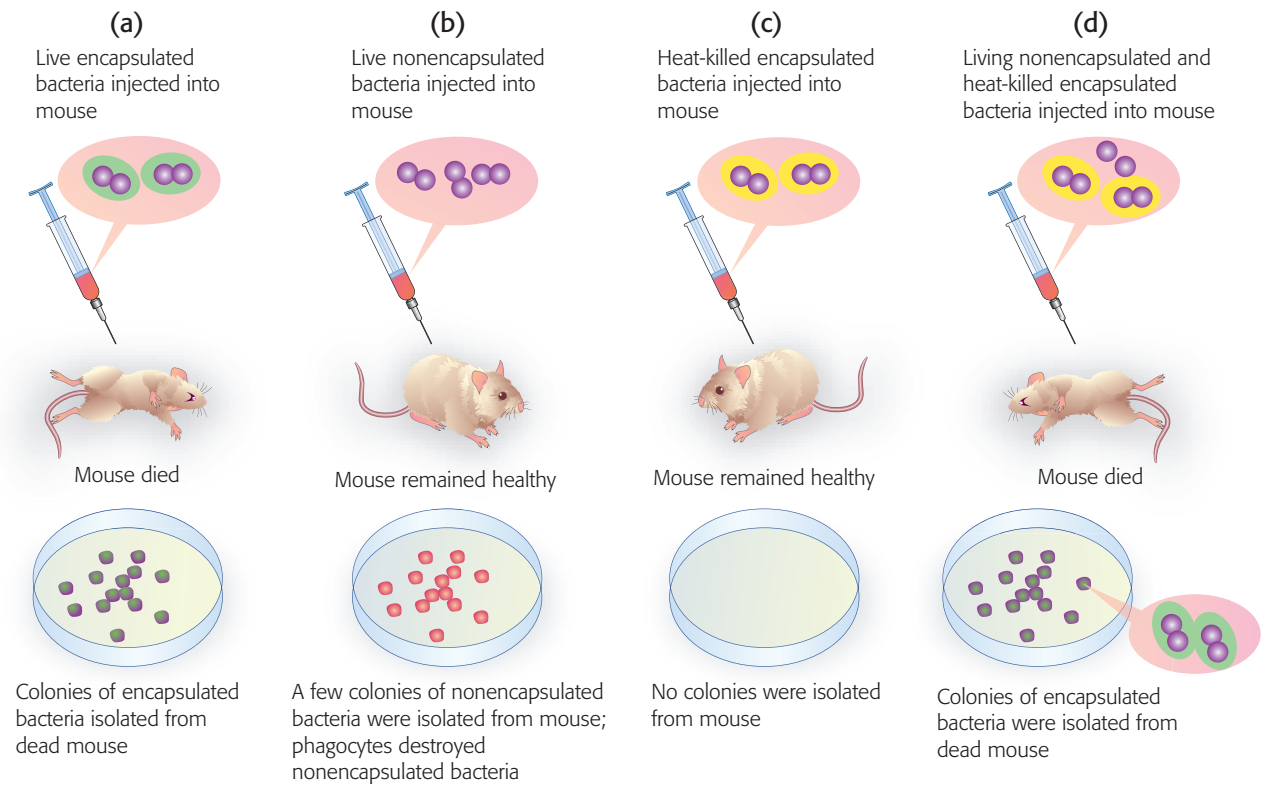


FIGURE 4.10

Transformation

The classic experiment by Griffith using pathogenic and non-pathogenic strains of Streptococcus pneumoniae *which showed that genetic information in the DNA could be transferred from one cell to another. Pathogenic encapsulated bacteria were isolated from a dead mouse, heat killed and mixed with live unencapsulated, non-pathogenic cells. When injected into an experimental mouse, the animal died, showing that the heat-treated DNA had entered and transformed the non-pathogenic cells.*

This showed that there must have been a heat-resistant 'factor' from the killed bacteria that carried the information for capsule formation and pathogenicity, and had somehow entered the non-pathogenic cells and 'transformed' them into a pathogenic strain. In the 1940s this 'factor' was identified as DNA, which is not destroyed by heat. The phenomenon that had occurred was termed **transformation**. As a result of these experiments, nucleic acid was identified as the carrier of genetic information.

Considerable research has been carried out since these early experiments and it is now known that, under certain conditions, live bacteria are able to take up fragments of DNA from their environment and incorporate them into their cellular DNA, thus 'transforming' their genetic properties. These fragments usually come from the lysis of dead cells. Not all bacteria are capable of undergoing transformation and it usually occurs only between cells of the same genera. *Bacillus*, *Haemophilus*, *Streptococcus* and *Staphylococcus* are some of the genera that have been shown to readily undergo transformation. Other cells have walls that do not easily allow the entry of large DNA molecules. The transformed cell, containing the recombinant DNA, continues to replicate and so the transferred genes are passed on to subsequent generations.

Transduction

Bacteriophage, or **phage**, is the name given to viruses that attack bacteria. Viruses are very simple, consisting of genetic material, RNA or DNA, enclosed in a protein coat. As discussed in Chapter 5, viruses are quite specific for the cell they infect and this specificity can be used to target a particular strain of bacteria.

Lytic cycle and generalised transduction

When a bacterium is infected by a phage, the phage first attaches to a specific receptor site on the outside of the cell. It then injects its DNA into the cell, leaving its protein coat outside. The nature of viral replication is such that the virus uses the host cell's metabolic machinery in order to replicate. Once inside the cell, the phage DNA directs the synthesis of phage nucleic acid and proteins and assembles them into new phage particles. Eventually, the infected cell ruptures (lyses), releasing the new phage particles, and the cell is destroyed. This is termed a **lytic cycle**. During this process, phage enzymes break the bacterial chromosomal DNA into small fragments.

When the newly synthesised phage particles are being assembled, some of the bacterial DNA fragments may be included at the expense of some of the phage DNA. Thus, when the phage particle is released and infects another cell, it may take with it some bacterial DNA that has been accidentally included. This phenomenon is referred to as **generalised transduction**. It involves the transfer of genetic material from one bacterial cell to another by a lytic phage and allows the transfer of bacterial characteristics from a donor to a recipient cell. Because the phage is carrying slightly altered DNA, some phage genes may be lacking and it may not kill the cell it attacks. Thus the phage DNA becomes incorporated into the recipient bacterial cell, which acquires new characteristics from the donor cell (see Figure 4.11).

Specialised transduction

A different type of transduction, **specialised transduction**, occurs under some circumstances. **Temperate phages** are phages which, instead of lysing the host cell, insert their DNA into the bacterial chromosome. The inserted phage DNA is called a **prophage** and replicates along with the bacterial DNA, a state called **lysogeny**. The phage DNA may confer additional characteristics on the infected cell. For example, pathogenic strains of *Corynebacterium diphtheriae* contain a temperate phage that carries the gene

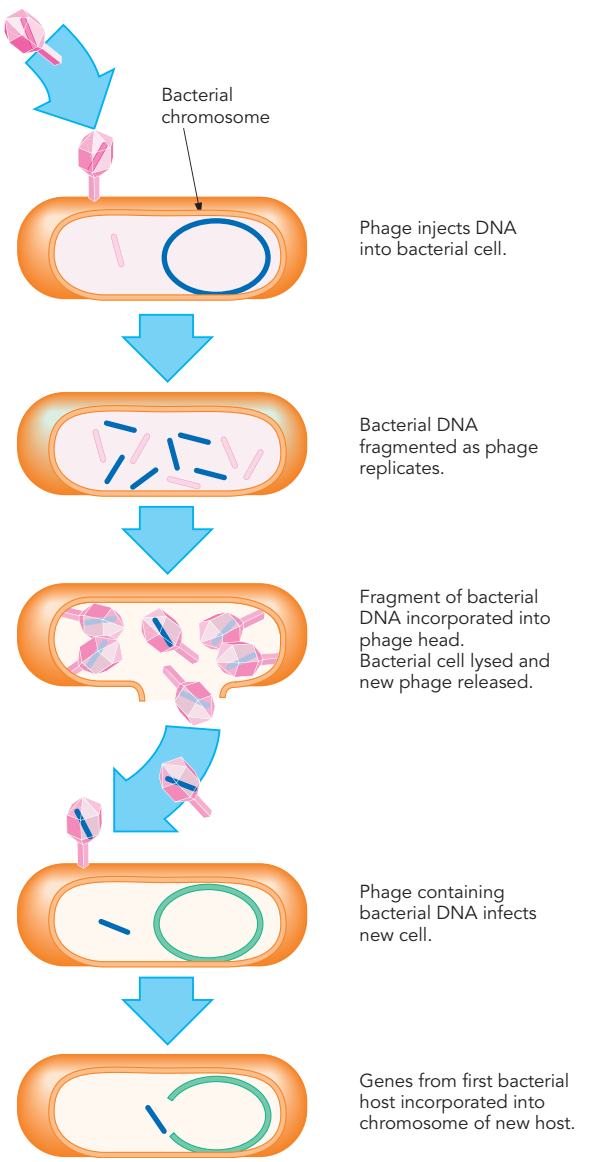


FIGURE 4.11

Generalised transduction

Bacteriophage infection of a host bacterium breaks the bacterial chromosome into fragments, which are carried (transduced) to the next bacterium the phage infects.

for toxin production. The toxin is largely responsible for the symptoms of diphtheria. Strains that do not carry the phage are not pathogenic.

A bacterial cell infected with a temperate phage may revert spontaneously to a lytic cycle, in response to an external stimulus. The phage DNA is then replicated, producing phage DNA and proteins and assembling them into new phage particles. These particles may contain fragments of bacterial DNA that will be transferred to the next bacterial cell the phage infects. The difference between these two types of transduction is that, in specialised transduction, the bacterial DNA carried to the new cell will usually be the DNA that was *adjacent to* the prophage DNA when it was in the lysogenic state, whereas in generalised transduction, random fragments of DNA are transferred. This phenomenon can be used to provide information about the location of specific genes on the bacterial chromosome, and can also be used to select particular genes to be transferred.

Transduction has been used by scientists to manipulate the DNA composition of bacterial cells. Bacteria that have a desired characteristic are infected with phage and the phage allowed to reproduce. Then the newly synthesised phages are transferred to another bacterial cell, which can be tested to see whether the desired characteristic has been transferred—that is, whether transduction has occurred.

Conjugation

Conjugation is another important method whereby genetic information is transferred from one bacterial cell to another. It differs from transformation and transduction in that it requires the two bacterial cells, the donor and the recipient, to come into actual contact with each other. This is achieved by the formation of a **sex pilus**, a tubular structure that forms a bridge or channel between two cells to allow the passage of pieces of DNA from one cell to the other (see Figure 4.12d).

Very often, the DNA that is transferred is in the form of a plasmid. As mentioned earlier, **plasmids** are small, circular, extra-chromosomal pieces of DNA that occur in some bacteria and replicate independently of the bacterial chromosome. They are not essential for growth of the cell, but have been shown to code for a number of other important characteristics.

The first plasmid to be described was the **F^+ plasmid** of *E. coli*. 'F' stands for fertility factor and refers to its ability to direct the formation of a sex pilus. Cells containing a plasmid that codes for a sex pilus are called 'F^+' and those that cannot form a pilus are 'F^-'.

Figure 4.12 illustrates the mechanism whereby the F^+ plasmid transfers its genes to another cell during conjugation. In the recipient cell, the plasmid either continues to replicate independently of the chromosomal DNA or it may fuse (recombine) with the chromosome. If this occurs, the recombinant DNA will then carry the genes for conjugation. These cells are called **Hfr strains** (**H**igh **f**requency of **r**ecombination) because they exhibit a very high frequency of bacterial chromosomal transfer. Hfr cells are capable of acting as donor cells in conjugation processes with new recipient cells. During this process, a copy of part of the chromosomal DNA from the donor cell is usually transferred and incorporated into the recipient cell DNA, although the F factor is not usually transferred completely so the recipient cell remains F^-.

Transfer of plasmids during conjugation is an important method of transfer of genes from one bacterial cell to another. Plasmids have been shown to contain the genes for a number of important properties. Some carry genes for the production of toxins or other virulence factors, including the tetanus neurotoxin and the toxin from *Staphylococcus aureus* that causes skin peeling (exfoliation) in 'scalded skin syndrome' (see Chapter 10). A plasmid-containing strain of *E. coli* is responsible for the toxin production associated with 'traveller's diarrhoea'.

Resistance plasmids

For clinical microbiologists, the most important plasmids are those carrying **resistance factors** (**R**). These are genes that enable the cell to display resistance to various antibiotics and/or heavy metals either by interfering with the entry or action of the antibiotic or by destruction of the antibiotic molecule. For example, most strains of *Staphylococcus aureus* currently isolated in hospitals carry an R plasmid with a gene that codes for the formation of the enzyme beta-lactamase, which destroys penicillin.

Resistance plasmids may carry genes for resistance to more than one antibiotic and can be rapidly passed from cell to cell, as described above. Transfer can also occur between closely related genera, which gives rise to populations of organisms that are highly resistant to a range of antibiotics. Whereas mutation usually gives rise to resistance to one antibiotic at a time, plasmid transfer allows for the development of resistance to several antibiotics in one step. This is of increasing importance in the hospital environment where there is widespread use of antibiotics and an increasing occurrence of resistant organisms (see Chapter 12).

GENETIC ENGINEERING

The above description of the ways in which genetic information can be transferred between bacterial cells is given as an introduction to the subject of genetic engineering. The discovery of the ways in which genes are split and re-assembled in Nature opened up the possibility that these same processes could be used to manipulate genetic information in the laboratory. Scientists have now developed laboratory techniques that enable them to transfer genes from one cell to another and to manipulate the activities of microbial cells in order to obtain desired products. These techniques are based on the types of transfers described above and use many of the enzymes derived from bacterial cells.

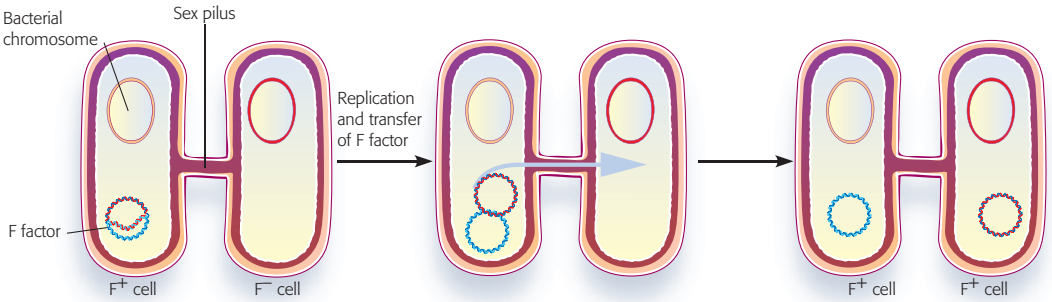
Recombinant DNA technology

The procedures used in genetic engineering result in the formation of DNA molecules that have been modified in some

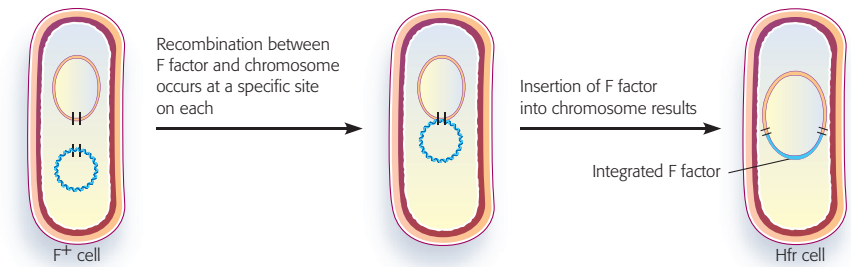
way; that is, they have become 'recombinant DNA' (rDNA). **Recombinant DNA** is a term widely used to refer to DNA that has been produced using DNA from two different sources. It can mean:

- DNA produced by spontaneous crossing over during cell division; or
- DNA produced in the laboratory by genetic engineering.

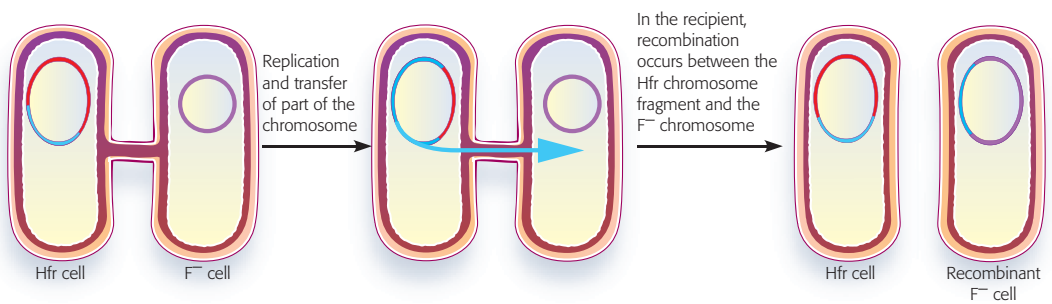
Scientists have developed ways of using genetic engineering to benefit humans. For example, a gene from a eucaryotic cell can be inserted into a bacterial cell. The recipient bacterial cell containing the recombinant DNA can then be induced



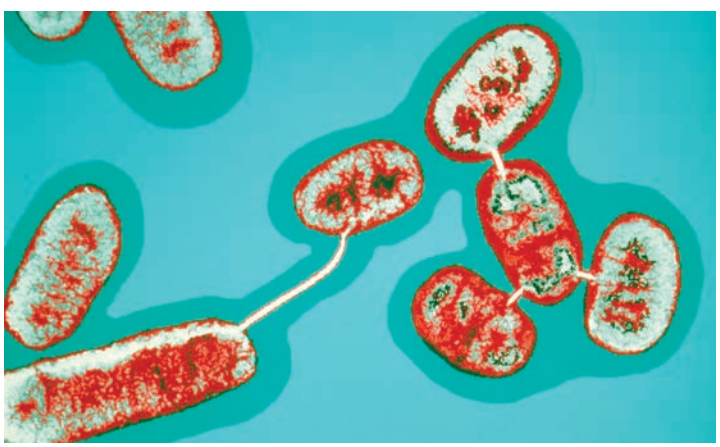
(a) When an F factor (a plasmid) is transferred from a donor (F^+) to a recipient (F^-), the F^- cell is converted into an F^+ cell.



(b) When an F factor becomes integrated into the chromosome of an F^+ cell, it makes the cell a high frequency of recombination (Hfr) cell.



(c) When an Hfr donor passes a portion of its chromosome into an F^- recipient, a recombinant F^- cell results.



(d)

FIGURE 4.12

(a)–(c) Diagrammatic representation of conjugation; (d) coloured transmission electron micrograph of sex pilus formation in *E. coli*.

Source: (d) Dr Linda Stannard, UCT/Science Photo Library.

Genetic engineering

SPOTLIGHT ON

One of the first examples of the use of biotechnology was the production of human insulin by cells of the bacterium *E. coli*. The gene for insulin production was isolated from human pancreatic cells and inserted into the bacterium, which then 'expressed' the foreign gene and synthesised insulin. The insulin is then extracted and purified from the culture medium for use by diabetic patients. This form of insulin is cheaper and safer than the insulin that used to be extracted from pig pancreas.

Vaccines such as the one for hepatitis B, which contains the hepatitis B surface antigen (HBsAg), have also been genetically engineered. The gene responsible for the synthesis of one particular sequence of the hepatitis B viral protein coat (the 'surface antigen') is introduced into a yeast cell. The yeast produces multiple copies of this fragment of the protein coat, which are then isolated, purified and used as a vaccine in humans to stimulate production of protective anti-hepatitis-B antibodies.

There are several advantages to the use of recombinant DNA in the production of compounds such as hormones or vaccines for human use. Previously, many of these compounds had to be derived from human or animal tissue. The presence of animal proteins often gave rise to an adverse immunogenic reaction (see Chapter 9). The need to use blood or tissue from human sources limited the amount of the substance that could be produced and increased the cost. There was also a slight risk of transferring another human pathogen that might be present in the extracts. There is some evidence that this occurred with the use of preparations of human growth hormone from pituitary extracts from cadavers. Some patients treated with these hormone preparations have developed Creutzfeldt-Jakob syndrome, a disease of the nervous system (see Spotlight box: Prions, in Chapter 11). The use of purified genetically engineered products, synthesised by microbial cells, minimises the risk of adverse effects from preparations contaminated with human pathogens or other substances that may cause hypersensitivity reactions.

to express this gene. Because of the rapid rate of growth of bacterial cells, large amounts of the substance coded for in the eucaryotic gene can be produced quickly in a relatively pure form.

Biotechnology is the term used for the many and varied techniques involving manipulation of genes or genetic information. Table 4.2 lists some of the microbial products of biotechnology.

Restriction enzymes

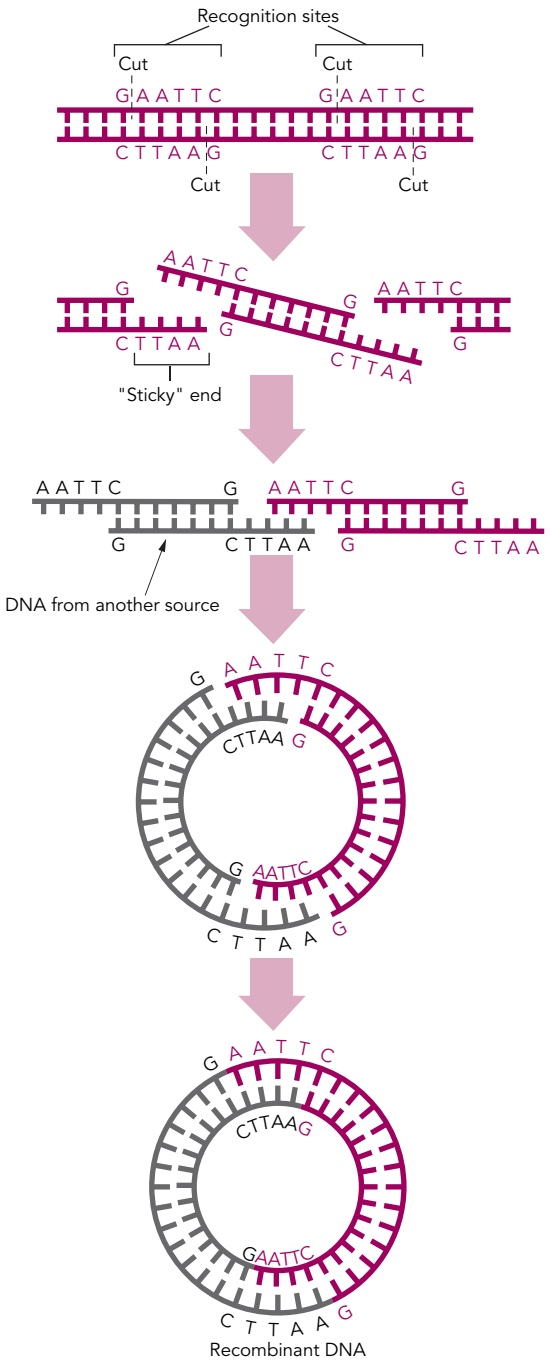
Of particular use in genetic engineering is a group of enzymes found only in bacteria, called **restriction endonucleases**. Each of these enzymes is able to recognise a particular sequence of nucleotides in the DNA molecule and hydrolyse (break) each strand at a specific point in the sequence. Because the DNA is a double strand, the break is always jagged, exposing a 'sticky' end. The enzymes are usually named after the bacteria from

TABLE 4.2 Microbial products of genetic engineering

PRODUCT	ORGANISM	USES
Medical		
Insulin	*Escherichia coli*	Treatment for diabetes
Interleukin 2 (IL-2)	*E. coli*	Stimulates immune system
Interferon α and γ	*E. coli* and *Saccharomyces cerevisiae*	Possible therapy for viral diseases
Tumour necrosis factor	*E. coli*	Attacks cancer cells
Human growth hormone	*E. coli*	Corrects growth deficiencies
Colony stimulating factor (CSF)	*E. coli* and *S. cerevisiae*	Stimulation of immune system and treatment of infections
Vaccines		
Hepatitis B	*S. cerevisiae*	HBsAg for vaccine
Cervical cancer	*S. cerevisiae*	Prevention of HPV-related cervical cancer
Modified organisms		
Genetically modified plants	*Pseudomonas syringae* *Bacillus thuringiensis*	Prevents frost damage to plants Resistance to insect pests and herbicides

which they were isolated. For example, Eco I comes from *Escherichia coli* (*E. coli*) and hydrolyses the sequence GAATTC between guanine and adenine, giving rise to two fragments each with the sequence AATT on the end of a single strand (the 'sticky' end). New pieces of DNA (genes) can be inserted into these breaks (see Figure 4.13).

Genetic engineering often involves the use of plasmids as vectors, or carriers, to introduce a foreign piece of DNA carrying the desired gene into a cell. A restriction enzyme is used to cut both the plasmid and the foreign DNA. This gives rise to fragments of DNA from both sources which have corresponding sticky ends, with the sequence coding for the particular gene somewhere in between. When these are mixed, some of the foreign DNA can be inserted into the plasmid. An enzyme called DNA ligase is added to join the nucleotides together and the result is a plasmid vector carrying the desired gene. In the laboratory the plasmid is usually introduced into the recipient cell by transformation (see Figure 4.14). If the host cell does not normally take up DNA, the cell wall can be modified by chemical treatments.



(a) Recognition sites of this particular restriction enzyme in double-stranded DNA are shown in red. Arrows indicate where the cuts occur.

(b) Cuts by this restriction enzyme produce a fragment with two sticky ends.

(c) When two fragments of DNA cut by the same restriction enzyme come together, they can join by base pairing.

(d) A circle can be formed – as for a plasmid.

(e) The enzyme DNA ligase is used to unite the backbones of the two DNA fragments, producing a molecule of recombinant DNA. (It may be linear or circular.)

FIGURE 4.13

The role of restriction enzymes in making recombinant DNA

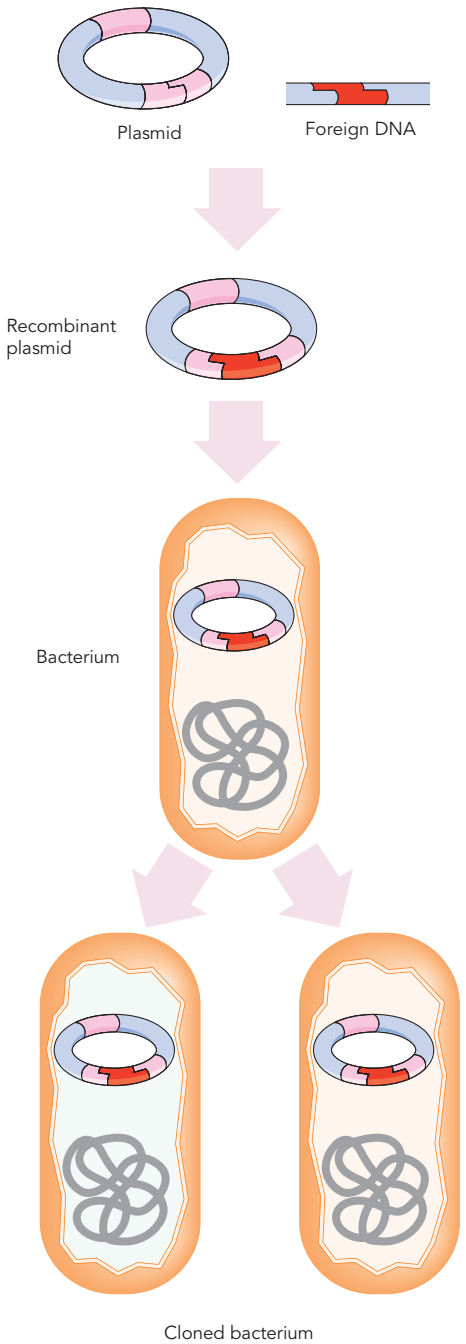


FIGURE 4.14

Insertion of foreign genes into a bacterium using a plasmid as the vector

Foreign DNA and plasmid DNA are cut with a restriction enzyme. The foreign DNA is inserted into the plasmid. The recombinant plasmid is introduced into a new bacterial cell which acquires the characteristics coded for by the foreign DNA and can be cloned.

Viruses are sometimes used as vectors to transfer information into animal cells.

METHODS OF DNA ANALYSIS

The techniques developed for genetic engineering are also of use in identifying nucleic acid from various sources. 'Typing' refers to the analysis of DNA or RNA from different 'types' of bacteria and viruses. This is increasingly being used in diagnostic microbiology laboratories and is described in more detail in Chapter 15. Analysis of DNA is used to identify microorganisms that may be difficult or slow to grow in the laboratory. Often a new species of bacteria is discovered and can be identified because it has a different DNA composition from any known bacteria. For example, DNA typing has been used to distinguish between different species of *Chlamydia*, an organism that cannot be grown in the laboratory. These studies have identified *Chlamydophila pneumoniae* as a significant cause of pneumonia.

DNA analysis can be used in epidemiological studies to trace the occurrence of a particular strain of bacteria. It is also used for the identification of human tissue. No two humans (except identical twins) look exactly alike. This is because every person has a different complement of DNA in his or her genes. DNA fingerprinting is used in forensic science to compare, for example, the DNA in a sample of semen with the DNA in a blood sample to help establish the identity of a criminal or determine paternity.

DNA fingerprinting

Methods have been developed to compare DNA samples obtained from different sources. The DNA analysis involves the use of special enzymes to cut the DNA sample into small fragments (**RFLPs—restriction fragment length polymorphisms**), which are then separated by gel electrophoresis. In electrophoresis, the sample containing the fragments of DNA is placed in a special compartment or 'well' in slab of agarose gel in a buffer solution, and an electric current passed through the gel. **Pulse field gel electrophoresis** (**PFGE**) is one method that provides good separation. The electric charge on the fragments of DNA allows them to move through the gel and separate into bands. The separation that is achieved is related to their size, the larger ones moving more slowly and the smaller ones migrating quickly through the gel to the opposite pole. A pattern, or fingerprint, of the DNA is obtained which is visualised by staining or using radioactive probes (see Figure 4.15, page 82).

This technique is very useful in epidemiological investigations and to monitor hospital infection control practices. Isolates of bacteria obtained from different sources can be compared to see whether they are the same strain (see Case History 4.1).

Sequence analysis

Very often, a genetic characteristic can be ascribed to a particular nucleotide sequence or gene. Techniques are now available which allow the determination of the sequence of nucleotides in a piece of DNA. In other words, it is now possible to detect the presence or absence of a particular gene. This method was developed in experiments with microorganisms—the complete sequence of nucleotides in the chromosome of *E. coli* (its 'genetic map') being one of the first to be completed. The development of automated

CASE HISTORY 4.1

Tracing the source of hospital infection

Many chronically ill patients have multiple hospital admissions. Often they are found to be colonised with pathogenic bacteria, but it is not known whether the infection was acquired in the hospital, in the community or in another health facility. In these cases, DNA typing can be used to differentiate between similar strains of the same bacteria.

A patient suffering from cystic fibrosis was admitted for a lung transplant in a large teaching hospital, but was found to be colonised with antibiotic resistant *Staphylococcus aureus* (MRSA) and the operation had to be postponed. In order to determine the source of the MRSA infection, Pulse Field Gel Electrophoresis of DNA from the patient's MRSA isolate was carried out and compared with the other MRSA strains circulating in the hospital at that time (see Figure 4.15).

Questions

1. Which two bands are identical in this PFGE test?
2. What is the significance of the result? Is it likely that the patient acquired this infection in this hospital?
3. Explain how this kind of test can be used to track the spread of an infection around the hospital.

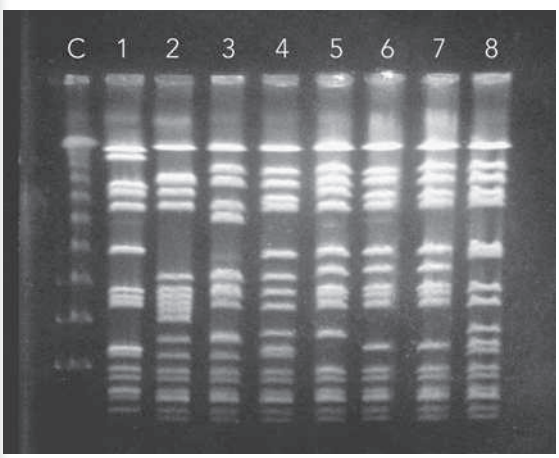


FIGURE 4.15

Pulse Field Gel Electrophoresis of DNA from different isolates of MRSA
Samples 1–4: July 1996 hospital isolates
Sample 5: October 1996 hospital isolate
Sample 6: December 1996 patient's isolate
Sample 7: November 1996 patient's isolate
Sample 8: November 1996 hospital isolate

Source: Dr Penny Bishop.

sequencing machines has enabled these studies to be extended to an analysis of the genetic composition of higher organisms, including humans.

Analysis of ribosomal RNA has been used for the classification of organisms into domains and kingdoms, as described in Chapter 3.

Amplification of DNA

The determination of the nucleotide sequence in DNA samples requires relatively large amounts of DNA, which may not always be available. In this case, a method of DNA amplification such as the **polymerase chain reaction** (**PCR**) is used. In this technique, fragments of the DNA of interest are copied until enough molecules are obtained to carry out an analysis such as fingerprinting or sequencing (described above). PCR can make billions of copies of a particular nucleotide sequence in just a few hours. This process is illustrated in Figure 4.16.

The piece of DNA to be copied is heated so that the double helix separates into single strands. The solution is then cooled and the four DNA nucleotides and enzymes are added. The nucleotides are incorporated, pairing with their corresponding bases on the exposed single strand. The new DNA strand is then 'zipped up' by the action of a polymerase enzyme, effectively doubling the amount of DNA each time.

PCR must be carried out under very strictly controlled laboratory conditions, as it is an extremely sensitive technique. It is important to ensure that the equipment and solutions are not contaminated with any external DNA from another source. Other amplification methods have been developed (see Chapter 15).

DNA probes

Another important technique is the use of DNA probes to see whether a particular nucleic acid sequence is present in a sample. This is very useful in diagnostic microbiology. Some microorganisms are either very difficult to grow, or grow very slowly in the laboratory. A number of DNA 'probes' have been developed commercially and can be used to identify an organism or detect its presence in a clinical specimen or tissue. The **probe** consists of a small fragment of DNA containing a nucleotide sequence which is specific to the particular organism being looked for. In the '**dot blot**' method, the DNA to be tested is first amplified by PCR if necessary, applied to a filter paper and heated to pull the strands apart. The probe, which has been labelled with a radioactive or fluorescent marker, is then introduced and the DNA strands allowed to rejoin. If the complementary sequence of nucleotides is present in the test DNA, then the probe will combine with it, giving a radioactive or fluorescent product that can be detected by X-ray film or visualised with UV light.

Southern blots

Southern blot is another technique used to determine whether a particular nucleotide sequence is present in a DNA sample. The DNA is hydrolysed and then subjected to gel

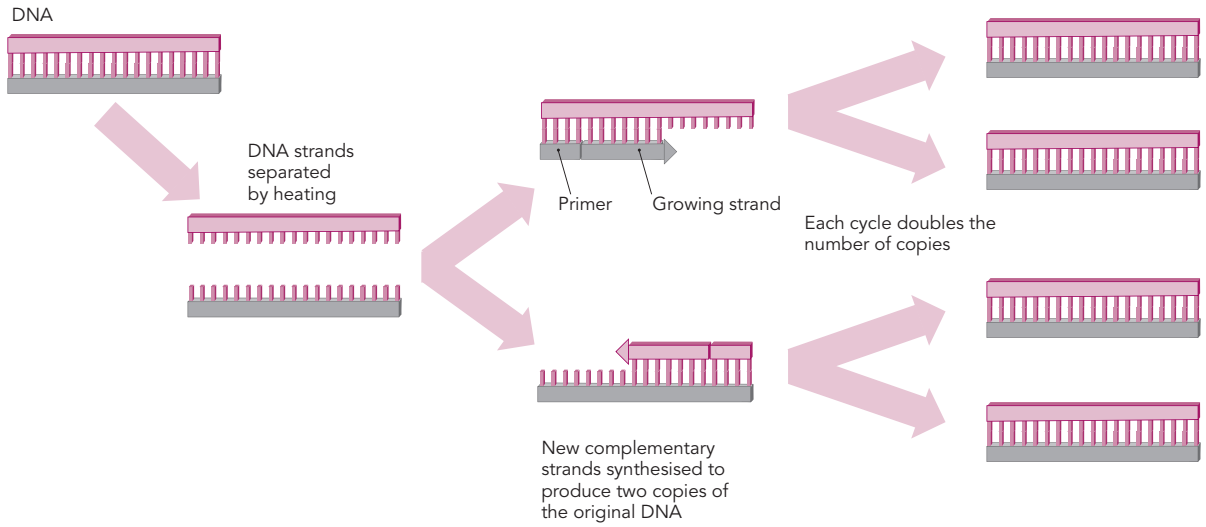


FIGURE 4.16

Polymerase chain reaction

electrophoresis. The fragments obtained are transferred to a nitrocellulose membrane by 'blotting' and the membrane is then treated with a radioactive probe. If the desired sequence of nucleotides is present the probe will bind to it and can be detected by exposure to an X-ray film. This method is useful when trying to identify a particular nucleotide sequence (gene) in a complex mixture.

THE FUTURE OF BIOTECHNOLOGY

In the future there will be increasing use of nucleic acid amplification and detection techniques to test for the presence of bacteria in a sample when the concentration of cells is too low to be detected by any other means. This is already having a major impact on diagnostic microbiology. As more probes become available commercially, these methods are being used increasingly in clinical laboratories to reduce the time required for identification (see Chapter 15). In agriculture, recombinant DNA technology is being used to produce genetically modified (GM) crops that have an increased yield and resistance to insects, frost or drought.

THE ETHICS OF GENETIC ENGINEERING

The last 20 years have seen an explosion in our knowledge of genes and our ability to manipulate genetic characteristics. However, this has been accompanied by many legal and ethical questions. Should scientists be allowed to release genetically modified organisms into the environment? Are genetically modified crops safe to eat? Should doctors manipulate the genes of unborn babies?

The use of techniques of DNA analysis means that it is now possible to test people for genetic defects, to help in the identification of human remains by comparison with the DNA of living relatives, to solve cases of missing persons and to assist in criminal forensic investigations. However, confidentiality issues are a major cause of concern.

The use of bacteria to produce vaccines and other substances of benefit to humans is expanding at an incredible rate. One of the strategies currently being investigated to produce an AIDS vaccine involves the use of recombinant DNA technology to modify the *Vaccinia* virus to carry HIV antigen. This creates a virus with the antigenic properties of HIV but not HIV-DNA, so it should theoretically stimulate the immune response without causing disease. Many people oppose these experiments as they believe it is interfering with Nature. Others believe that the use of genetically modified crops is the only way to feed the world's expanding population.

SUMMARY

GENES
- The characteristics of bacterial cells are determined by their genetic material, or genes, which are linear sequences of nucleotides of DNA.

STRUCTURE OF DNA
- DNA consists of two strands of nucleotides twisted around each other to form a double helix.
- In protein synthesis, the genetic information contained in the DNA is first transcribed to messenger RNA, which then acts as a template for the synthesis of proteins.
- Transfer RNA transports amino acids to the mRNA template to ensure the correct sequence of amino acids occurs in the protein molecule.

MUTATION
- Mutation (which can occur spontaneously or as a result of exposure to chemicals or radiation) is a change in the nucleotide bases in the DNA that gives rise to different properties in the cell.
- Favourable mutations, such as the development of drug resistance, allow some bacteria to survive instead of others.
- Mutations in viruses may give rise to types with different specificities and immunological properties.

TRANSFER OF GENETIC INFORMATION
- Genetic recombination refers to the exchange of pieces of DNA between two DNA strands.
- Transformation, transduction and conjugation are different ways in which one bacterial strain acquires the characteristics of another strain.

GENETIC ENGINEERING
- Recombinant DNA technology is being used in a number of ways to develop products that are of benefit to humans.
- Biotechnology is used to determine genetic characteristics and identify samples based on their DNA composition.

METHODS OF DNA ANALYSIS
- A number of new techniques of DNA analysis have been developed, including fingerprinting, nucleic acid probes, sequence analysis and PCR for amplification of small amounts of DNA.

STUDY QUESTIONS

1. Describe the structure of DNA. How does it differ from RNA?
2. How are proteins synthesised in bacterial cells?
3. What is meant by the 'genetic code'?
4. What important role does tRNA play in protein synthesis?
5. What happens when a mutation takes place in a bacterial cell?
6. What is meant by 'recombinant DNA'?

TEST YOUR UNDERSTANDING

1. Explain the way in which genetic information is passed from one generation to another.
2. How is the information for antibiotic resistance transferred from one bacterial cell to another? What are the implications for the control of infectious diseases?
3. How does the use of nucleic acid analysis (NAA) contribute to the identification of bacteria?
4. Describe the ways in which recombinant DNA technology is being used to benefit humans.

FURTHER READING

Visit the website <http://learn.genetics.utah.edu>.

CHAPTER

5

Viruses and viral diseases

CHAPTER FOCUS

- What are the unique characteristics of viruses?
- How do viruses replicate inside cells?
- What are the different clinical outcomes of viral infection in humans?
- What are the major routes of transmission of viral diseases?
- How are viral diseases diagnosed, treated and prevented?

INTRODUCTION

Viruses are responsible for many of the significant diseases that affect our society, and new viral diseases such as AIDS, SARS and swine flu are constantly emerging. Most of the viruses responsible for these diseases have been identified in the last 60 years. Before then, many unexplained illnesses were attributed to the effects of toxins or poisons, or to exposure to 'bad air'.

The word **virus** means venom, or poison, and the original name given to the infective agents that caused these diseases was 'filterable viruses'. This was because samples obtained from diseased animals or plants were still infectious after being passed through a filter that was known to retain bacteria. There are many different kinds of viruses and they cause disease not only in humans but also in other animals, plants and even bacteria. In this chapter we describe the unique properties of viruses and examine the ways in which they cause disease in a susceptible host.

CHARACTERISTICS OF VIRUSES

Viruses are among the smallest infectious agents known. They range in size from the tiny polio virus (20 nm diameter) to the large pox virus, which is 400 nm in diameter. This is still smaller than even the smallest bacteria, which are 1000–2000 nm (1–2 μm) in diameter. For comparison, human erythrocytes are 7–8 μm in diameter. Viruses cannot be seen under the light microscope, requiring the higher resolution of electron microscopy for their structure to be visible.

Viruses lack most of the enzymes necessary for the metabolism and synthesis of complex molecules and so can replicate (grow) only inside a living (host) cell. Viruses use the metabolic machinery of the host cell and, as a result, have been called *obligate intracellular parasites*. In most instances, viruses damage the cells in which they replicate, causing the cells either to burst (lyse) or to die gradually.

STRUCTURE OF VIRUSES

Viruses have a simple structure consisting of only one type of nucleic acid enclosed in a protein coat called a **capsid**. Some of the more complex viruses have, in addition, an inner protein core enclosing the nucleic acid. Others have an additional outer layer called an envelope (see Figure 5.1). Viruses are not strictly considered to be living cells as they are unable to carry out any metabolic functions without the involvement of a host cell. Each virus has a distinctive shape and structure that can be used in classification (see Figure 5.2).

Nucleic acid

Each virus possesses only one type of nucleic acid, which carries its genetic information. This is single-stranded RNA (ssRNA), double-stranded RNA (dsRNA), single-stranded DNA (ssDNA) or double-stranded DNA (dsDNA). The

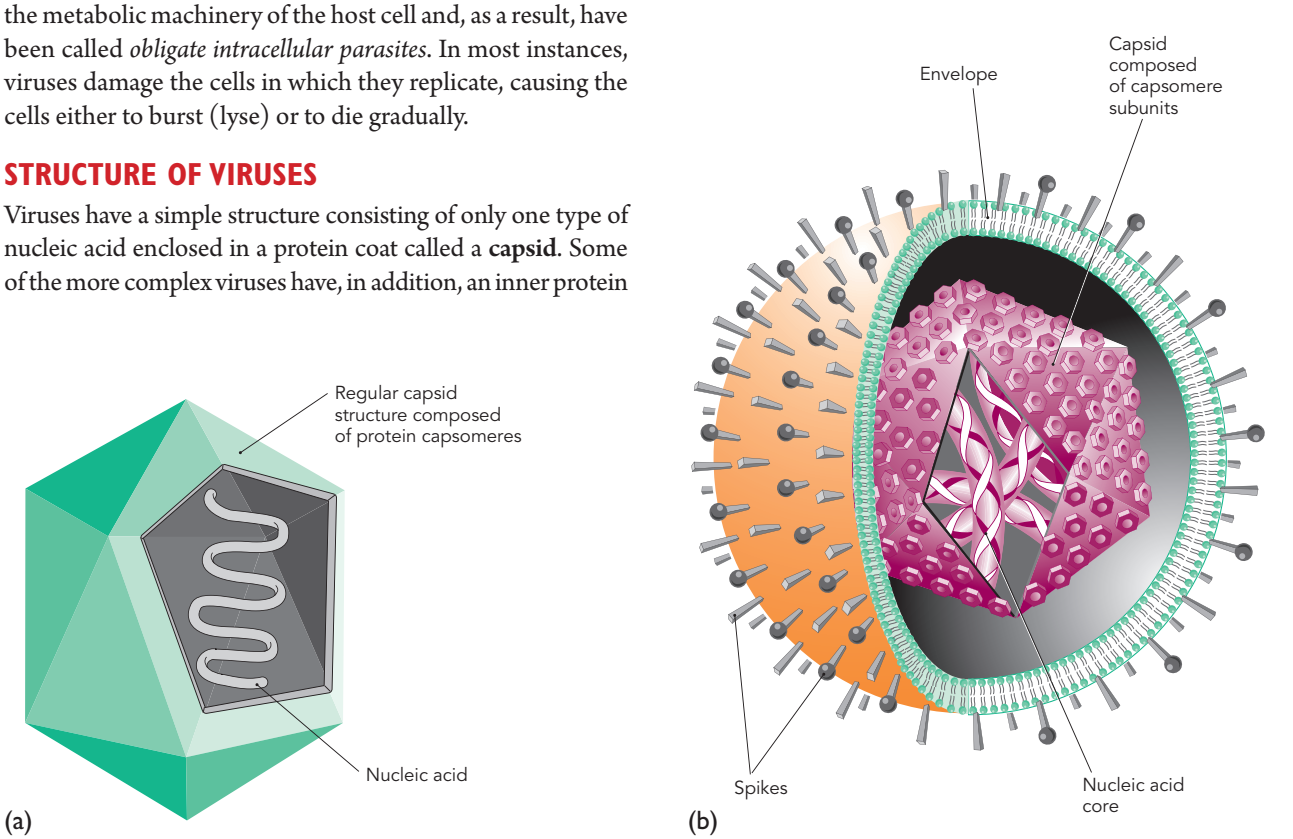


FIGURE 5.1

Schematic diagram of a virus

(a) Naked virus; (b) enveloped virus.

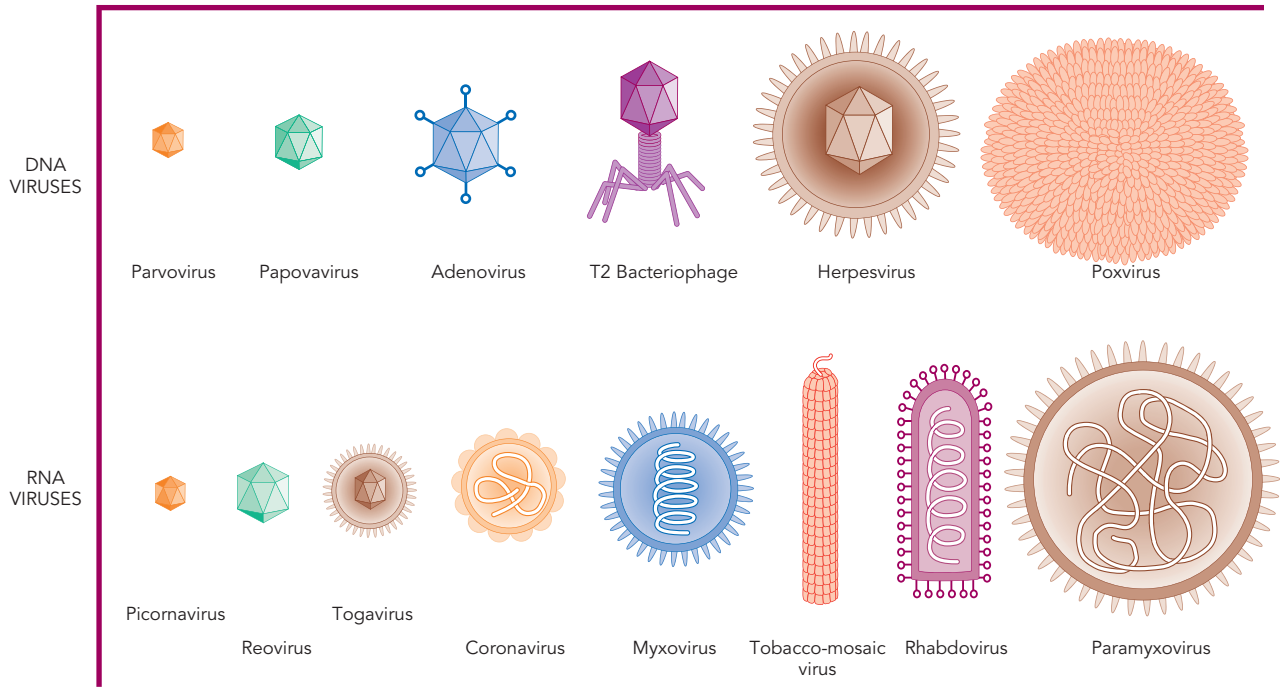


FIGURE 5.2

Relative sizes and shapes of different viruses

size of the viral genome is limited and does not carry all the information necessary for the synthesis of a complete virus. Instead, the genome consists of the genes that code for structural viral proteins and a limited number of viral enzymes, as well as regulatory genes that direct the host cell pathways to produce new viral particles.

The viral capsid

The protein coat covering the nucleic acid, the **capsid,** is made up of repeating protein units called **capsomeres**. These are often arranged in symmetrical patterns which enable the purified virus particles to be crystallised. Capsids occur in distinctive shapes (see Figure 5.2). Many consist of a regular polyhedron such as the icosahedral (20-sided) structure found in, for example, the adenovirus. Others are helical, forming hollow cylinders which may be either rigid or flexible. Some (e.g. the pox viruses) have a complex structure.

Although viral capsids contain only a minimum number of protein molecules, the capsid proteins have a number of important functions. They may have antigenic properties (i.e. they possess a distinctive molecular structure which stimulates the production of a corresponding antibody by the host's immune system) and they participate in the attachment and entry of the virus particle to the host cell during infection (see below). They also protect the nucleic acid against inactivation by nuclease enzymes.

Envelope

Some viruses are surrounded by an envelope. This usually consists of a lipid bilayer derived from the membrane of the host cell, into which some specific viral proteins and glycoproteins have been inserted. The viral glycoproteins may appear as 'spikes' protruding from the envelope (see Figure 5.3). The structure of the envelope assists the virus to attach to the host cell. For example, the spikes on the influenza virus enable it to attach to the surface receptors on the cells of the respiratory epithelium and also to similar receptors on red blood cells, causing agglutination.

Viruses that lack envelopes are called **naked viruses**. They are generally more resistant to environmental conditions

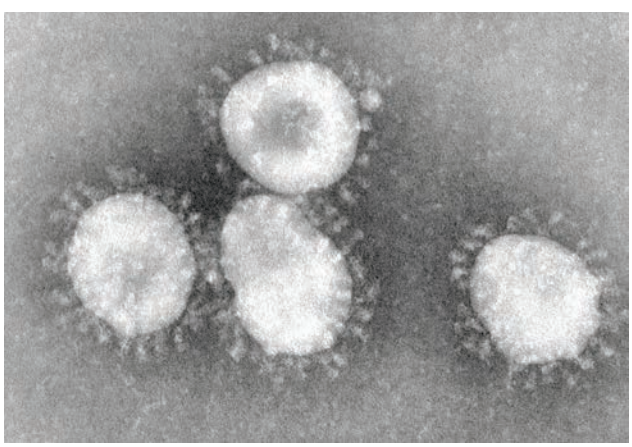


FIGURE 5.3

Transmission electron micrograph of a Corona virus, showing distinctive spikes on the outer envelope (magnification × 200 000)

Source: Centers for Disease Control (CDC)/Dr Fred Murphy.

than enveloped viruses because the envelope is easily damaged by chemicals or extremes of pH or temperature.

The surface glycoproteins of viral envelopes participate in the attachment of the viral particle to the specific receptor site on the susceptible cell. They also determine the antigenic characteristics of the virus. The immune system of the host produces antibodies directed against the proteins and glycoproteins present on the viral surface (see Chapter 9).

Viral enzymes

Viruses do not have all the enzymes required to carry out metabolic processes outside the host cell. However, some viruses contain genes for the production of enzymes that are important for the infective process. Most of these are involved with nucleic acid transcription—for example, reverse transcriptase carries out the initial step in the replication of the retroviruses, the synthesis of DNA from viral RNA. Neuraminidase, an enzyme located in the spike of the influenza virus, is important for the release of new viral particles. These enzymes are not generally found in the host cell so they are good targets for antiviral therapy (see Chapter 12).

Terminology

A number of different terms are used to describe viruses.

- **Virion** is used to describe an entire mature virus particle consisting either of nucleic acid and capsid (naked virus) or nucleic acid, capsid and envelope (enveloped virus). Virions are found outside the host cell and are capable of transmission from one host to another.
- **Virus**, or **viral particle**, refers to the intracellular infectious particle consisting of nucleic acid and a protein coat.
- **Nucleocapsid** refers to the protein–nucleic acid complex of the virus—it may also be a subunit of a more complex virus.
- The **viral genome** is the genetic information of the virus, either RNA or DNA.
- **Viroids** are infectious agents that lack a capsid and consist only of a closed circular RNA molecule. They are usually plant pathogens and are not discussed further in this text.
- **Prions** are unusual infective particles that consist only of protein.

Prions

Prions were first thought to be a kind of virus; however, they appear to consist only of protein, without any nucleic acid. They are infective particles that have been implicated in some unusual transmissible neurodegenerative disorders, known as **transmissible spongiform encephalopathies** (**TSEs**). These include kuru and Creutzfeldt-Jakob disease (CJD) in humans, scrapie in sheep, and bovine spongiform encephalopathy (BSE), or 'mad cow disease' (see Chapter 20, page 530). It is thought that the diseases they cause are due to a conformational change in the proteins in brain cells, initiated by the prion protein. Prions are particularly resistant to procedures that normally destroy microbial cells, such as treatment with disinfectants and autoclaving.

CLASSIFICATION OF VIRUSES

The classification of viruses, based on the disease they produce or the part of the body they affect, is useful but not completely satisfactory for the scientist who is interested in other properties. Viruses with very different structures may cause diseases that are superficially similar. For example, hepatitis A, B, C, D and E all affect the liver but belong to quite different viral families.

New techniques in immunology and molecular biology are constantly providing information about the structure and composition of viruses. This may provide different grounds for classification in the future. At present, viruses are separated into major groups or families, based on the type of nucleic acid they contain, their shape and structure and their method of replication.

Table 5.1 lists the major families of viruses responsible for human infections.

HOST RANGE AND SPECIFICITY

Viruses are highly selective for the range of hosts and the type of cells they infect. In general, plant viruses infect only plants, bacterial viruses infect only bacteria, and animal viruses infect only animals. Within these broad categories, most viruses will attack only one type of animal or plant (its host range) and may even be specific for only one kind of cell or tissue within that organism (**tissue tropism**). However, some viruses are able to cross the species barrier and infect birds as well as animals (e.g. influenza A).

The ability of a virus to infect a particular kind of cell is dependent on the presence of an attachment site for the virus on the surface of the host cell. **Ligands** (chemical groups) in specific proteins (**viral attachment proteins**) on the surface of the virion bind to receptor molecules on the surface of the plasma membrane of the target cell. For some viruses the chemical structure of the ligands and corresponding receptor molecules has been determined. For example, orthomyxoviruses bind to a terminal sialic acid molecule located on a side chain of a membrane glycolipid or glycoprotein. The gp120 attachment protein of the human immunodeficiency virus, HIV, binds to the CD4 receptor molecule on the membrane of T4 lymphocytes.

The recognition of specific receptors by particular viruses is not absolute. Sometimes, unrelated viruses use the same receptor, which means that a cell with that receptor may be vulnerable to attack by a number of different viruses. On the other hand, similar viruses may use quite different receptors. In addition, certain viruses are able to recognise more than one kind of receptor. If the receptors are on different types of cells, this will extend the tissue tropism (i.e. the range of cells the virus can attack) or even the host range of the virus. For example, the rabies virus can attach to the acetyl choline (ACh) receptor as well as sialyted gangliosides, and this greatly extends its range of susceptible cell types and allows it to cross the species barrier. The more common the

TABLE 5.1 Viruses that affect humans classified into families by chemical and physical properties

VIRAL FAMILY	CAPSID SYMMETRY	VIRION NAKED OR ENVELOPED	SIZE (nm)	NUCLEIC ACID
DNA viruses				
Parvoviridae	Icosahedral	Naked	18–26	ssDNA
Polyomaviridae	Icosahedral	Naked	40	dsDNA (circular)
Papillomaviridae	Icosahedral	Naked	55	dsDNA (circular)
Adenoviridae	Icosahedral	Naked	70–90	dsDNA
Herpes viridae	Icosahedral	Enveloped	150–200	dsDNA
Hepadna viridae	Icosahedral	Enveloped	40–48	dsDNA (circular)
Poxviridae	Complex		230 x 400	dsDNA
RNA viruses				
Reoviridae	Icosahedral	Naked	60–80	dsRNA (segmented)
Picornaviridae	Icosahedral	Naked	20–30	ssRNA
Calciviridae	Icosahedral	Naked	27–40	ssRNA
Astroviridae	Icosahedral	Naked	28–30	
Togaviridae	Icosahedral	Enveloped	50–70	ssRNA
Flaviviridae	Unknown	Enveloped	45–60	ssRNA
Arenaviridae	or complex	Enveloped	50–300	ssRNA (segmented)
Coronaviridae		Enveloped	120–160	ssRNA
Retroviridae		Enveloped	80–100	ssRNA (diploid)
Bunyaviridae	Helical	Enveloped	80–100	ssRNA (segmented)
Bornaviridae	Helical	Enveloped	80–125	ssRNA
Orthomyxoviridae	Helical	Enveloped	80–120	ssRNA (segmented)
Paramyxoviridae	Helical	Enveloped	150–300	ssRNA
Rhabdoviridae	Helical	Enveloped	75 x 80	ssRNA
Filoviridae	Helical/filamentous	Enveloped	80 x 1000	ssRNA

Source: Data derived from G.F. Brooks, J.S. Butel and L.N. Ornston 2004, *Jawetz, Melnick & Adelberg's Medical Microbiology* (New York: McGraw-Hill).

receptor molecule, the wider the range of cells the virus can attack.

In general, it appears that viruses have evolved in such a way as to make maximum use of the available glycoprotein molecules on the host cell membrane as their receptors. It should be noted, however, that these molecules are an integral part of the host cell membrane and have functions other than merely serving as attachment sites for viruses. The presence or absence of these receptor molecules is a fundamental determinant of the susceptibility of the host cell to viral attack. The polio virus, for example, binds to specific receptors that are present in primate cells but not in other animals. Hence the polio virus can attack humans and monkeys, but not mice. Even then, replication of the polio virus occurs only in neuronal and muscle cells so it appears that the existence of appropriate receptors on primate cells is not the only determinant of successful infection. Other kinds of primate cells may be non-permissive for other reasons.

It is thought that, after penetration of the host cell by the virus, viral replication depends on the activity of a number of regulatory factors, determined by the host cell genome and viral genome, and variously called promoters, enhancers or transcriptional activators. These regulatory factors may be restricted to certain types of cells and tissues where they exert an important role as an additional determinant of successful viral infection.

VIRAL REPLICATION

As was mentioned earlier, viruses do not contain the genetic information for all the enzymes necessary for the synthesis of new viral particles. Instead, they use the host cell DNA and some host cell enzymes to produce viral components. The assembly of the new components into viral particles is under the direction of viral genes.

Replication of bacterial viruses

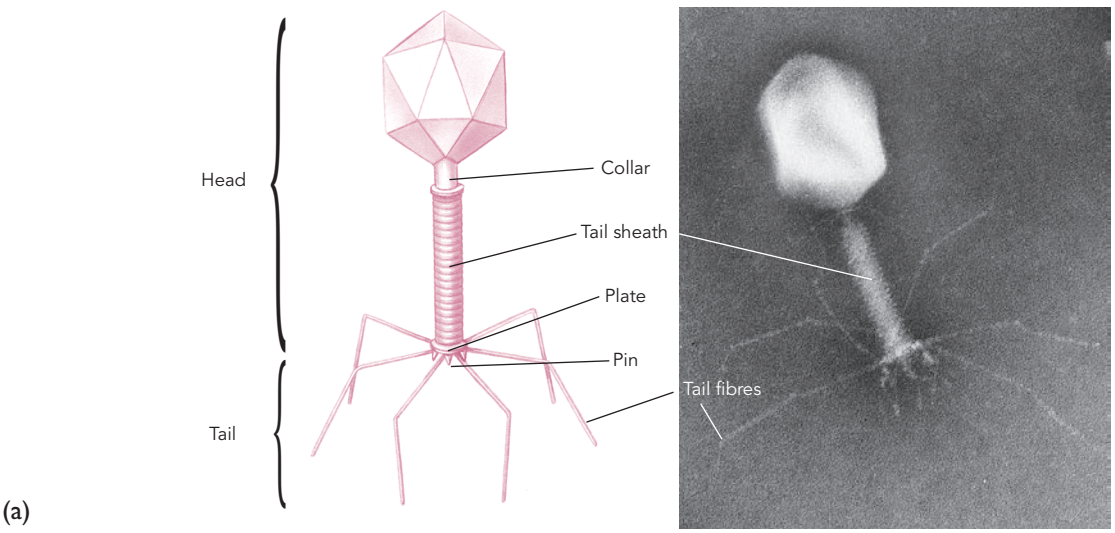
Although we are not primarily concerned in this text with the infection of bacteria by viruses, a brief description is included here as they provide a simple model for investigating the mechanisms of viral infection. Viruses that infect bacteria are called **bacteriophage**, or **phage**. They have been extensively studied, and have provided much information about the important processes that occur during the infection of animal cells. A bacterial culture contains many millions of

identical cells. It is easy to infect a culture of bacteria with phage and observe the results in a uniform population.

Phage exert the same specificity for their host cell as the viruses that infect higher organisms. This property is often used to differentiate strains of bacteria, using a technique known as phage typing (see Figure 3.16, Chapter 3, page 59). The first step in the infection of a bacterium by a phage involves the attachment of the phage to a specific receptor site on the bacterial cell wall. Since the bacterial cell is relatively small, only the viral nucleic acid is injected into the bacterium during infection. This is illustrated in Figure 5.4.

Once the phage DNA has entered the cell, a number of different events may occur. The most usual outcome is that the phage takes over the cell machinery, produces many new phage particles and then causes lysis of the cell as the new phage are released into the surrounding medium. This is called the **lytic cycle**.

Sometimes, after the phage enters the cell, instead of reproducing itself, the phage DNA is incorporated into the host cell DNA. In this state, called **lysogeny**, the phage is **latent** and does not produce new phage or cause lysis. The phage DNA that has been inserted into the bacterial chromosome is called a **prophage**. When the bacterium divides, the phage DNA is also copied, with the result that all the bacterial daughter cells contain the phage genes. One possible outcome of lysogeny is that the host cell may exhibit new properties.



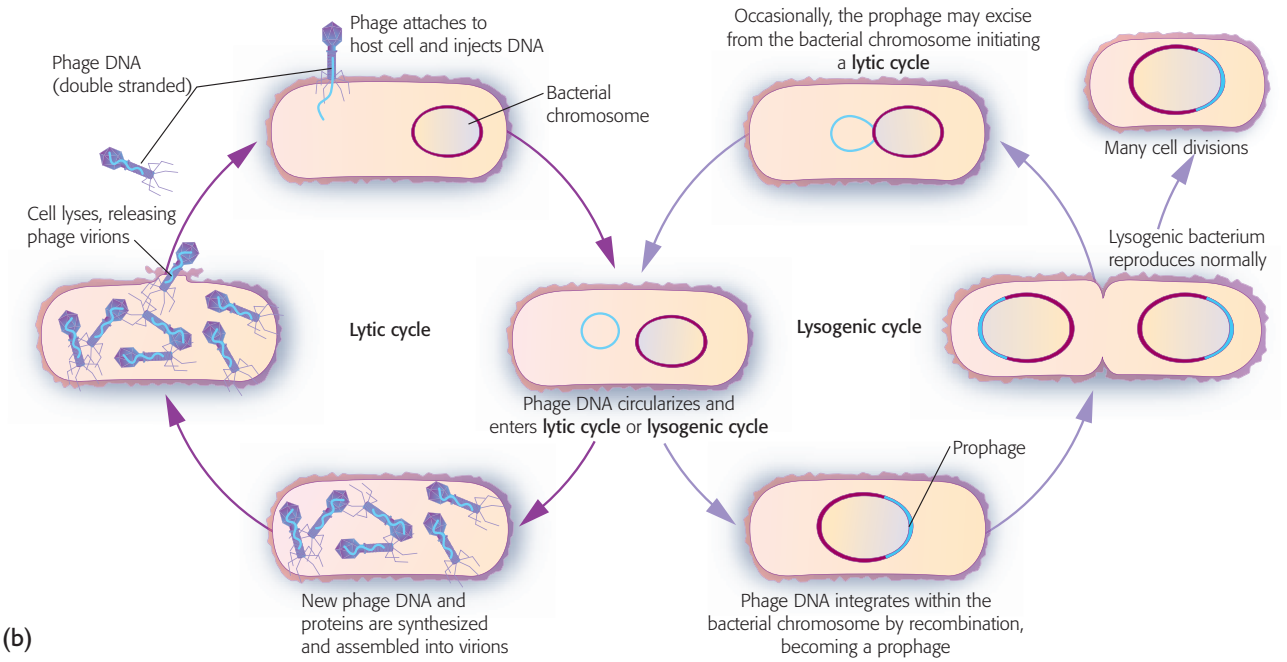


FIGURE 5.4

Bacteriophage

(a) Diagram and electron micrograph of T-even bacteriophage; (b) diagrammatic representation of lytic and lysogenic cycles of bacteriophage infection.

For example, *Corynebacterium diphtheriae*, the bacterium that causes diphtheria, is only pathogenic when it is carrying the prophage that has the gene for the production of the diphtheria toxin. Scarlet fever, a possible complication of a streptococcal infection, occurs only when the particular strain of streptococcus is carrying the gene for the erythrogenic scarlet fever toxin on its prophage DNA.

Lysogeny can also give rise to **specialised transduction**, in which bacterial genes are transferred with the phage DNA to another bacterial cell (see Chapter 4). A similar process to lysogeny can occur in animal cells, and may involve the transformation of the animal cell by the incorporation of viral DNA into the host cell genome. This usually results in the production of abnormal or cancerous cells. However, it also has the potential to be used clinically or therapeutically. Research is continuing into virus-mediated gene therapy, which would allow the insertion of a desired gene into a cell that has a defective gene.

Replication of animal viruses

Of more interest to health professionals is the sequence of events that takes place when an animal (human) cell is infected by a virus. This process can be broken down into six steps.

1. *Adsorption*. The first step in viral infection is the attachment of the virion to a specific receptor site on the outside of the host cell. The part of the viral capsid or envelope that binds to the host has a specific chemical structure and is often a glycoprotein spike or other part of the viral antigen.
2. *Penetration*. After the virion has attached to the host cell, the whole virion enters the cell by one of three processes—direct penetration, membrane fusion, or phagocytosis (a process in which the host cell membrane engulfs the viral particle). Animal cells are so much larger than viruses that there is plenty of room for the whole virion to enter.
3. *Uncoating*. The virus is then dismantled, the envelope and capsid removed and broken down into their component amino acids, and the nucleic acid is released into the cytoplasm.

   Adsorption, penetration and uncoating occur very rapidly, usually taking only a few minutes.
4. *Synthesis*. The nucleic acid is then copied many times, the viral genes directing the synthesis of viral messenger RNA and new viral capsid proteins and enzymes. The process of nucleic acid replication and protein synthesis differs for different viruses, depending on the type of nucleic acid involved—single- or double-stranded RNA or DNA (see below).
5. *Assembly*. The viral nucleic acid and the newly synthesised capsomeres are assembled in either the nucleus (for DNA viruses) or the cytoplasm (RNA viruses) of the cell.
6. *Release*. Naked viruses are released from the host cell, usually resulting in cell lysis and death. Enveloped viruses 'bud' out through the cell membrane, acquiring their envelope from the host cell membrane, which has been modified by the insertion of viral glycoproteins (see Figure 5.5).

   The complete cycle may occupy several hours.

When many viral particles are synthesised and released at once, lysis and death of the host cell inevitably occurs. Sometimes, virus formation is slower and the new virions are released continuously. This does not necessarily cause the immediate death of the cell.

Synthesis of viral particles

DNA viruses

Synthesis of new viral components takes place in the nucleus and cytoplasm of the host cell. The actual method of viral replication varies, depending on whether the viral nucleic acid is RNA or DNA, and whether it is single- or double-stranded. The replication of double-stranded DNA (dsDNA) viruses essentially follows the usual pathways for nucleic acid and protein synthesis as described in Chapter 4, the host cell providing most of the necessary enzymes. The first step is the integration of the viral DNA into the host cell genome where it directs the synthesis of new viral DNA, viral messenger RNA and proteins. These move from the nucleus to the cytoplasm where they are assembled into new viral particles. This process is illustrated in Figure 5.6 for a DNA-containing virus.

RNA viruses

The details of replication of the RNA viruses are quite complex and are different for each family, so are not all illustrated here. Most RNA viruses contain single strands of RNA (ssRNA) which are described as either positive or negative sense strands. The single RNA strands synthesise new viral RNA and viral protein via a series of steps involving positive and negative sense RNA strands. Positive (+) sense strands can function directly as messenger RNA (i.e. as a template for protein synthesis). Negative (–) sense

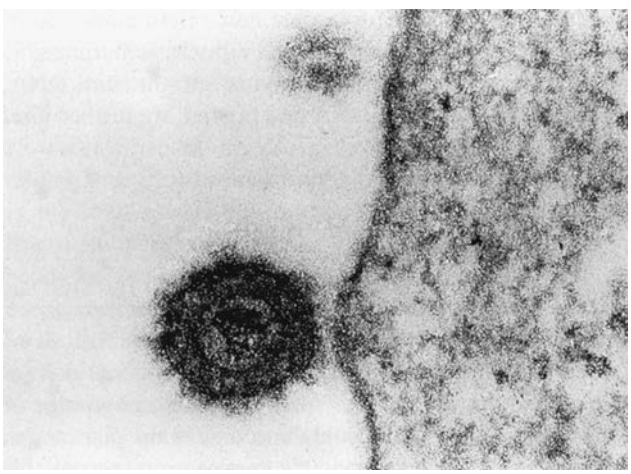


FIGURE 5.5

Herpes budding out of cell

Source: Dr Penny Bishop.

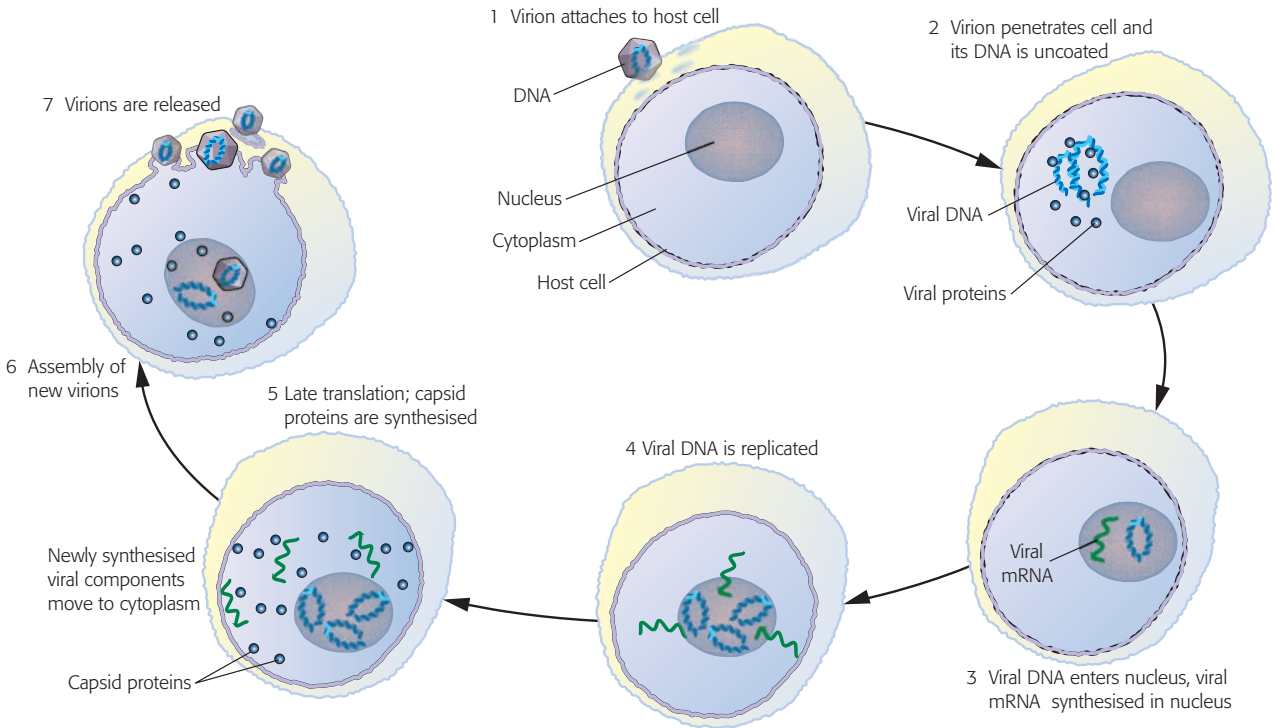


FIGURE 5.6

Multiplication of a DNA-containing virus

strands must first synthesise a +ve sense strand. The viruses containing double-stranded RNA (dsRNA) first synthesise viral messenger RNA, which then directs the synthesis of the viral enzymes needed to make new dsRNA for assembly into new viral particles.

Retroviruses

A completely different pathway of synthesis occurs in an important family of single-stranded RNA viruses known as **retroviruses**. Replication in these viruses initially involves a reverse process whereby viral DNA is first synthesised from the single-stranded viral RNA, using a viral enzyme called **reverse transcriptase**. The viral DNA is then transcribed into viral messenger RNA, protein and new viral RNA. A number of very important pathogens belong to this group, including the human immunodeficiency virus, HIV. Figure 5.7 illustrates the steps involved in the synthesis of a retrovirus. Figure 5.8 shows the release of mature HIV particles from an infected cell.

An understanding of the different pathways of viral synthesis allows scientists to identify critical steps that could be the target for antiviral drugs (see below and Chapter 12).

PATHOGENESIS OF VIRAL INFECTIONS IN HUMANS

Viruses are the causative agent of a number of serious infectious diseases. It is important for health professionals to understand the characteristics of viral infections in humans and their possible outcomes in order to appreciate the implications for transmission, treatment and prevention of viral disease. A knowledge of the methods of viral replication and the course of viral diseases can aid in the development of suitable treatments or preventative measures.

Viral disease is a result of viral infection—the signs and symptoms of the disease make up a syndrome that is typical of a particular viral infection. The severity of the symptoms and the outcome of the infection may vary and are determined by the type of virus and the susceptibility of the host. Some viruses produce a range of disease symptoms (e.g. measles) and the same apparent disease can be produced by a number of different viruses (e.g. the common cold is caused by adenoviruses, rhinoviruses, parainfluenza viruses, enteroviruses, etc.).

Table 5.2 is a list of some common viral infections of humans. New viruses are constantly being described, so a table such as this requires continual updating. The diseases listed are described in detail in other chapters of this book under the affected body system (see references in table). For arboviral infections, see Table 5.4 on page 102.

HOST RESPONSE TO VIRAL INFECTION

The human body has a complex system of non-specific and specific immune responses to invasion by foreign organisms. This is described in detail in Chapter 9.

The body's response to viral infection varies depending on the type of virus that is present and the susceptibility of the host. After gaining entry to the body, the virus is usually spread

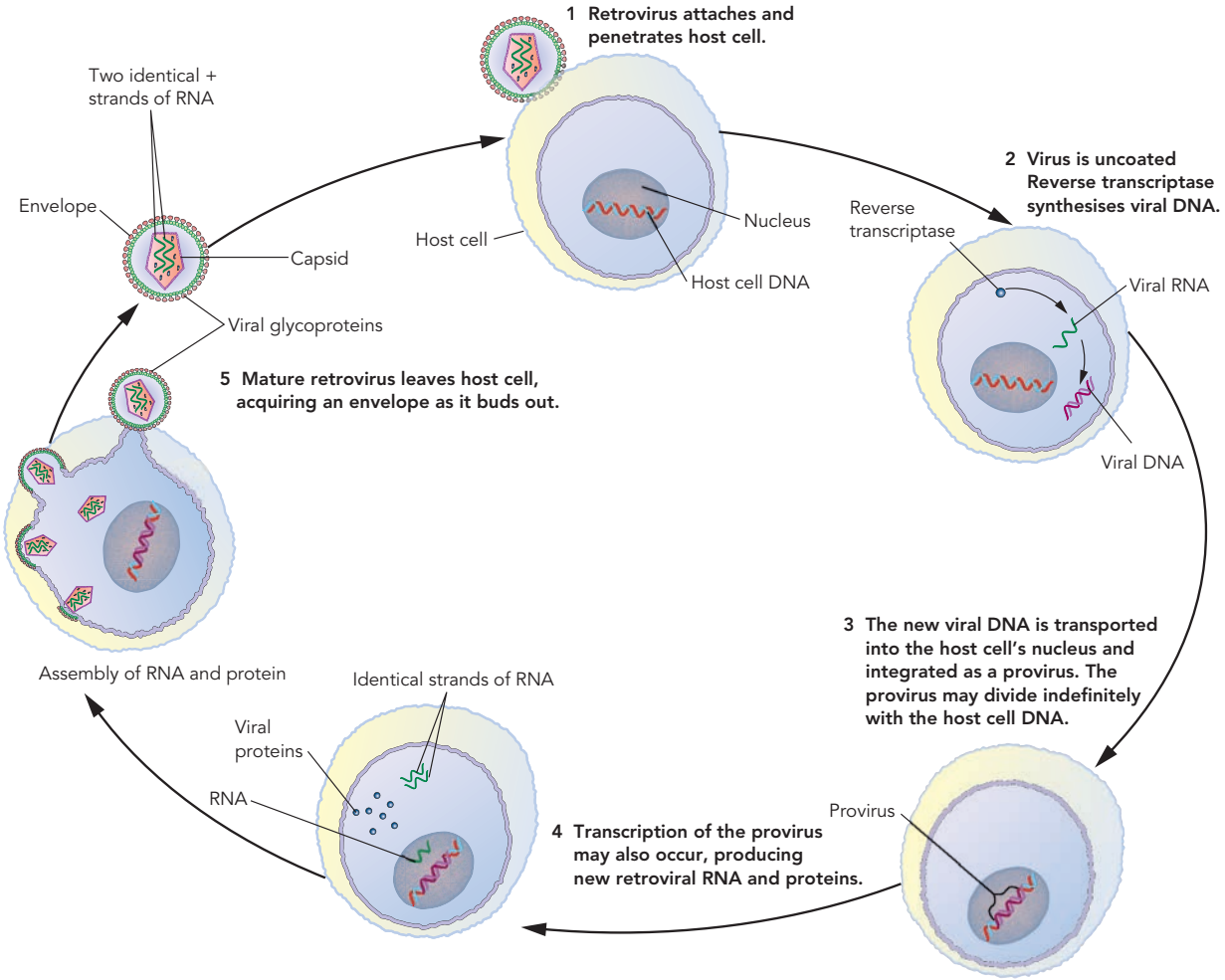


FIGURE 5.7

Retroviral infection and multiplication

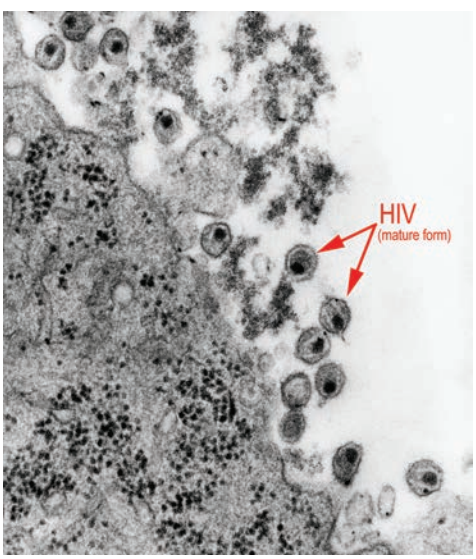


FIGURE 5.8

TEM of the release of newly synthesised mature HIV virions

Source: Centers for Disease Control (CDC).

via the bloodstream or lymphatic system. In the early stages, large numbers of viral particles are present in the bloodstream—a state known as **viraemia**. They may be present in the plasma or associated with a particular blood cell type.

Viral infection provokes several kinds of response by the host's immune system. The first is the production and release of interferon from infected cells. There are three different classes of interferons (α, β and γ), specific for the cells that produce them but not for the kind of virus. They are called **cytokines** (proteins which modulate the immune response). Interferon binds to receptors on uninfected cells to prevent further viral replication (see below).

The immune response also includes:

- activation of the cytotoxic T lymphocytes that identify and destroy infected cells
- production of lymphokines and other cytokines
- activation of B lymphocytes and the production of antibodies, which bind to and inactivate free viral particles
- production of memory cells to protect against re-infection (see Chapter 9).

TABLE 5.2 **Some common viral infections of humans**

DISEASE	VIRUS	FAMILY	MAJOR ORGANS AFFECTED	REFERENCE
Bronchiolitis	Respiratory syncytial virus Human metapneumovirus	Paramyxoviridae	Respiratory tract	Chap. 17 p. 424
Chickenpox	Varicella zoster	Herpes viridae	Generalised symptoms/skin rash	Chap. 16 p. 397
Common cold	Rhinovirus Adenovirus	Picornaviridae Adenoviridae	Respiratory tract	Chap. 17 p. 416
CMV	Cytomegalovirus	Herpes viridae	Generalised/salivary glands	Chap. 19 p. 497
Ebola, Marburg*		Filoviridae	Haemorrhagic	Chap 19 p. 499
Encephalitis	Various/some arthropod-borne		Nervous system/brain	Chap. 20 p. 521
Gastroenteritis	Rotavirus Norovirus Enterovirus	Reoviridae Calciviridae Picornaviridae	Gastrointestinal system	Chap. 18 p. 452
Glandular fever (infectious mononucleosis)	Epstein-Barr	Herpes viridae	Immune system	Chap. 19 p. 496
Hepatitis	Hepatitis A Hepatitis B Hepatitis C Hepatitis D Hepatitis E	Picornaviridae Hepadnaviridae Flaviviridae ? Calciviridae	Liver Liver Liver Liver Liver	Chap. 18 pp. 463–73
Herpes	Herpes simplex type 1, type 2	Herpes viridae	Skin	Chap. 16 p. 399 Chap. 21 p. 549
HIV/AIDS	Human immunodeficiency virus	Retroviridae	T4 cells of immune system	Chap. 19 p. 488
Influenza	Influenza type A, type B Parainfluenza	Orthomyxoviridae Paramyxoviridae	Generalised/respiratory system	Chap. 17 p. 424
Lassa fever*	Lassa	Arenaviridae	Haemorrhagic	Chap. 19 p. 499
Measles	Rubeola	Paramyxoviridae	Generalised/skin rash	Chap. 16 p. 396
Meningitis	Various		Nervous system/meninges	Chap. 20 p. 518
Mumps	Mumps	Paramyxoviridae	Salivary glands	Chap. 20 p. 487
Polio	Polio	Picornaviridae	Nervous system	Chap. 20 p. 529
Rabies	Rabies, Lyssa	Rhabdoviridae	Nervous system	Chap. 20 p. 527
Rubella (German measles)	Rubella	Togaviridae	Generalised/skin rash affects foetus	Chap. 16 p. 395
SARS	Coronavirus	Coronaviridae	Severe acute respiratory syndrome	Chap. 17 p. 434
Shingles	Herpes zoster (latent form of varicella zoster)	Herpes viridae	Skin/nervous system	Chap. 16 pp. 397–8
Warts	Papilloma	Papillomaviridae	Skin/mucous membranes	Chap. 16 p. 398
Yellow fever†	Yellow fever	Flaviviridae	Generalised/ liver	Chap. 19 p. 498

* Blood-borne haemorrhagic diseases, not seen in Australia

† Mosquito-borne disease endemic to South America and tropical areas

The activation of the immune system and the production of interferon and cytokines are responsible for many of the symptoms associated with viral infections—for example, swollen glands (lymphadenopathy), a general feeling of malaise, headache and myalgia (muscle aches). Treatment of most viral infections is aimed at relieving these symptoms. Bed rest and administration of analgesics (for pain) and antipyretics (for fever) are the main methods available. Recovery from the disease depends largely on the ability of the host's immune system to recognise the foreign invader and produce antibodies and T cells to combat it.

The age of the patient and the status of the immune system are important determinants of the patient's susceptibility to viral infections. It is well known that the immune system matures in the first year of life and that it is less effective in the elderly. On the other hand, there seems to be an age-related difference in susceptibility to some viral infections. The so-called childhood diseases, such as mumps and chickenpox, are usually much more severe in adults.

During pregnancy, the mother's immune system is also somewhat compromised to accommodate the foetus. This may explain the ability of viruses such as rubella and cytomegalovirus to cross the placenta and cause congenital defects. A very high level of maternal mortality is associated with hepatitis E infection in pregnancy.

VIRAL EVASION MECHANISMS

Viruses are true parasites and need to establish a balance with their host and its immune system or they would not survive. They use various strategies to escape detection and to avoid killing their host, thus ensuring their own survival. These include 'hiding' in infected cells, so that the viral antigen is not expressed on the outside of the infected cell and is thus protected from attack by cytotoxic T cells. HIV attacks and destroys the T cells of the cell-mediated immune system and also 'hides' in macrophages. Other viruses become latent (e.g. the herpes zoster virus persists in nerve cells) and manipulate the host immune system. These strategies are described in more detail in following sections.

Viral mutation

Mutation is another way that viruses are able to evade the immune system. Mutation involves a change in the genes that determine the properties of the virus such as envelope structure. Some viruses continually mutate and change their appearance (outer structure) so that previously formed antibodies are no longer effective. One of the most important of these is influenza A, the main cause of seasonal influenza. The virus is an enveloped virus and has glycoprotein 'spikes' of haemagglutinin (H) and neuraminidase (N) on its outer layer (see Figure 5.9). These spikes have antigenic properties. Haemagglutinin is responsible for the attachment of the virus to the surface of the target cell. Neuraminidase is required for the release of new viral particles. The different strains are identified by assigning numbers to the spikes—for example, H5N1 is the strain responsible for avian or 'bird flu'. The virus is able to infect other animals, including domestic pigs and birds, and during passage through these animals it can undergo genetic re-assortment (antigenic shift) to produce new virus types (see Spotlight box, page 96, and Chapter 17, pages 424–6).

OUTCOMES OF VIRAL INFECTION

The familiar acute, highly contagious viral illness that follows a defined course, characterised by recognisable signs and symptoms, and leads to complete recovery is only one of the outcomes of viral infection. Some viral infections do not produce recognisable symptoms of disease at all. Viral infections can be described in a number of ways: as acute, subclinical, persistent, latent, chronic (carrier), slow or oncogenic (see Figure 5.11). We now look at each of these outcomes of viral infection in more detail.

Acute lytic infection

An acute lytic infection is typified by a disease with well-defined, recognisable symptoms—for example, the common cold, mumps or influenza. The virion gains entry to the host, attaches to its specific target cell, enters and takes over the host cell machinery, making many copies of the virus. The release of the new virions causes cell lysis and death. The new viral particles then infect neighbouring cells, producing typical disease symptoms. Eventually, the host immune response is successful in eliminating the invading virus and producing antibodies, and immunological memory is established to protect against further infections by the same virus.

For many diseases (e.g. mumps) this acute infection will give rise to long-lasting immunity. However, some viruses, such as those responsible for influenza, have the ability to mutate and alter the structure of their outer envelope or

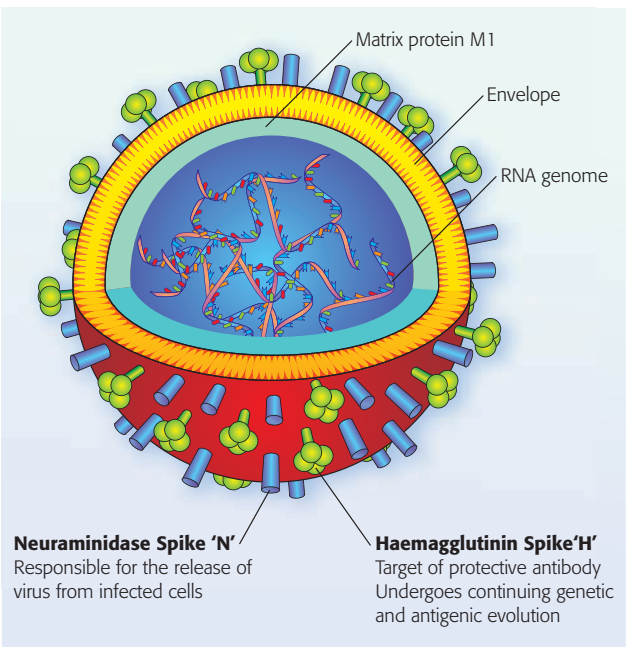


FIGURE 5.9

Diagrammatic representation of influenza A virus

SPOTLIGHT ON

Influenza A

The outbreak of 'swine flu' that started in Mexico in 2009 and spread worldwide caused alarm because it was highly transmissible and appeared to be quite unlike any of the strains of influenza that were circulating globally at that time. Health services in every country were mobilised to institute quarantine regulations to contain the spread of the disease, identify the virus and develop a vaccine. It was identified as influenza A, H1N1, similar to the strain of influenza that had resulted in the deaths of between 25 and 40 million people in the 1918–19 pandemic (see Figure 5.10). However, although the disease was highly infectious, it was not as severe as the 1918 pandemic and the death toll was limited. Modern technology was able to produce a vaccine for global distribution within months of the virus being identified, and it is now included in the seasonal influenza vaccine that is made available each year.

Another strain of influenza A, avian influenza A virus, H5N1, or 'bird flu', first appeared in commercial poultry in China in 1996 and spread to Hong Kong, where it caused the deaths of 6 people out of 18 who were infected. In an attempt to contain the disease, millions of chickens were slaughtered. This was the first report of an avian virus that could directly infect humans. Since then the virus has spread worldwide, carried by migratory birds. There have been more than 500 reported cases with a nearly 60 per cent mortality rate, mainly in Indonesia and Egypt where there is close contact between humans and poultry. At present, H5N1 virus does not seem to have a high affinity for human respiratory epithelial cells, so the likelihood of human infection is low. However, if the virus mutates so that human-to-human transmission is possible, the risk of a global pandemic is high.

The emergence of the highly infectious H1N1 has raised concerns that these two strains could possibly combine, re-assort and produce a highly infectious strain with a high mortality rate at some time in the future. This might occur in Asian countries where farming practices allow close contact between humans and animals.

Vaccination is the best method of prevention or treatment. The influenza vaccine is prepared each year to protect against the strains of virus that are known to be circulating in the community at that time. The new antiviral drugs oseltamivir (Tamiflu®) and zanamivir (Relenza®) are neuraminidase inhibitors. They have been used successfully to shorten the duration and reduce the complication rates of influenza, but some resistance is developing and there is a difference of opinion as to whether they should be used prophylactically or only after the infection occurs.

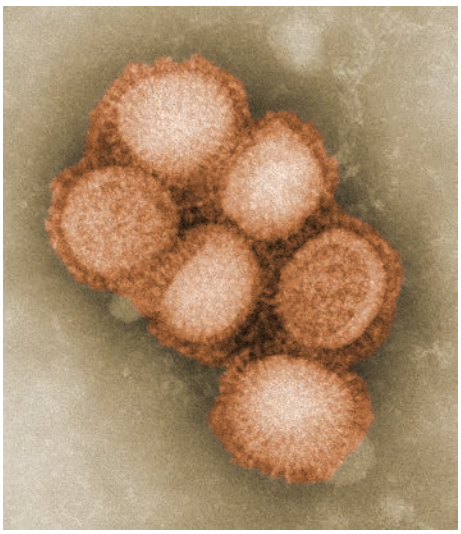


FIGURE 5.10

Coloured transmission electron micrograph of influenza A, H1N1 virus, responsible for swine flu

Source: Centers for Disease Control (CDC)/C. S. Goldsmith and A. Balish.

capsid. This means that the antibodies produced against one particular strain of influenza virus will not recognise and be effective against another strain. (See Chapter 10 for discussion of antigen-antibody reactions.) Exposure to this new or altered strain of influenza virus will *apparently* give rise to the same disease again. In other cases, a person may appear to suffer from the common cold more than once. However, cold symptoms may be caused by any one of about 100 different viruses. Exposure to each of these viruses will provide long-lasting protection from re-infection with the same virus, but not from other cold viruses.

Subclinical infections

Very often a person is infected by a virus but does not exhibit any recognisable clinical signs or symptoms. A general feeling of malaise, slight fever or **lymphadenopathy** ('swollen glands') may be the only signs that the patient is unwell. In this type of infection, the patient recovers, often unaware of having had any disease. Frequently, the only evidence of infection is that an immune response has been provoked, resulting in the production of specific antibodies and a cellular immune response to the virus. The antibodies can be detected by serological examination of the patient's blood. Depending on the level of antibodies present (the **antibody titre**), the patient may be considered to be immune—that is, protected from future attacks by the same virus.

Rubella (German measles) can be a very mild disease and is often subclinical. However, when this disease occurs during the first trimester of pregnancy it can cause serious congenital

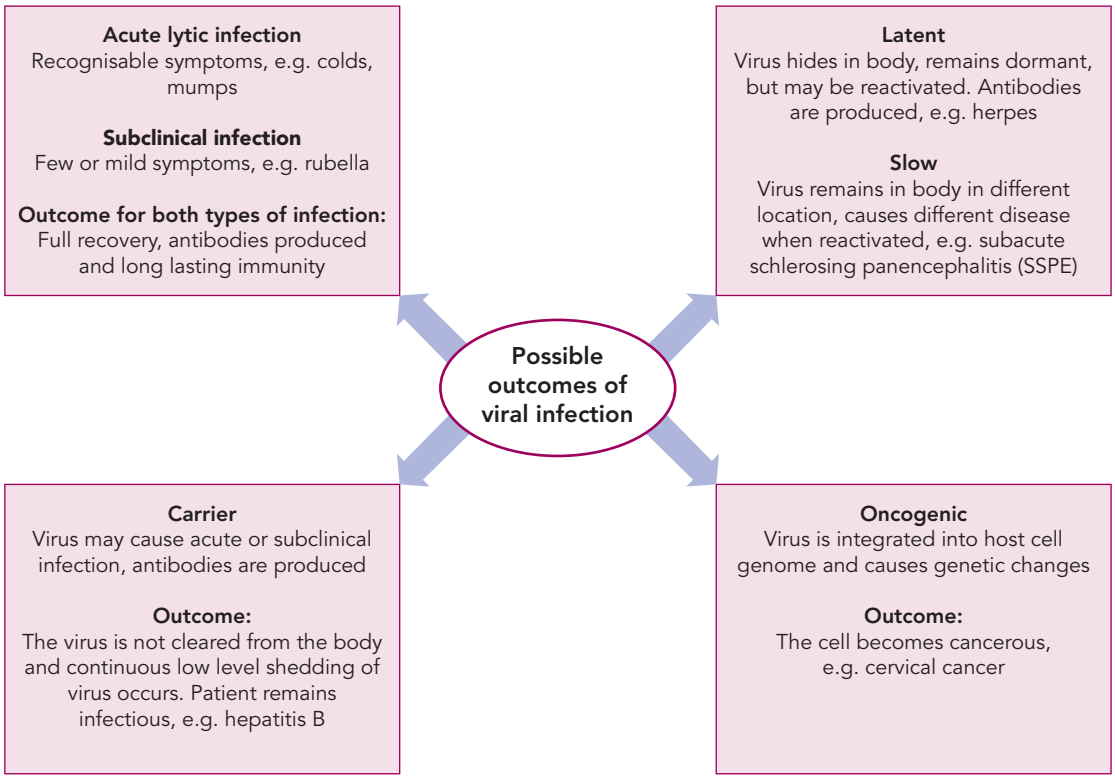


FIGURE 5.11

Outcome of viral infection

abnormalities in the foetus, even though the mother may experience few, if any, symptoms. A vaccine to protect against rubella is available and a national program of vaccination was introduced in Australia in 1971. Similarly, cytomegalovirus infection produces only very mild cold-like symptoms, but can cause serious congenital defects in a developing foetus. Possible exposure of the mother to these viruses during pregnancy can be determined by measuring the level of recently formed antibodies (IgM) in the mother's serum, although additional tests are needed to clearly define the risks to the unborn baby (see Chapter 9). There is no vaccine for CMV.

Polio is another disease that can occur subclinically, as well as in the more acute paralytic form. When the polio virus was first isolated and tests were developed for the detection of antibodies, it was found that a large percentage of the population was carrying antibodies to the virus without any previous history of the disease. It has been suggested that this was most probably the result of a subclinical infection in an earlier childhood exposure.

Other viral diseases that are known to occur subclinically include glandular fever (Epstein-Barr virus) and hepatitis B and C. These diseases can also have serious outcomes.

PERSISTENCE OF VIRAL INFECTIONS

Many viral infections are self-limiting—that is, the host immune system overcomes the virus and it is completely eliminated from the body. However, sometimes the virus persists in the human host for long periods. This persistence may have a number of different outcomes, variously described as latent, chronic or slow infections. Oncogenic viruses also persist and transform the host cells into cancer cells.

Latent viral infections

In some cases the virus remains latent (dormant) in some of the host cells and can be reactivated at a later stage, causing a recurrence of the disease. Latent infections are caused by many of the viruses in the herpes family as well as the human immunodeficiency virus, HIV.

The virus generally produces an initial recognisable disease, followed by apparent recovery. However, instead of the virus being completely cleared from the host by the immune system, some viral particles remain. They often 'hide' in cells that are not the usual target of attack. Reactivation of the virus may occur when the physiological state of the host is altered. This may be due to conditions of physical or psychological stress, immunosuppression or other illnesses.

The most familiar example of a latent infection is the blisters produced by herpes simplex virus (HSV), types 1 and 2. Many patients only ever report one initial attack of herpes. However, others suffer repeated attacks associated with colds ('cold sores', 'fever blisters'), exposure to UV light (sunburn) or other stress factors. The virus has been shown to be dormant in the nerve cells of the sensory ganglia. Other herpes viruses also produce latent infections. These include the varicella zoster virus which is responsible for **varicella** (chickenpox) and **zoster** (shingles). After an attack

of chickenpox, the virus may remain dormant in the sensory nerve ganglia and give rise to shingles many years later.

Epstein-Barr virus (EBV), which is responsible for glandular fever, and cytomegalovirus (CMV) can also establish latent infections.

An important characteristic of latent infections is that they tend to be reactivated when the immune system is compromised. This may be a serious complication for elderly patients, for immunodeficient patients, or for those receiving immunosuppressive therapy or after transplant surgery. Infections such as shingles (reactivated varicella zoster virus, VZV) and cold sores (reactivated herpes simplex virus, HSV) are common in these patients.

Slow infections

In some types of latent infection, the virus responsible for the disease persists in the host and, some years after the initial infection, symptoms of a new disease occur, usually slowly and over a long period. These symptoms are not the same as the original disease. **Subacute sclerosing panencephalitis** (**SSPE**) is a rare late complication of measles infection, and usually occurs in teenagers and young adults. It has been attributed to the persistence of the measles virus, which lodges in the brain cells. The disease is characterised by slow (1–2 years), progressive mental deterioration and degeneration of the nervous system, and is invariably fatal.

Chronic viral infections (carrier state)

An important outcome of viral disease occurs when the virus remains in the host and gives rise to a continuous low level of viral production and shedding—the carrier state. This can occur after either an acute illness or a subclinical infection. Carriers do not have any disease symptoms and are often unaware that they are carrying the virus and are therefore infectious to others.

Hepatitis B virus is a good example. Infection with this virus can have a number of outcomes. It may cause an acute infection, leading either to complete recovery and development of immunity or, occasionally (in 1–2 per cent of cases), to a fulminant infection resulting in death. However, in 5–10 per cent of adult patients the result of infection is a mild or subclinical disease that gives rise to a chronic carrier state. The risk of becoming a carrier decreases with age. Hepatitis B can also be acquired by vertical transmission from a carrier mother to the baby. The baby may not exhibit any symptoms of hepatitis, but has an 80–90 per cent risk of becoming a carrier. Serological testing for the presence of the various hepatitis B antigens and antibodies is used to establish the infectious status of mother and baby. Treatment of the baby at birth with anti-hepatitis globulin, followed by hepatitis B vaccination, can prevent development of the carrier state in the baby. (See Chapter 18 for a discussion of hepatitis and the significance of the presence of the various viral antigens.)

Chronic active hepatitis B infection can cause long-term liver damage, **cirrhosis**, and in some cases liver cancer.

Hepatitis C is another virus that can persist as a chronic infection. A test for hepatitis C has only been available since 1990 and there is still much to be learnt about this virus. However, it is estimated that more than 75 per cent of people who contract this virus become carriers. Hepatitis C also has long-term sequelae and can lead to liver cancer. There is still no vaccine for hepatitis C (see Case History 5.1).

CASE HISTORY 5.1

Risks associated with needlestick injuries

On a busy Friday night, a nurse in the accident and emergency department of a large Sydney teaching hospital suffered a needlestick injury while attending to a patient who had been admitted with a drug overdose. The patient subsequently tested positive for hepatitis C.

Questions

1. What should the nurse have done after suffering a needlestick injury?
2. What are the risks associated with the injury?
3. How is hepatitis C transmitted?
4. What are the symptoms/prognosis/treatment for someone infected with hepatitis C?

Human immunodeficiency virus (HIV) is the primary cause of **AIDS** (**A**cquired **I**mmune **D**eficiency **S**yndrome). It is described as a chronic infection because the virus is considered to be transmissible throughout the illness.

Oncogenic viruses

A number of viruses have been shown to cause tumours in animals and to be able to transform human cells into cancer cells in tissue culture. These viruses are termed **oncogenic**. Cancer cells are cells that have lost control of normal regulation of growth processes. One of the most important of these regulatory mechanisms is **contact inhibition**. Transformed cells replicate in an uncontrolled way, piling up on top of each other to produce a mass of cells (tumour). Some viruses, as well as a number of other agents, including mutagenic chemicals and ionising radiation, have been shown to be able to alter cellular DNA in such a way that the cell multiplies uncontrollably. This phenomenon is called **neoplasia** and the tumour formed is a **neoplasm**. There are two main ways in which it is thought that a virus can alter normal cells:

1. The oncogenic virus introduces a new 'transforming' gene into the cell.
2. The virus induces or alters the expression of a pre-existing cellular gene (a 'proto' oncogene).

In both cases the DNA from the oncogenic virus is integrated into the host cell genome and replicates with the host cell DNA in a similar manner to lysogeny in bacteria.

Various types of cancers have been shown to be associated with a previous or current viral infection. They are listed in Table 5.3. Of these, human papillomavirus (HPV) is particularly important as it is sexually transmitted and quite widespread. There is a large amount of evidence linking certain types of HPV to the occurrence of cervical cancer. The virus induces characteristic changes in the epithelial cells of the cervix. These can be detected by microscopic examination of a smear of cells obtained from the cervix (the Papanicolaou test—Pap test), and appear even before the first evidence of malignancy (see Case History 5.2). More recently, HPV has also been associated with oropharyngeal cancers.

Other important oncogenic viruses are hepatitis B, hepatitis C, human herpes virus 8 (HHV8), human T cell lymphotrophic virus (HTLV1) and Epstein-Barr virus (EBV).

TRANSMISSION OF VIRAL DISEASES

Effective prevention and control of viral diseases depends on an understanding of the mechanisms of transmission of these infectious agents. Infections are transmitted from one host to another when complete viral particles (virions), shed by the infected host, reach a susceptible host. Most viruses do not survive for long periods outside living cells, so transmission usually requires direct transfer of body fluids or tissues, close contact between hosts, or transmission via an insect vector. There are some exceptions: hepatitis A and the Norwalk viruses can survive for days on surfaces; hepatitis B is also quite resistant.

Human reservoirs

Humans are the main reservoir for viral diseases. Diseases are usually spread from one host to another in infected body fluids or secretions. The rate and number of virus particles being shed by the host varies during the course of the infection. Very often virus production and shedding is highest just before recognisable clinical symptoms develop (the **prodromal period**). This fact helps to explain the occurrence of epidemics, since transmission can take place before patients are aware they have the disease (e.g. chickenpox outbreaks in schools).

Animal reservoirs

Few viruses cause disease in both humans and other animals because viruses are host-specific. The viruses that do cross the species barrier usually occur under conditions that allow transmission from the animal reservoir to humans, and use receptor molecules that are found in a wide variety of cells. One of these is the rabies virus, which is widespread throughout the animal kingdom and can be transmitted to humans by the bite of an infected animal.

Other viruses that persist in nature in an animal reservoir can be transmitted to humans by close contact with the animal or infected droppings. Among these, the hantavirus, which was responsible for a serious outbreak of an influenza-like disease in New Mexico in 1993, was linked to a plague of deer mice. Hendra virus (previously named equine morbillivirus), which was identified as responsible for the death of a horse trainer and 14 racehorses in Australia in 1994 and has since caused three more human deaths, is another example of a virus that can apparently cross the species barrier. Another serious outbreak in 2011 resulted in the deaths of more than 20 horses. The natural reservoir for the virus is fruit bats, but the virus can infect horses and other animals, including domestic cats and dogs as well as humans (see Spotlight box: Viral zoonoses linked to flying foxes, Chapter 8, page 160). Bat lyssavirus, ABL, is a rabies-like virus that is also found in fruit bats. Rabies vaccine is used to treat people infected with lyssavirus.

Menangle virus, which has been found in New South Wales, and Nipah virus, which occurs in Malaysia, are also carried by fruit bats and infect both pigs and humans. West Nile virus and the avian influenza virus, H5N1, are recently identified viruses that infect birds and humans. H1N1 affects pigs and humans.

Some viruses that persist in animal or bird reservoirs are transmitted to other animals or humans by the bite of insects such as mosquitoes. These viruses cause a subclinical infection in the animal host and multiply in both the invertebrate vector and vertebrate host. Some of these cause serious infections in humans. They are called **arboviruses** and are described later in this chapter.

Airborne transmission

Viruses tend to be shed mainly from the area of the body where the infection is localised. Thus, infection of the mucosal

TABLE 5.3 Human cancers related to viral infections

CANCER	VIRUS	FAMILY	GENOME
Adult T cell leukaemia	HTLV-I	Retroviridae	ssRNA
Skin carcinomas, genital carcinomas, oropharyngeal carcinomas	Papilloma, HPV 5, HPV 16,18	Papillomaviridae Papillomaviridae	DNA DNA
Kaposi's sarcoma	HHV 8	Herpes viridae	DNA
Hepatocellular carcinoma	Hepatitis B Hepatitis C	Hepadnaviridae Flaviviridae	DNA RNA
Nasopharyngeal carcinoma	Epstein-Barr	Herpes viridae	DNA
Burkitt's lymphoma	Epstein-Barr	Herpes viridae	DNA

CASE HISTORY 5.2

Cervical cancer

Mrs Y, a 32-year-old mother of two children, was admitted to hospital with advanced cervical carcinoma. Although she had been sexually active since she was 20, she had never had a Pap smear. A hysterectomy was performed to remove the diseased uterus, followed by radiation treatment and chemotherapy. However, the cancer had already spread through her body and she died 18 months later.

Comment

In Australia there is an average of 1000 new cases of cervical cancer and 350 deaths each year. More than 80 per cent of cases are associated with a previous infection by human papillomavirus (HPV), the cause of genital warts. All women who have ever been sexually active are at risk of developing cervical cancer, even if they have never suffered from genital warts. The risk is highest in women with multiple partners who do not use protection such as condoms or if they have other sexually transmitted infections.

There are more than 100 different types of human papillomaviruses that attack skin and mucosal cells. About 30 types have been associated with genital infections. Of these, types 16 and 18 are most frequently associated with abnormal Pap smears. Type 16 is most commonly associated with cancer in Australia, and type 18 has been shown to have the highest risk of recurrence. Other types that have been implicated in various parts of the world are types 31, 33, 35, 39, 45, 51, 52 and 56.

HPV induces characteristic changes in the epithelial cells of the cervix. The changes progress through stages described as Low-grade or High-grade Squamous Intraepithelial Lesions, LSIL or HSIL (previously called cervical intraepithelial neoplasias (CIN) I, II and III and carcinoma *in situ* (CIS)). HSIL is a precancerous condition and can lead to invasive squamous cell carcinoma. The stages are described as:

- LSIL: minor dysplasia
- HSIL: severe dysplasia—precursor to cancer.

If abnormal cells are detected by a Pap smear, the patient is referred for colposcopy. This involves examination of the cervix after a painless application of 3 per cent acetic acid (vinegar). The lesion appears as a flat white plaque and can be removed by laser treatment. If detected early, the treatment is successful, but the woman is advised to have regular follow-up Pap smears. If the disease has progressed further, a cone biopsy can be performed. Early treatment of cervical cancer is very successful and in all countries where routine Pap smears are available the mortality from the disease has been reduced.

The Pap test is not always accurate and incorrect results may sometimes occur because of poor sampling or from laboratory error. Approximately 20 per cent of tests may return false negative results. However, the benefit of regular screening far outweighs problems with diagnosis. Recently, some laboratories have introduced computerised scanning of microscope slides to improve the accuracy of diagnosis. Women are advised to have a Pap test at least every two years, or more frequently if there are abnormal symptoms, as the disease can be cured if detected early. Women who live in rural and remote areas or who are immigrants to Australia are less likely to be aware of, or to comply with, screening programs. Some states have established a central register to encourage women to return for regular checkups.

A vaccine to prevent HPV infection and cancer was developed in Australia by a team led by Dr Ian Frazer. The vaccine protects women against certain strains of the human papillomavirus (HPV), which are known to cause 7 out of 10 cases of cervical cancer and 9 out of 10 cases of genital warts. It is now licensed for use and the Australian government is providing the vaccine 'Gardasil' through the National HPV Vaccination Program, including a free school-based program for all 12–13-year-old girls. However, the vaccine does not yet replace the need for regular Pap tests.

Questions

1. Which group of people are most at risk of contracting HPV?
2. Name three strategies that should be used to avoid the risk of cervical cancer.
3. Why should women continue to have Pap tests even though a vaccine is now available?

cells of the upper respiratory tract by cold viruses (rhinoviruses, coronaviruses) results in virions being shed in aerosol droplets when the patient coughs or sneezes. These viruses do not survive for long outside the body. Other viruses that enter via the respiratory route are shed in the same way.

Viral infections, such as chickenpox and herpes, that produce pustules or vesicles on the skin also shed virions into the air from these lesions. Transmission of herpes is thought to mainly occur when visible vesicles are present, although there is some evidence that, in genital herpes, virus shedding and transmission can occur when there are no apparent lesions.

Faecal–oral transmission

Microorganisms that cause gastrointestinal infections are usually shed in the faeces. Many viruses are transmitted by faecal contamination of food or water—for example, hepatitis A, rotavirus and enteroviruses. Among these, rotavirus is of special concern as it can cause rapid, severe dehydration and death in infants. Special care must be taken to prevent transmission of rotavirus among babies and small children in hospitals and childcare centres. A vaccine for rotavirus has now been licensed and included in the Australian vaccination schedule.

Outbreaks of food poisoning are often caused by noroviruses (previously called Norwalk-like viruses) (see Chapter 18, page 453). They are transmitted by faecal–oral contact and are often responsible for extensive outbreaks of gastrointestinal disease in institutions or on cruise ships. They are unusual in that they have been shown to persist on surfaces for extended periods and can easily be spread person to person in aerosols. Viral shedding may continue after symptoms disappear. Environmental cleaning and handwashing is most important to prevent the spread of the virus. Exposure to noroviruses does not always give protection against re-infection, as different strains circulate at different times.

The polio virus (another enterovirus) is also shed in faeces. Despite a worldwide vaccination campaign to eradicate polio, it is still endemic in some parts of the world (see Spotlight box: Eradication of polio, Chapter 14, page 339). Viruses transmitted by the faecal–oral route are still a cause for concern in areas where sanitation is poor and they remain a major cause of death in developing countries.

Transmission in body fluids

In recent years considerable attention has focused on viruses that can be transmitted in blood or other body substances. These include hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV). When a previously unknown and potentially fatal virus (HIV) was discovered in the 1980s, it drew attention to the possibility that there may be other unidentified viruses present in body substances. Following the discovery of the accidental transmission of HIV in contaminated blood and body fluids, a system of infection control procedures designed to minimise the risks of transmission of viral infections has been developed to prevent the spread of these viruses in hospitals and healthcare facilities (see Chapter 13).

Occasionally, viruses can be transmitted by organ transplantation if adequate screening procedures are not used on the donor organs (see Case History 5.3).

Transmission by vectors—arboviral diseases

An important method of transmission of some viruses is by **arthropod vectors**—that is, by insects such as mosquitoes and other arthropods which carry the virus from an infected host to a susceptible human. These viruses are called **arboviruses** (**ar**thropod-**bo**rne). The insect ingests the virus from an infected human or animal reservoir while the insect is feeding. The virus replicates in the gut of the insect vector, spreads to the salivary glands and is injected into a susceptible host during the next blood meal. The replicative phase of the virus in the insect may take several days and the rate is affected by the ambient temperature.

There are approximately 80 arboviruses known that are capable of infecting humans, although only a limited number occur in Australia. Most arboviruses exist in Nature in animal hosts that have usually sustained a subclinical infection and developed immunity. One of the most common arthropod vectors is the mosquito, and specific breeds of mosquito act as vectors for different viruses. For an outbreak of a viral disease to

CASE HISTORY 5.3

Viral transmission in organ transplants

In January 2007, in Victoria, a 57-year-old organ donor died of a brain haemorrhage. His liver and kidneys were transplanted into three different female recipients. The women all died within a week of each other, about a month after receiving the transplants. Health authorities suspected that the donor organs had carried an infection. Genetic testing of tissue samples identified a previously unknown virus belonging to the Arena family. Arena viruses are a family of mainly rodent viruses that occasionally affect humans but usually cause only minor symptoms. Organ transplant recipients, however, do not have the immunity to fight the infection. Authorities in the United States found the new virus was related to the lymphocytic choriomeningitis virus (LCMV), which caused similar transplant deaths in the US in 2003 and 2005. Health authorities in Australia have announced they will consider the introduction of routine testing for the virus in donor organs in future.

Questions

1. What tests are usually carried out on organs before a transplant takes place? (*Hint:* See Chapter 14, 'Screening procedures'.)
2. Could these deaths have been avoided?
3. Why were the transplant patients vulnerable to this virus? (*Hint:* See Chapter 13, 'Patient susceptibility'.)

occur a pool of infected hosts must be present in the community, together with favourable breeding conditions for the particular type of mosquito. Control is best achieved by mosquito eradication programs.

Some important viral diseases that are endemic to Australia and transmitted by mosquitoes include Ross River fever, Murray Valley encephalitis, Barmah Forest fever and dengue fever. Of these, Ross River fever is the most widespread and the mosquito vector (*Aedes* species) is found throughout the eastern states. Other serious arboviral diseases found outside Australia are yellow fever, Lassa fever and Japanese encephalitis. Recently, cases of chikungunya virus have been reported in Melbourne. It is characterised by fever, headache and joint pain. The cases were acquired overseas, but as the virus is carried by *Aedes* species it could become established in Australia. Epidemics have occurred in Africa, Asia and Indian Ocean islands.

Table 5.4 lists the major arboviral infections occurring in Australia (see also Spotlight box: Arboviruses endemic to Australia, Chapter 14, page 355).

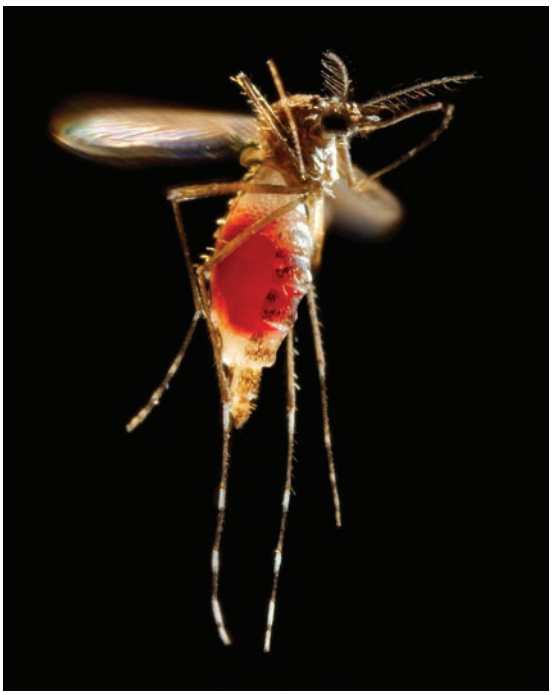


FIGURE 5.12

Mosquito after a blood meal

Source: CDC/Professor Frank Hadley Collins, Director, Center for Global Health and Infectious Diseases, University of Notre Dame.

Foetal and neonatal transmission

Viral infections in the mother can have serious repercussions for the foetus or neonate. These include teratogenic (mutational) effects causing premature delivery or congenital abnormalities, vertical transmission of infection to the neonate, and the acquisition of viral diseases during birth.

Teratogenic effects

Several viruses have been proven to cause serious damage to the unborn foetus. One of the earliest syndromes to be recognised was the effect of rubella (German measles) virus on the foetus during the first trimester of pregnancy (see Figure 5.13). The connection was first observed in Sydney in 1941, when a severe epidemic of German measles was followed by a very high incidence of congenital abnormalities. These included congenital heart defects, total or partial blindness, and growth or mental retardation. Work carried out by Sir Norman Gregg at the Royal Alexandra Hospital for Children in Camperdown, Sydney, correctly identified the rubella virus as the causative agent. A vaccine to prevent rubella is now available and is given routinely in combination with measles and mumps vaccine (MMR) to all children (male and female) at 12 months of age, and again at 4 years of age. Some recent cases of congenital rubella in Australia have occurred, mainly in unvaccinated immigrant women. Despite the negative publicity linking the MMR vaccine to autism, extensive research has proved that there is no causal link.

Another virus that has been shown to cross the placenta and cause congenital abnormalities is cytomegalovirus (CMV). The extent of damage caused by CMV depends on the state of gestation at which infection occurs and is worse if the mother acquires the infection for the first time during pregnancy. *In utero* infections may result in death or premature birth. Growth retardation and developmental malformations such as congenital heart defects, cataracts, deafness and central nervous system defects may also result. No vaccine is available for CMV. Despite a high level of immunity in the community from previous, mainly subclinical, exposure, CMV remains the most common cause of congenital malformations in Australia and other developed countries.

TABLE 5.4 **Major arboviral infections in Australia**

FAMILY	VIRUS	MOSQUITO VECTOR	REFERENCE
Flaviviridae	Murray Valley encephalitis (MVE)	*Culex annulirostris*	Chapter 20 p. 522
	Kunjin (KUN)	*Culex annulirostris*	Chapter 20 p. 522
	Dengue	*Aedes aegypti*	Chapter 19 p. 494
Togaviridae (Alphavirus)	Ross River (RR)	25 species, including *Aedes vigilax* and *Aedes camptorynchus*	Chapter 19 p. 494
	Barmah Forest (BAR)	*Culex annulirostris*	Chapter 19 p. 494

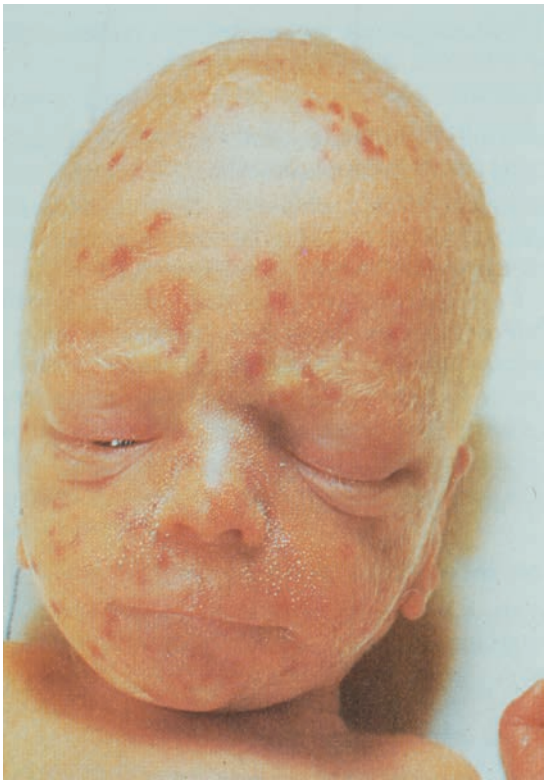


FIGURE 5.13

Newborn infant showing signs of congenital rubella syndrome

Source: Audiovisual Services, Royal Prince Alfred Hospital.

Recent studies have shown that there is also a slightly increased risk of congenital defects if the mother acquires chickenpox during pregnancy. Another viral infection, parvovirus B19, is generally a mild disease but there is a slight risk of damage to the foetus if contracted during the first half of pregnancy. Viral infection during pregnancy is suspected of being associated with the occurrence of other birth defects, and of causing severe foetal damage leading to abortion. However, not enough data are available at present to link specific viruses with specific outcomes.

Vertical transmission of infection to the neonate

The blood-borne viruses hepatitis B and HIV can be transmitted vertically to the neonate.

If the mother is infected with hepatitis B (HBV), there is a significant risk of the infection being passed on to the baby. It is not certain whether the virus crosses the placenta during the last few weeks of pregnancy, or whether it is acquired by the neonate when blood from mother and baby mix at the time of delivery. Probably both situations occur. If there is no intervention, the baby has an 80–90 per cent risk of being infected and this often leads to a chronic infection. In Australia, babies born to mothers who are known hepatitis B carriers are given anti-hepatitis B immunoglobulin antibodies at birth. The hepatitis B antibodies neutralise any live virus particles transmitted at, or just prior to, birth. The baby is then started on a course of hepatitis B vaccination to prevent development of the disease by future exposure to the maternal carrier. Usually breast feeding is not recommended in this situation. Babies treated this way have a very good chance of not acquiring hepatitis B, and of not suffering any ill effects. Since 2001, all babies born in Australia have been given free hepatitis B vaccination, beginning at birth.

Babies born to HIV-positive mothers are usually antibody-positive at birth because they are carrying IgG antibodies, which have been transferred across the placenta from the mother. It will be 6–12 months before the maternal antibodies disappear from the neonate and it is therefore not possible, during this period, to test specifically for neonatal antibodies to find out whether the baby is infected with the virus. Tests for the virus itself are useful in detecting HIV in the baby, but may not be conclusive as the virus may be 'hiding' inside a white blood cell or lymph node, rather than being free in the bloodstream. Breast feeding by HIV-infected mothers is usually not recommended.

Present data indicate that the risk of vertical mother-to-baby transmission in Western countries is less than 2 per cent if mothers are treated with combination (three-drug) antiviral therapy during pregnancy, particularly just before birth (parturition). The risk is influenced by the stage of illness and level of viraemia in the mother. Unfortunately, drug treatment is expensive and not readily available in developing countries, where there has been an explosion of HIV infection in the heterosexual community and as many as 50 per cent of babies born to HIV-positive mothers are infected.

The risk of vertical transmission for hepatitis C is considered to be very low, although additional data are still being collected.

Viral diseases acquired at birth

Viruses transmitted to the baby during the birth process may also pose a serious risk to the neonate. The most serious of these is herpes simplex. If the mother has an active genital herpes infection at the time of birth, the baby may acquire the virus during passage through the birth canal. Usually the eyes are infected first, but in severe cases **herpes encephalopathy**, viral infection of the brain, may result. This can be fatal. If the mother has had a long-established genital herpes infection, the baby will already have maternal IgG antibodies that will provide some protection. The greatest risk to the neonate is from a recently acquired active maternal infection. In such cases, lower segment caesarean section (LSCS) is often performed to avoid exposure of the baby to the virus during passage through the birth canal. Infected babies are treated with intravenous aciclovir, but relapses can occur even after treatment.

DIAGNOSIS OF VIRAL INFECTIONS

Most viral infections are diagnosed by signs and symptoms. Very often, a general feeling of malaise and slight fever is suddenly explained when a distinctive rash appears (e.g. chickenpox). Usually, by the time these symptoms appear in an acute infection, the body's immune system has already responded. Treatment of the patient is therefore largely symptomatic.

Sometimes, however, it is important to have an accurate diagnosis as early as possible. It may be necessary to determine the level of infectivity of the patient and the consequent risk of transmission to others. Blood samples or specimens from the affected tissues of the patient are taken, and various methods are used to detect and identify the virus. Significant developments in the diagnosis of viral infections have occurred in recent years, due to the development of techniques for nucleic acid amplification and identification. These are discussed in detail in Chapter 15.

A brief overview of the use of each technique is given here.

Immunofluorescence

If large numbers of viral particles are present in the specimen, they can often be detected by reaction with a fluorescent labelled antibody, followed by observation with a fluorescence microscope. This method can give a quick definitive answer in 2–3 hours, but is limited to viruses for which appropriate labelled antibodies are commercially available (see Figure 5.14).

Nucleic acid analysis

Tests for the presence of live viruses in serum or tissue may require the use of nucleic acid amplification and detection techniques (e.g. PCR) and DNA or RNA typing. The use of DNA or RNA probes (i.e. specific nucleotide sequences) can detect the viral nucleic acid sequences which correspond to the presence of viral particles in clinical specimens. For example, probes for the detection of the various types of human papillomavirus in cervical cells are available commercially. The use of these probes can confirm the presence or absence of the viral types (16,18) linked to cervical cancer in abnormal Pap smears. Patients who test positive for hepatitis C antibodies can be tested for HCV-RNA to find out whether the virus is present in their blood and to determine their level of infectivity.

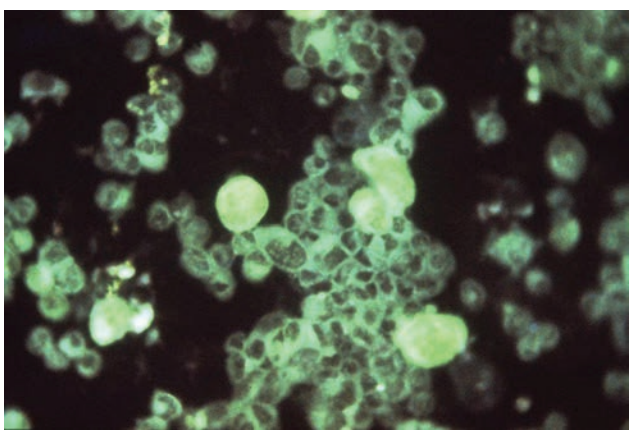


FIGURE 5.14

Detection of virus particles by immunofluorescence: cytomegalovirus in a sample of human embryonic lung tissue

Source: Photography courtesy of Dr A. Smithyman, Cellabs Pty Ltd, Sydney.

Serology

Serology is the detection of viral antigens and antibodies in body tissues. It requires the presence of a measurable level of antigen or antibody in serum. A blood sample is taken from the patient and tested for the presence of antibodies to the suspected virus. The presence of specific IgM antibodies generally indicates a recent infection, although in some cases (e.g. CMV) they can persist for months to years in serum. IgG antibodies indicate that the infection occurred at an earlier time. The method is limited by the time (several days) required after infection for the body to mount an immune response and produce detectable antibodies. It is mainly useful for the determination of a patient's immune status, in screening blood and tissue products, and in epidemiological studies. The most common method involves the use of EIA (enzyme immunoassay), an automated colour reaction.

Cell culture

Under certain conditions, viruses can be identified using the technique of cell culture (see below). When insufficient virus particles are present in the specimen to detect by immunofluorescence, a sample is usually inoculated into a cell culture and grown for 2–5 days. The cells are then observed for **cytopathic effects** (**CPE**) (see Figure 5.15). This method is slow and mainly used now for confirmation of other test results, for research, or if other identification methods have not been successful.

The cell culture can also be tested for the presence of a specific virus by immunological methods and molecular methods (see Chapter 15). These include the direct enzyme-linked immunosorbent assay (**ELISA**), which produces colour reactions linked to antibody-antigen reactions, and the immunofluorescent antibody method described above.

PRODUCTION OF VIRUSES IN THE LABORATORY

Culture of viruses in the laboratory is more difficult, expensive and time-consuming than the culture of bacteria,

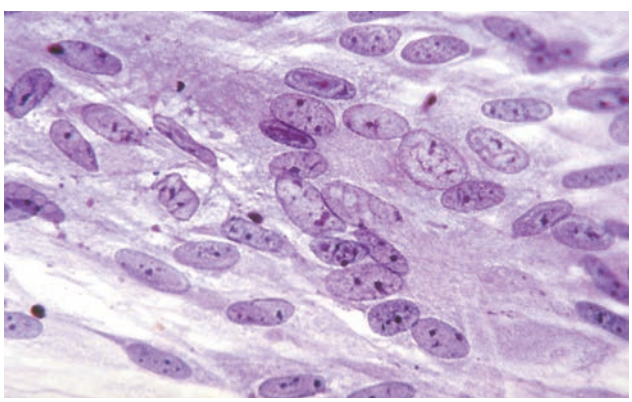


FIGURE 5.15

Cytopathic effects of viral infections in cell culture

This photomicrograph reveals the presence of multinucleated giant cells along with intranuclear inclusions in cell cultures inoculated with varicella virus. Magnified 500x.

Source: Department of Infectious Diseases, University of Sydney.

because viruses must be grown inside a living cell. This characteristic places severe limitations on the methods that can be used to cultivate viruses. In general, viruses are grown in the laboratory for one of the following reasons:

- diagnosis of infection
- research
- preparation of a vaccine.

The actual method employed depends on the number of viral particles required and the availability of suitable cell cultures. Early attempts to grow viruses relied on the use of animals or embryonated eggs. The inoculation of viral specimens into live animals is costly and in many cases inconclusive. It is used mainly for the primary isolation of 'new' viruses or for studies of *in vivo* pathogenesis or oncogenesis.

Growth of viruses in embryonated eggs is a convenient and cheap alternative to whole-animal experiments for viruses such as influenza. Several different membranes in the egg are able to support the growth of viruses. The effects of viral infection are measured by death of the embryo or production of typical lesions or other damage. Early vaccine preparations were made from viruses harvested from egg cultures. However, there were problems associated with this method as the vaccine sometimes contained residual egg proteins, which provoked an allergic response in some individuals. Most viral cultures are now carried out using the techniques of cell culture (tissue culture). Some vaccines (e.g. hepatitis B) are produced by genetic engineering (see page 77).

Cell cultures are preparations of animal (usually human) cells grown in a special cell-culture medium under strictly controlled aseptic conditions. The cells adhere to the walls of the flask and spread out to form a confluent monolayer. Viral specimens are inoculated into the culture and the effect of the virus on the host cell can be observed microscopically. The changes that occur in infected cells are called **cytopathic effects** and are usually typical of the infecting virus. These effects include:

- production of 'inclusion bodies'—granules in the cytoplasm of the infected cell
- fusion of several cells, forming giant, multi-nucleated or 'syncytial' cells
- transformation of the cell into a spindle-shaped cell that does not exhibit contact inhibition
- cell death.

Cell culture is the main method used in specialist virology laboratories. The cells are grown in special culture flasks, and may also be grown in small volumes in the wells of microtitre plates. Different dilutions of the virus preparation are inoculated into the wells to observe the cytopathic effects produced by the virus infection and to quantify the amount of virus present (see Figure 5.15 and Chapter 15). After the virus has been allowed to multiply in the cells, it may also be further identified by the ELISA test or by reaction with specific fluorescent antibodies, as described above.

PREVENTION OF VIRAL DISEASE

A good understanding of viral properties is necessary in order to stop transmission and to devise strategies to prevent and treat viral diseases. Vaccination is the most effective method of prevention.

Vaccination

The observation by Edward Jenner in the 18th century that milkmaids who had recovered from cowpox did not usually contract smallpox laid the basis for our modern vaccination programs. Vaccination (or immunisation) is the major weapon available against viral disease, and is the means whereby smallpox has been eradicated worldwide.

Vaccination involves the exposure of a person to a modified form of the pathogen in order to elicit an immune response without causing the disease. The aim is to produce antibodies and B lymphocyte and T lymphocyte memory cells specific to the pathogen, without producing any clinical symptoms of disease. These antibodies and memory cells protect the patient against subsequent exposure to the virulent form of the disease-causing agent. The mechanism of the immune response is discussed in Chapter 9.

It has been possible to derive attenuated (weakened) forms of many viruses for use in vaccines. Other vaccines consist of killed or inactivated viral preparations which retain their antigenic properties; that is, they are still able to stimulate an immune response. Recent developments in genetic engineering have seen the production of vaccines consisting of fragments of the virus produced by cloning a part of the viral DNA in a yeast or bacterial cell. Hepatitis B vaccine is one example of this type of vaccine. One of the major advantages (apart from the ease of production) is that there is no risk of causing the actual disease since the vaccine consists of only part of the viral coat.

Prevention of viral disease by mass immunisation campaigns is an important aspect of primary healthcare. Table 5.5 lists the viral diseases for which vaccines are currently available. Because of the ability of the influenza virus to mutate, the composition of the influenza vaccine has to be modified each season to provide adequate protection against the strains of the virus circulating in the community. Figure 5.16 illustrates the dramatic fall in the incidence of some important diseases since the introduction of vaccines.

It is theoretically possible to use effective vaccination campaigns to eradicate viral diseases such as measles and polio, for which humans are the only reservoir and which are caused by viruses that do not survive for long periods outside the human body. Smallpox has officially been eradicated worldwide. Polio has been eradicated from North America and the Western Pacific region, which includes Australia (see Spotlight box: Eradication of polio, Chapter 14, page 339 and Chapter 20, page 529). The campaign against measles has not been as successful, due partly to poor compliance with vaccination programs (see Chapter 14).

Inactivation of viruses

Viral infections are readily transmitted from person to person. There is great variability in the length of time that a virus particle (virion) can survive outside the host cell, as well as in the range of physical and chemical conditions it can withstand. In order to prevent transmission of viral

TABLE 5.5 **Viral diseases for which vaccines are available**

DISEASE	RECOMMENDED SCHEDULE IN AUSTRALIA
Chickenpox (Varicella: VZV)	Infants at 18 months. Recommended for healthcare workers and 'at risk' individuals.
Hepatitis A	Three doses: two 1 month apart, and a booster at any age.
Hepatitis B	Three doses: 1, 2, 6 months apart, can be given at any age. Now included in childhood immunisation schedule at birth, 2, 4, 6, and 12 months with a booster at 10–13 years.
Rotavirus	Three doses: 2, 4, 6 months.
Influenza	Different vaccine produced each year for prevalent strain. Recommended for elderly and 'at risk' patients.
Measles	MMR: two doses—12 months, 4 years.
Mumps	MMR: two doses—12 months, 4 years.
Rubella	MMR: two doses—12 months, 4 years. Booster may be required before becoming pregnant. Antibody titre should be checked.
Polio	Salk IPV: 2, 4, 6 months; boosters at 4 years.
Human papillomavirus (HPV)	12–13 years
Rabies	Usually only after exposure to rabies virus, or bat lyssavirus.
Yellow fever	Recommended when travelling to endemic countries.
Japanese encephalitis	Recommended when travelling to endemic countries.

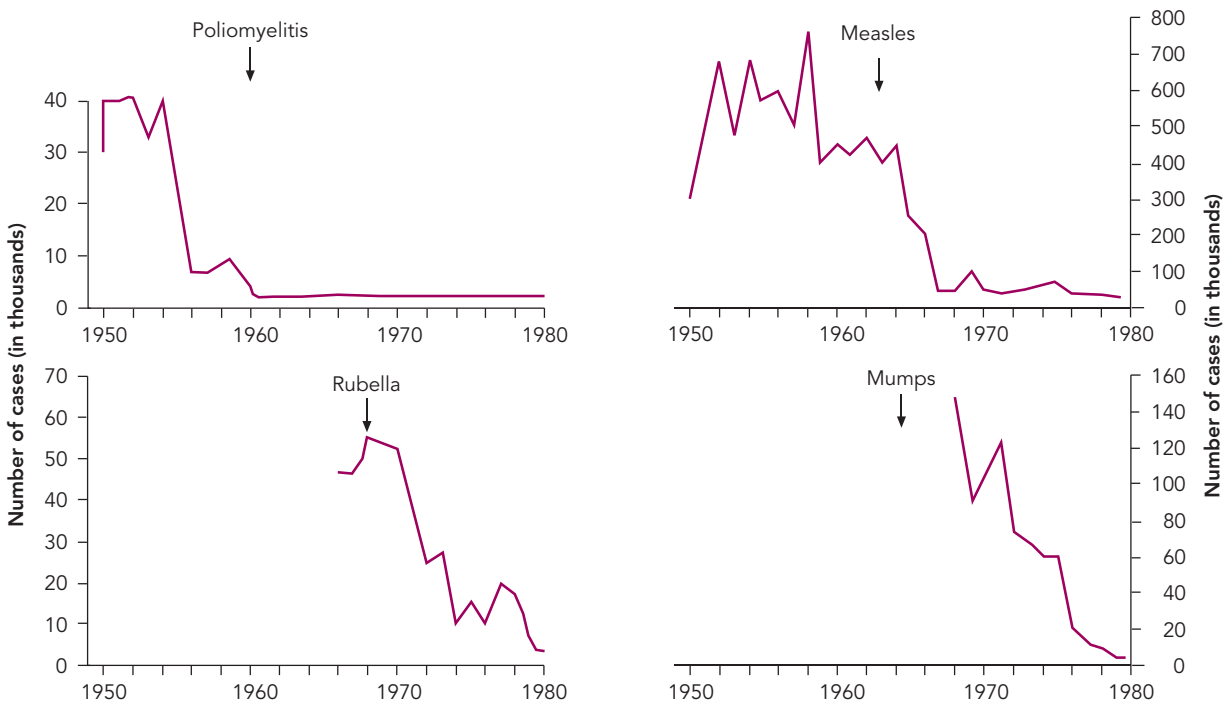


FIGURE 5.16

Effect of introduction of vaccines on incidence of viral diseases

The fall in incidence of poliomyelitis, measles, rubella and mumps in the United States following the introduction of vaccination against diseases (arrows). Inactivated poliovirus vaccine was introduced in 1954, live vaccines in 1963. The other three—for measles (introduced in 1963), mumps (1967) and rubella (1969)—are live vaccines.

Source: D.O. White and F. Fenner 1994, *Medical Virology*, 4th ed. (Academic Press). Reproduced with permission from Elsevier.

infections it is necessary to be familiar with methods that can be used to inactivate them.

- Most viruses are destroyed by heating at 60°C for 30 minutes, although some, such as the hepatitis B and papova viruses, can withstand much higher temperatures.
- Many viruses can survive for several days in the cold (4°C).

- Some viruses are stable in the presence of salt solutions and buffers, which means that the virus can persist in food or body substances for significant periods of time. Viruses are usually stable at neutral pH (pH 5–9), but most are destroyed by alkaline conditions. Some viruses, like the enteroviruses, are resistant to acid pH, which allows them to survive the acidity of the stomach.
- Disinfectants such as quaternary ammonium salts, organic iodine compounds and alcohols are not very effective against viruses. Higher concentrations of chlorine are needed to kill viruses than are required to kill bacteria. Viruses have been treated with the chemical formaldehyde in the preparation of some viral vaccines, such as the Salk polio vaccine, as the antigenic properties of the capsid proteins are retained but the virus is no longer infective.

Methods of sterilisation and disinfection are described further in Chapter 11.

TREATMENT OF VIRAL INFECTIONS

Antiviral drugs

Viral infections provoke a number of responses in the host, which are usually treated symptomatically—bed rest, fluids, analgesics. There are very few effective antiviral drugs available, and they are of most use in chronic infections.

An understanding of the methods of viral replication is important in order to appreciate the difficulties associated with finding suitable antiviral drugs. One of the basic requirements for a safe, effective drug is **selective toxicity**—that is, the ability to kill the pathogen without harming the host cell. Since viruses use the host cell machinery to replicate, any drug that inhibits or interferes with viral multiplication is also likely to have an adverse effect on the host cell. Most drugs that interfere with viral replication are too toxic to be used therapeutically. It is therefore necessary to identify steps in the replicative process that are unique to the virus, or to identify and target one of the few virus-specific enzyme reactions. This is why antibacterial drugs are not effective against viruses.

A limited number of antiviral drugs have been developed for herpes viruses, influenza viruses, HIV and hepatitis C.

At present, the most successful antiviral drugs are those that are analogues of the various nucleotide bases found in viral DNA—that is, they have a structure that is similar, but not identical, to the naturally occurring base. They can be incorporated by the viral enzymes into viral DNA, but the DNA is inactive and cannot be replicated. Other possible antiviral targets include the attachment site of the virus to the host cell, the translation of viral mRNA to viral proteins, and assembly and release of new virions.

Zidovudine (AZT), an analogue of thymine, was one of the first nucleotide analogues to be used; it is incorporated into the DNA of the AIDS virus by an enzyme unique to retroviruses, called **reverse transcriptase**. This enzyme is used by the virus to synthesise DNA from RNA. Incorporation of the analogue into the viral DNA gives rise to an inactive DNA which cannot be replicated and so prevents further viral replication. However, although zidovudine is readily incorporated into viral DNA by reverse transcriptase, it is also incorporated to some extent by DNA polymerase into normal cell DNA, causing severe side effects in the host, such as bone marrow depression. Other nucleotide analogues have also been used successfully.

Currently, the best results in the treatment of AIDS are being obtained with combination therapy using protease inhibitors as well as nucleotide analogues. This helps to minimise the problems of development of drug resistance.

Another useful drug is **aciclovir**, an analogue of guanosine. This compound is phosphorylated by thymidine kinase, an enzyme present only in herpes viruses, to produce an inactive nucleotide triphosphate that, when incorporated into viral DNA, interferes with DNA replication. Aciclovir has been used successfully for the treatment of a number of infections caused by the herpes family of viruses, including shingles and genital herpes.

Interferons are naturally occurring antiviral proteins that are produced by animal (host) cells in response to viral infection. There are several different interferons, commonly grouped into three classes: alpha interferon (α -IFN), beta interferon (β -IFN) and gamma interferon (γ -IFN). They appear to act by stimulating the synthesis of antiviral proteins which block the translation of viral messenger RNA into viral protein, thus preventing the further production and spread of virus after the initial infection has occurred. Interferons are host-cell specific, not virus-specific. There are, however, serious limitations in their therapeutic use since they have a very short half-life in the human body, and a number of toxic side effects. They have been used with some success in the treatment of chronic viral infections such as hairy cell leukaemia, hepatitis B and hepatitis C. Combination therapy of interferon plus ribavirin is being used with more success.

A major breakthrough in the development of antiviral drugs was the synthesis of compounds that specifically inhibit the neuraminidase enzyme of the influenza virus. This enzyme is involved in the release of new viral particles from infected cells. Two drugs are approved in Australia—**zanamivir**, which is administered by inhalation, and **oseltamivir**, which is taken orally. When given within the first 48 hours, use of these drugs can reduce the duration of the illness by 1–2 days.

Antiviral drugs are discussed in more detail in Chapter 12.

FUTURE DIRECTIONS IN VIRUS RESEARCH

In recent years a number of 'new' emerging viruses have been identified (see Chapter 1) and it has become apparent that there are many viruses yet to be discovered. Some of these may be responsible for presently unexplained illnesses. People in Africa were dying from 'wasting disease' long before HIV was identified as its cause, and 'transfusion hepatitis' was described long before it was shown to be caused by the hepatitis C virus. New techniques in molecular biology make it possible to identify a viral genome without isolating or culturing the virus itself, as has been the case for hepatitis C.

The ability to amplify fragments of viral RNA or DNA by the polymerase chain reaction (PCR) has far-reaching

consequences for diagnostic virology. The sensitivity of PCR means that it is easier to probe for the viral nucleic acid than to try to isolate the virus itself. The discovery of new viruses such as SARS, Hendra virus and bat lyssavirus indicates that there are probably many other still unidentified viruses.

Human metapneumovirus (**HMPV**) was only identified in respiratory secretions in 2001 but has since been isolated from stored clinical samples from as early as 1953. It is now recognised that, like respiratory syncytial virus (RSV), it is a significant cause of severe respiratory infections worldwide, especially in children. TTV (transfusion-transmitted virus) is another virus that was cloned from blood in 1997, but its effect (if any) on the human body is not yet clear.

Genetic analysis of a virus can yield information about its antigenic structure that may be useful in determining public health programs. For example, viruses that have stable antigens on their surface (e.g. measles and polio) are more easily controlled by vaccination (as the antibodies required for neutralisation also stay the same). Other viruses that exist as many genetic types (cold viruses, rhinoviruses) or change their outer coat frequently (influenza A) are difficult to control by vaccination. It is also difficult to develop a vaccine for viruses that persist and mutate in the host (e.g. HIV) because the immune response that is induced is effective only against the original virus structure.

Despite our detailed understanding of viral replication, we still have a lot to learn about how viruses cause disease. Cytopathic effects, leading to the destruction of infected cells, are obvious consequences, but there are more subtle physiological changes that appear to be due to non-cytocidal viruses. The ability of some viruses to evade host defences, to undergo specific mutations, or to lie dormant and be reactivated under certain conditions has far-reaching implications for human health in general. Persistent viral infections, such as measles and polio, which produce symptoms many years after the patient has apparently fully recovered from the disease, pose a particular challenge. The World Health Organization is mounting a worldwide campaign aimed at eradicating both these diseases. This requires education and the cooperation of both individuals and governments.

The significance of the role that viruses play in multifactorial diseases such as hepatocellular carcinoma has yet to be fully elucidated. One of the most interesting aspects of viral infection that needs further study is the involvement of viruses in autoimmune reactions and the possible viral suppression of the immune system with consequent reactivation of, or superinfection by, other microbial species.

SUMMARY

CHARACTERISTICS OF VIRUSES
- Viruses are the smallest type of infectious particle.
- Each virus possesses only one type of nucleic acid—RNA or DNA—enclosed in a capsid made up of capsomeres.
- Capsids occur in distinctive shapes (e.g. icosahedral, helical).
- Some viruses are enclosed by an envelope that helps the virus attach to the host cell.
- Viruses use the host cell enzymes for synthesis.

STRUCTURE OF VIRUSES
- Viruses are classified into groups or families based on the type of nucleic acid they contain, their shape, structure and method of replication.

HOST RANGE AND SPECIFICITY
- Viruses are specific for the type of organism and the type of cell they infect.
- This specificity depends on the presence of a suitable receptor for viral attachment to the susceptible cell.

VIRAL REPLICATION
- Viruses always replicate inside another living cell.
- Bacterial viruses called bacteriophage infect bacteria, resulting in lysis, lysogeny or specialised transduction.
- Viral infection of animal (human) cells involves six steps: adsorption, penetration, uncoating, synthesis, assembly and release.
- The method of replication inside the host cell depends on the type of nucleic acid the virus contains.

PATHOGENESIS OF VIRAL INFECTIONS IN HUMANS
- Many common human diseases are caused by viruses.

HOST RESPONSE TO VIRAL INFECTION
- Viral infections usually activate the immune system with the production of cytokines, T cells and B cells that produce antibodies to protect the host against subsequent infection.
- The susceptibility of a person to viral infection is influenced by age and immune status

VIRAL EVASION MECHANISMS
- Viruses survive in the body using various evasion strategies such as hiding in infected cells and mutation.

OUTCOMES OF VIRAL INFECTION
- Viral infections may be acute or subclinical.

PERSISTANCE OF VIRAL INFECTIONS
- Some viral infections persist in the human host and may be described as chronic, latent, slow or oncogenic.

TRANSMISSION OF VIRAL DISEASES
- Viruses are shed mainly from the area where the infection is localised.
- Control of the spread of virus disease is helped by an understanding of the method of transmission of the virus.
- Transmission may be via the airborne route as aerosol droplets, from vesicles or pustules, via the faecal–oral route, or in blood and other body fluids.
- A system of infection control procedures for the handling of blood and body substances has been introduced to prevent the transmission of blood-borne pathogens.

- Arboviruses are transmitted by insects, the most common of which is the mosquito.
- Some viruses are transferred vertically from mother to baby, transmission occurring *in utero*, during birth or through breast milk.
- Some viruses are teratogenic, causing congenital defects in the foetus when the mother contracts the infection during pregnancy.

DIAGNOSIS OF VIRAL INFECTIONS

- Viral diseases are diagnosed mainly by signs and symptoms.
- The presence of virus particles in tissues can often be detected in the early stages of infection by reaction with fluorescent antibodies.
- Viruses can be grown in cell culture which is observed for cytopathic changes.
- The presence of antibodies to the virus in the patient's blood indicates infection.
- Some viruses are identified by DNA or RNA typing, using nucleic acid probes.

PRODUCTION OF VIRUSES IN THE LABORATORY

- Viruses may be grown in the laboratory in animals, embryonated eggs or cell culture.

PREVENTION OF VIRAL DISEASE

- Vaccines are available for many serious viral diseases.
- Effective mass vaccination campaigns can lead to the eradication of some viral diseases.
- The spread of viruses can be prevented by destruction of live viral particles in various ways.
- Most viruses are destroyed by heat, detergents and chlorine.
- Treatment of viruses with formaldehyde destroys their infectivity but leaves their antigenicity intact.

TREATMENT OF VIRAL INFECTIONS

- It is difficult to find antiviral drugs that do not also harm the host cell.
- The most successful antiviral drugs are analogues of DNA bases which react with specific viral enzymes.
- Interferons are naturally occurring antiviral compounds.

STUDY QUESTIONS

1. Describe the structure of a virus.
2. What type of nucleic acid occurs in viruses?
3. Where do viruses replicate?
4. What is meant by 'tissue tropism'?
5. What is the difference between a capsomere and a capsid?
6. How does the newly synthesised virus acquire its envelope?
7. What kind of infectious particle is a prion?
8. What is a bacteriophage?
9. Differentiate between 'lysis' and 'lysogeny' when applied to viral infection of bacterial cells.
10. List the six steps involved in the replication of a virus in an animal cell.
11. What is meant by a 'subclinical infection'?
12. How do viruses cause latent infections?
13. Which viruses are transmitted mainly in body fluids?
14. What is meant by 'viral shedding'? When and where does it occur?
15. What is meant by 'an arbovirus'? Give examples.
16. Which viruses have teratogenic effects?
17. How are viral diseases diagnosed?
18. What are interferons?
19. What is meant by an 'attenuated' virus?
20. What is the most effective way of preventing viral diseases?

TEST YOUR UNDERSTANDING

1. How does the replication of the retroviruses differ from other RNA-containing viruses?
2. How is it possible to determine whether a person is a carrier or has had a subclinical viral infection?
3. What are the major advantages of being vaccinated against measles rather than acquiring the disease naturally?
4. How are viruses able to transform normal cells?
5. Why is it difficult to design antiviral drugs?

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CHAPTER

6

Eucaryotic microorganisms: fungi, protozoa and multicellular parasites

CHAPTER FOCUS

- What are the characteristics of eucaryotic organisms?
- What are the main properties of fungi?
- Which are the most important infections caused by fungi?
- What are the main characteristics of protozoa?
- How do parasitic diseases caused by protozoa and helminths differ from other infectious diseases?
- What factors affect a patient's susceptibility to fungal and protozoal diseases?
- What role do insects play in the spread of infectious diseases?

INTRODUCTION

Eucaryotic organisms include all organisms other than bacteria. The group comprises single-celled microorganisms (protozoa, yeasts and unicellular algae), multicellular organisms (some algae and fungi), and the more complex higher organisms, including insects, worms, plants and animals. They differ from procaryotic organisms (bacteria) in their structure and method of reproduction (see Table 1.1, Chapter 1, page 6).

Microscopic examination reveals that eucaryotic cells are larger, and structurally more complex, than procaryotic cells. They contain a defined nucleus enclosed by a membrane, as well as other **organelles**—specialised structures responsible for carrying out specific functions in the cell.

Reproduction in eucaryotic cells can be sexual or asexual. **Asexual reproduction** occurs in protozoa and some algae and fungi, and in normal cell division during growth of higher organisms. In this process, termed **mitosis**, the chromosomes replicate and two daughter nuclei are formed in a complex but organised process. The new nuclei move to opposite ends of the cell and the cell then splits into two identical daughter cells with identical sets of chromosomes.

Sexual reproduction in eucaryotic organisms involves the formation of gametes (sex cells) by the parent cells. Fusion of the gametes produces a new cell which has received half its genetic material (chromosomes) from each parent. The new cell therefore has some, but not all, characteristics of each parent. The cell division process involved in gamete production is termed **meiosis**. Meiotic division and fusion of gametes is then followed by mitosis. Sexual reproduction allows for mixing of genetic material (i.e. characteristics) to occur and produces genetic diversity in the offspring.

A number of diseases are caused by eucaryotic organisms that are either microscopic (e.g. fungal spores, yeasts and protozoa) or have a microscopic stage in their life cycle (e.g. some fungi, worms and other parasites). In general, algae do not cause disease in humans although some produce toxins that can have serious effects if ingested.

Protozoal diseases such as malaria and other parasitic diseases are responsible for millions of deaths annually, mainly in developing countries where the standards of healthcare and hygiene are lower than in developed counties.

Some eucaryotic microorganisms cause only minor diseases in healthy individuals, but can have serious effects on a person whose immune system is compromised in some way. They are then referred to as opportunistic pathogens. **Opportunistic infections** are defined as infections caused by microorganisms that would not normally cause disease in individuals whose immune system is intact, but may do so in people with altered defence mechanisms (see Chapter 7). Opportunistic infections caused by protozoa or fungi are a major cause of morbidity and mortality in AIDS patients.

Eucaryotic microorganisms have many similar properties to their host (human) cells, which means that it is often difficult to find drugs that can destroy them without having significant side effects on the host. This is of special importance in the treatment of systemic infections, when the infection is spread throughout the body and various body organs. In these cases, administration of an antimicrobial drug may mean that susceptible host cells are also damaged by the drug.

In this chapter we look at the properties of eucaryotic microorganisms and discuss briefly the diseases they cause and the implications for healthcare.

FUNGI

Characteristics of fungi

Fungi are a large, diverse group of eucaryotic organisms that are widely distributed in Nature. The study of fungi is called **mycology**. They exhibit a range of morphology (appearance). Fungi comprise two major groups—yeasts and moulds.

There are thousands of different types of fungi, ranging from microscopic single-celled yeasts to multicellular, filamentous moulds and large fleshy moulds, or mushrooms. Fungal cells are surrounded by a cell wall, consisting of carbohydrate, polysaccharides and lipids, and this determines their shape. They are distinguished from plants and algae in that they do not contain chlorophyll and cannot carry out photosynthesis. They differ from animal cells because they have cell walls.

Most fungi can grow under conditions where bacteria are not able to thrive. For example, many moulds grow on substances with a very low moisture content (e.g. bread and cereals) and are resistant to extremes of osmotic pressure (e.g. they are able to grow in high sugar or salt concentrations). Many prefer an acid pH and can tolerate cold conditions (e.g. the household refrigerator). They are described as **heterotrophic**—that is, they require an organic source of carbon for growth. Many of them are saprophytes, feeding off dead, woody plant materials that are not readily decomposed by bacteria. Thus, they play an important role in the decomposition of organic matter. However, fungal diseases of crops also cause important economic losses in agriculture. In the last decade the incidence of fungal diseases of crops such as potatoes, bananas, cotton and grapes has increased, partly due to globalisation and the spread of fungal spores.

Many fungi are beneficial to humans. Some species are a source of antibiotics (e.g. penicillin and cephalosporin) and other drugs (e.g. the immunosuppressive drug cyclosporin). Yeasts are important in the food industry for making bread, wine, beer and soy sauce. Moulds are used to give special flavour to some cheeses. The large fleshy mushrooms are a

source of food. However, fungi also account for a significant amount of food spoilage.

In this section we will focus on fungal diseases that affect humans.

Types of fungi

Yeasts

Yeasts are unicellular organisms, usually spherical to oval in shape and bounded by a cell wall. They are slightly larger than bacteria, varying from about 3 to 15 μm, and contain various subcellular organelles. Yeasts occur widely in the environment and are found on the surfaces of plants and fruit. They are able to ferment sugars with the production of alcohol. Strains of the yeast *Saccharomyces cerevisiae* are used in bread making and in wine and beer production. *S. cerevisiae* has also been used by molecular biologists for the production of genetically engineered products, such as the vaccine for hepatitis B (see Chapter 4). The yeast *Candida albicans* occurs as part of the normal flora in humans.

Reproduction in yeasts is mainly asexual, by a process called *budding*. A small outgrowth, or bud, appears on the parent cell; it gradually enlarges and then separates, forming a new daughter cell, which then rapidly increases in size (Figure 6.1). Some non-pathogenic yeasts exhibit sexual reproduction, in which two haploid cells fuse to form a zygote, followed by the production of spores.

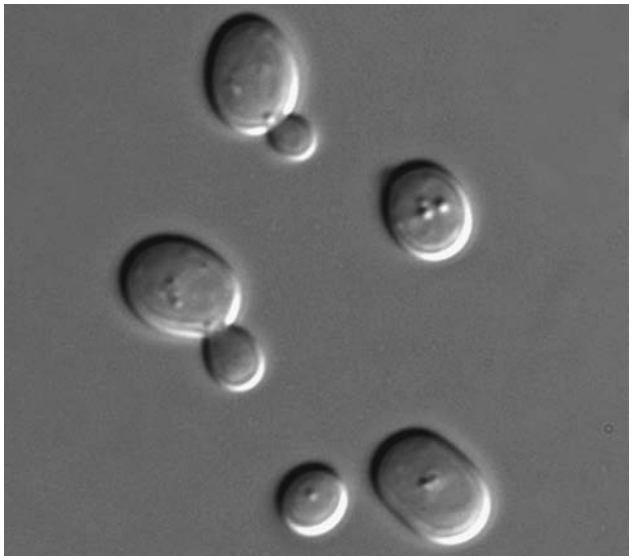


FIGURE 6.1

Yeast reproducing by budding

Circular scars can be seen on the surface of the larger cell, representing sites of previous budding.

Source: Masur on Wikimedia.

Moulds

Moulds are filamentous fungi that are capable of growth in many different habitats. They are commonly found growing on stale bread, cheese, cereals and vegetables. They consist of long filaments or **hyphae** (singular: hypha) which grow by extending the terminal cell at the tip of the filament. As they grow, the hyphae branch and form a dense mat of filaments called a **mycelium**, which is usually visible to the naked eye. The cells of this vegetative structure often contain more than one nucleus. After the dense mycelial mat has formed, **aerial hyphae** are produced and extend up into the air above the mycelial mat.

The most common method of reproduction in moulds is by the production of asexual spores in specialised structures on the ends or off the sides of these aerial hyphae. Asexual spores are produced by mitotic division of the parent cell. There are two main types (see Figure 6.2).

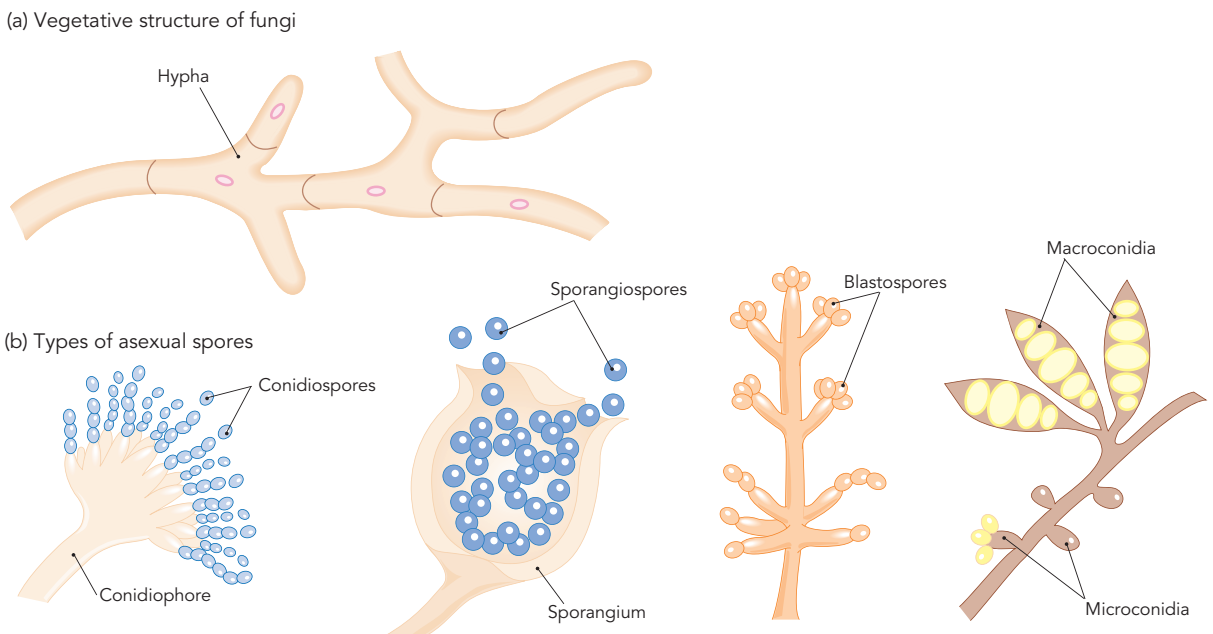


FIGURE 6.2

Structure of filamentous fungi

1. **Sporangiospores** are produced inside a sac, or **sporangium**, on the tip of a stalk, or **sporangiophore**. These spores are released when the sporangium ruptures.
2. **Conidia** (**conidiospores**) are not enclosed in a sac. They develop by being pinched off from the tip of an aerial hypha. Conidiospores are the most common type of asexual spore. They are usually pigmented, ranging in colour from blue or green to black or red, so the growing fungus changes in appearance from a white mat to its own particular colour with a dusty-coloured surface.

The structure of the spore-bearing hyphae is distinctive for each mould and is useful for identification. Their production is essential for survival. They are dispersed on air currents and carried to a new site where they germinate and grow into new hyphae. Some typical moulds are illustrated in Figure 6.3.

Mushrooms are filamentous fungi in which the aerial hyphae form a fleshy structure called a fruiting body. During most of its life, the mushroom exists as a mycelium of hyphae in the soil; when conditions are favourable, a fruiting body or 'mushroom' is formed. Spores are produced on the coloured gills underneath the cap, and are dispersed when the mushroom matures and dries. The various members of the mushroom family are an important food source.

Fungal infections

Although many fungi are responsible for plant infections, there are only about 200 species of fungi that actually cause disease in humans. Very few are truly pathogenic. The fungi that cause human disease belong to two main groups: (1) the

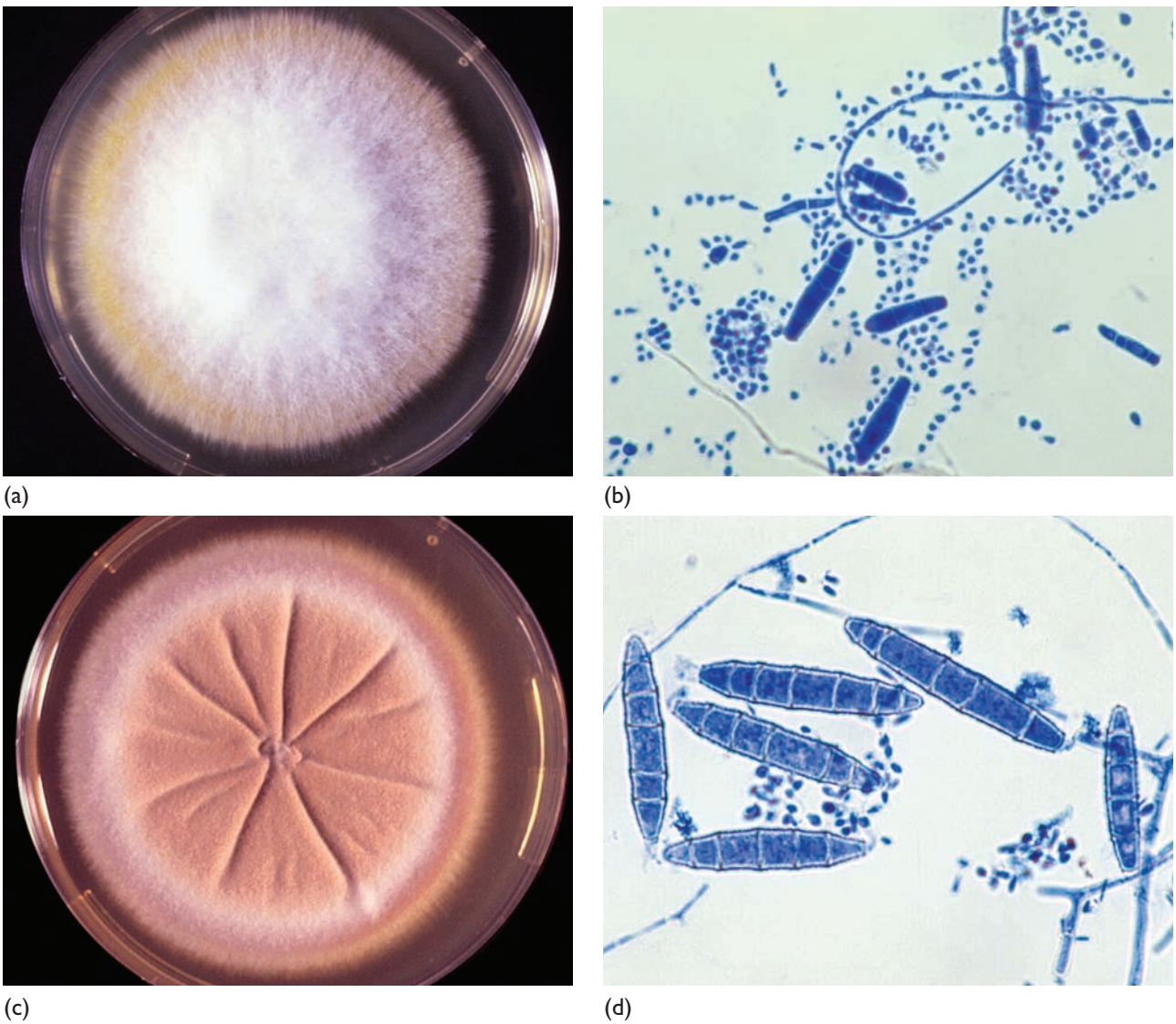


FIGURE 6.3

Structure of moulds

(a) Distinctive appearance on agar plate of dermatophyte; (b) microscopic appearance of spores of Trichophyton *mentagrophytes; (c) growth of* Microsporum cookei *on agar; (d) microscopic appearance of spores of* M. cookei.

Source: Published with permission Professor David Ellis, University of Adelaide.

yeasts, and (2) the **filamentous fungi**. Most fungal infections are cutaneous, affecting only the upper layers of the skin, or mucosa, and do not cause severe illness. However, when fungal infections become systemic, spreading throughout the body, they can cause life-threatening conditions.

For many years, infections caused by fungi were regarded as a nuisance rather than life-threatening. More recently, however, fungal infections have become increasingly important, especially when they occur in patients with serious underlying diseases or compromised host defences. Such people are not necessarily confined to hospital. Many of them live in the community. They include transplant patients who are being treated with immunosuppressive drugs, patients with leukaemia, other cancer patients and diabetics, as well as people suffering from immune disorders such as AIDS. All these individuals are likely to suffer severe morbidity (illness) if they contract a fungal infection, even though it may not be serious in a person with a competent immune system.

An infection caused by a fungus is called a **mycosis**. Depending on its location in the body, it may be classified as **superficial**, **cutaneous**, **subcutaneous** or **systemic**. Most superficial and cutaneous infections are not life-threatening, although they are often resistant to treatment and tend to persist for long periods with associated inflammation and discomfort. Some examples are given here—a full description can be found in Unit Four of this book under the different body systems.

Superficial mycoses

The most common of the superficial mycoses is pityriasis versicolor (**tinea versicolor**), caused by the fungus *Malassezia furfur* (see Figure 6.4). It is a mild infection of the skin and produces lesions that range from white, or non-pigmented (usually seen in darker-skinned people—e.g. in New Guinea it is colloquially called 'white spot'), through to brown, which is more common in fair-skinned people. The lesions are purely cosmetic. No inflammatory response is involved and the infection responds to topical antifungal treatment, such as selenium.

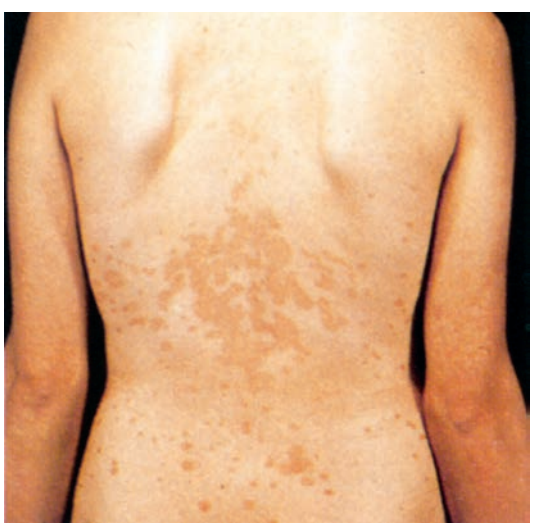


FIGURE 6.4

Pityriasis versicolor

Distinctive rash caused by the dermatophyte Malassezia furfur.

Source: © Royal Prince Alfred Hospital, Audio Visual Services.

Cutaneous mycoses

Cutaneous mycoses comprise the familiar skin infections commonly referred to as **tinea** or **ringworm** (see also Chapter 16). They are caused by fungi called **dermatophytes**, which invade 'dead' keratinised tissue (skin, hair and nails) using keratin as a nutrient source. The most important of these are organisms belonging to the genera *Microsporum*, *Trichophyton* and *Epidermophyton*.

Cutaneous fungal infections are transmitted by person-to-person contact or by the shedding of infected skin scales or hair clippings. Tinea infections can occur on any part of the body and are named accordingly.

- **Tinea pedis** (**athlete's foot**) is the most common dermatophyte infection. Usually, the skin between the toes becomes infected, followed by the development of small vesicles that burst and cause peeling and cracking of the skin (Figure 6.5). *Tinea pedis* is frequently contracted from fungal cells present in communal showers and change rooms.
- **Tinea corporis** may occur anywhere on the body. The lesion usually consists of a central scaly patch surrounded by a circular border of vesicles that are red and inflamed, giving the appearance of a ring—hence the name 'ringworm' (see Figure 6.6).
- **Tinea cruris**—'jock itch'—occurs in the groin.
- **Tinea capitis** is the name given to infections on the scalp. These may also involve the roots of the hair.

Subcutaneous mycoses

Subcutaneous infections occur when a fungus penetrates beneath the skin and establishes an infection. They are

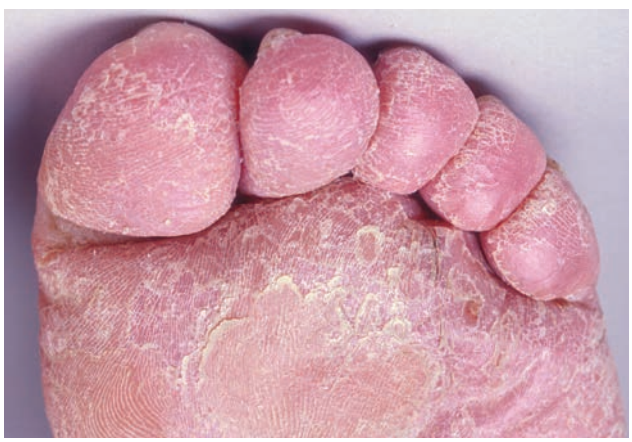


FIGURE 6.5

Tinea pedis showing severe scaling

*Ringworm of the foot, or athlete's foot (*Tinea pedis*). Moisture between the toes favours fungal infections.*

Source: © The University of Adelaide. Reproduced with permission.

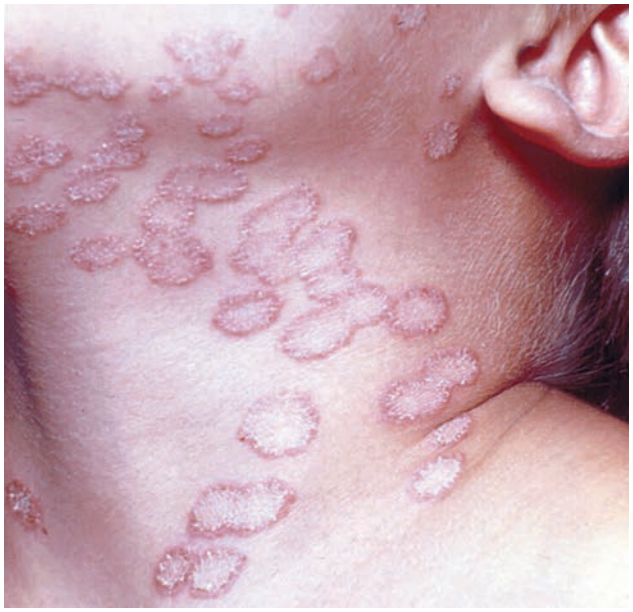


FIGURE 6.6

Tinea corporis, or ringworm

The infection has circular scaly lesions with distinct raised erythmatous borders.

Source: © The University of Adelaide. Reproduced with permission.

usually caused by soil or plant microorganisms that are introduced when the skin is broken or damaged, such as when a thorn or splinter penetrates the skin while gardening. Many of the fungi associated with plants cause this type of infection—for example, *Sporothrix schenckii*, which lives on wood and plants and produces a chronic granulomatous infection that can spread along the lymphatic system.

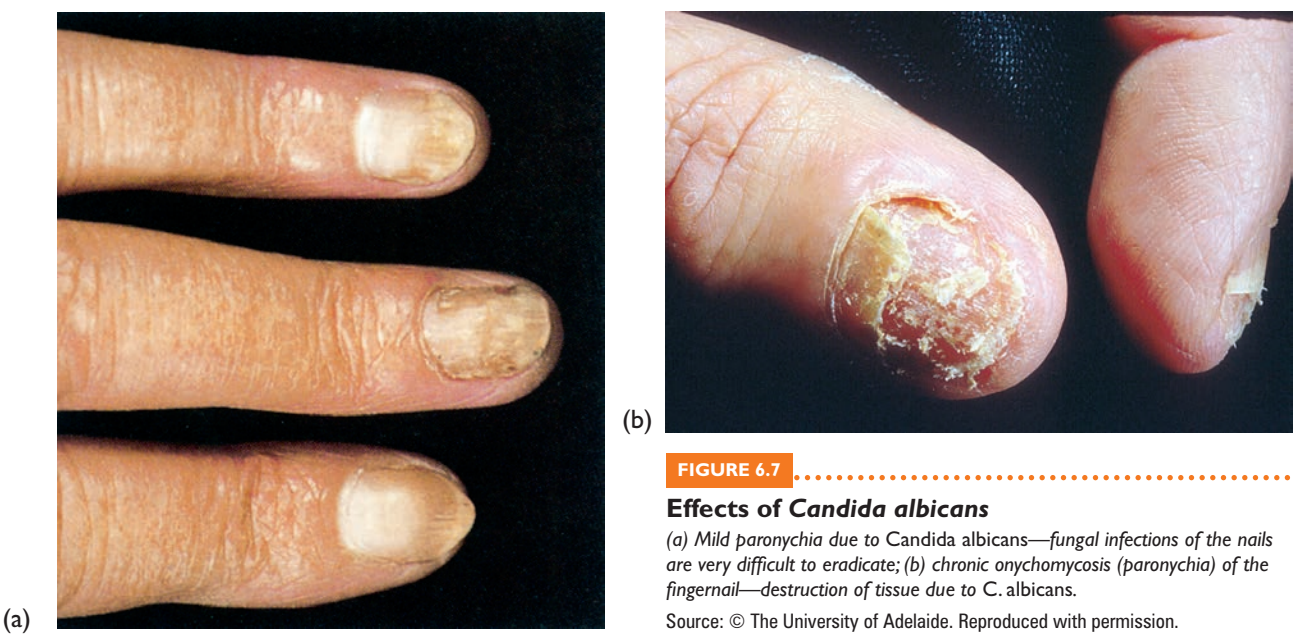
Paronychia, or **onychomycosis**, is an infection that occurs under the fingernail or toenail. It is caused by any of the soil or plant fungi, but can also be caused by the yeast *Candida albicans* (Figure 6.7). Subcutaneous mycoses are difficult to treat and can persist for long periods.

Systemic mycoses

Systemic (deep) fungal infections may occur in healthy individuals when they are invaded by pathogenic fungi. Immunocompromised patients are particularly susceptible to opportunistic fungi (see below). Very few fungi are truly pathogenic; that is, very few will cause an infection in an individual with normal immune defences. Table 6.1 contains a list of true pathogenic fungi that can give rise to systemic infections. They are predominantly dimorphic, free-living soil organisms and tend to be restricted to defined geographical habitats. These fungi are a common source of infection in the United States. They are rarely seen in Australia, with the exception of *Histoplasma*, a few cases of which have been reported.

In endemic areas these pathogenic fungi are usually introduced into the body by inhalation of spores. In the majority of cases the result is a mild lung infection. In a small percentage of cases, the fungus may be disseminated (spread) through the body, affecting other body organs and progressing to a full-blown, sometimes fatal, disease. Patients' ability to withstand invasion by these fungi depends on the integrity of their cell-mediated immune response (see Chapter 9). Although these fungi may infect any individual exposed to them, they have much more serious consequences in immunosuppressed patients and are commonly found as opportunistic infections in AIDS patients in the United States.

Apart from the pathogens described above, the major cause of systemic mycoses is opportunistic fungi, which occur mainly in immunodeficient patients. Table 6.1 also lists the opportunistic infections that can become systemic.



(a) (b)

FIGURE 6.7

Effects of *Candida albicans*

(a) Mild paronychia due to Candida albicans*—fungal infections of the nails are very difficult to eradicate; (b) chronic onychomycosis (paronychia) of the fingernail—destruction of tissue due to* C. albicans.

Source: © The University of Adelaide. Reproduced with permission.

TABLE 6.1 **Systemic fungal infections**

ORGANISM	DISEASE	DISTRIBUTION/HABITAT
Pathogenic fungi		
Blastomyces dermatitidis	Blastomycosis	North America
Coccidioides immitis	Coccidioidomycosis	Soil; south-west United States, Mexico
Histoplasma capsulatum	Histoplasmosis	Central and eastern United States, South America, Africa
Paracoccidioides brasiliensis	Paracoccidioidomycosis	Soil; Latin America
Opportunistic fungi		
Candida spp.	Candidiasis	Normal human flora
Aspergillus spp.	Aspergillosis	Ubiquitous; soil, grains
Cryptococcus neoformans	Cryptococcal pneumonia Cryptococcal meningitis	Birds
Rhizopus, Mucor	Zygomycosis	Soil, plants
Scedosporium spp.		Environment, dust

Opportunistic fungal infections

Candida infections

A number of fungi are opportunistic pathogens; that is, they cause disease only when the host defences are weakened. Among these, the yeast *Candida albicans* and related *Candida* species are the most common.

A number of pathogenic strains exhibit **dimorphism**—that is, the ability to grow in two different forms. Dimorphic fungi are usually filamentous at room temperature but grow as yeast cells in the body or when incubated in the laboratory at 35°C on an enriched medium such as blood agar. This property allows them to thrive at body temperatures which would normally be too high for fungal growth.

C. albicans, the organism responsible for thrush, exhibits several forms of growth at 28°C and also in the host tissues. At 28°C it may exhibit a single-celled budding yeast form, or produce rudimentary filaments called pseudohyphae, or even true **hyphae**. The production of pseudohyphae appears to aid in the invasion of the mucocutaneous host tissues and indicates a pathogenic, rather than commensal, role for the organism (see Figure 6.8).

Candida is a **commensal** of human mucosal surfaces, especially the mouth, vagina and intestinal tract, but may become pathogenic when the body's defence mechanisms are impaired or when the balance of microbial flora is disrupted.

Candida infections may be either **mucocutaneous** or **systemic**. **Thrush** is the name commonly given to mucocutaneous infections of the mouth or vagina. Outbreaks of thrush are usually indicative of a local or systemic weakness in the immune system. A number of factors can contribute to the proliferation of *Candida* and production of disease (candidiasis). For example, oral thrush is frequently seen in neonates (especially low birth-weight infants) where the immune system is immature and the population of protective normal flora has not yet been established. Oral thrush is commonly associated with the use of corticosteroid puffers for asthma. Thrush often occurs after extended treatment with broad spectrum antibiotics, which alters the composition of the normal bacterial flora.

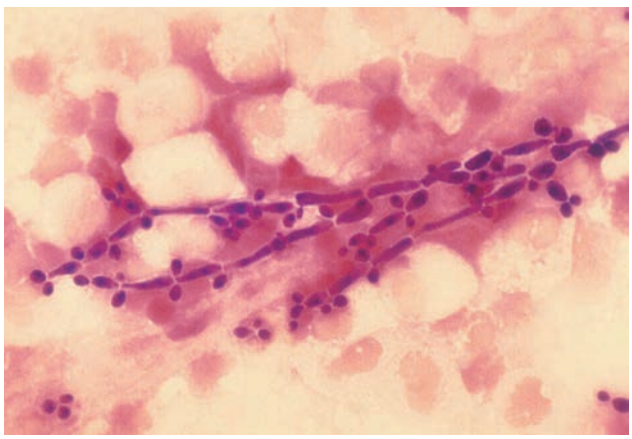


FIGURE 6.8

***Candida albicans* in tissue phase showing blastoconidia budding from pseudohyphae**

Chronic mucocutaneous candidiasis (**CMC**), which presents first in childhood but is also seen in adults, is usually an indication of an underlying deficiency in cellular immunity (see Figure 6.9).

Vaginal candidiasis in adult females is often associated with diabetes, pregnancy or the use of the contraceptive pill. Alterations in the body's physiological state, such as hormonal imbalance or stress, appear to favour the growth of the yeast cells. Prolonged antibiotic therapy can also destroy the normal flora in the vagina. Species of *Lactobacillus*, a normal inhabitant of the vagina, help to maintain an acid environment, limiting the growth of *Candida*, which prefers a neutral pH (pH 7). Destruction of the lactobacilli by prolonged broad spectrum antibiotic therapy allows the pH to rise, and an overgrowth or **superinfection** of *Candida* may result.

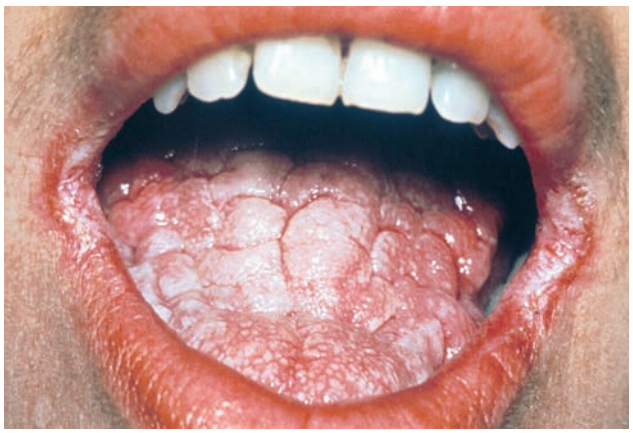


FIGURE 6.9

Chronic oral mucocutaneous candidiasis of tongue and mouth in adult with underlying immunodeficiency

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Candida is often responsible for skin infections in obese or diabetic patients. The yeast flourishes in the warm moist parts of the body (axillae, skin folds, under the breasts in females), causing inflammation and red, weeping vesicles. It can also be a cause of skin infections in elderly patients, who may have problems with hygiene and adequate care. Nappy rash in babies due to the constant moisture on the skin can also be caused by *Candida* (see Figure 6.10).

Mucocutaneous candidiasis can be treated with topical antifungal preparations, although some species (e.g. *Candida glabrata*) are exhibiting increased drug resistance.

Systemic candidiasis (**candidaemia**) is an invasive fungal infection that may present as a healthcare-associated infection (HCAI). It generally occurs in association with another severe underlying disease such as leukaemia or other cancer, and has a mortality rate as high as 40 per cent. The incidence of systemic infection is also high in patients subjected to traumatic medical procedures, such as organ transplantation with consequent immunosuppressive therapy. Invasive procedures, such as intravenous therapy with **total parenteral nutrition** (**TPN**), or **endotracheal intubation**, increase the risk of systemic infections. *Candida* infections are frequently associated with central lines and Hickman catheters. Severely compromised babies in intensive care may develop systemic *Candida* infections, often with a significant mortality rate.

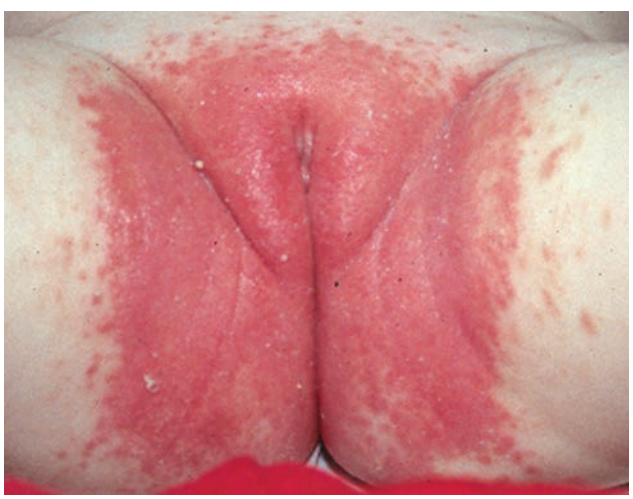


FIGURE 6.10

Nappy rash due to *Candida albicans*

Source: Dr Gayle Fischer.

The ability of *Candida* to develop into a systemic infection is related to the immune status of the patient. *Candida* typically grows in the extracellular spaces and is subject to phagocytosis by neutrophils and macrophages. **Neutropenia** (a deficiency of neutrophils) appears to render a patient more susceptible to systemic *Candida* invasion. However, *Candida* infections also occur in association with a reduction in T cells and the cell-mediated immune response (see Chapter 9). Mucocutaneous candidiasis is commonly seen in patients with the human immunodeficiency virus (HIV positive), but it does not appear to be readily disseminated in patients who have progressed to AIDS.

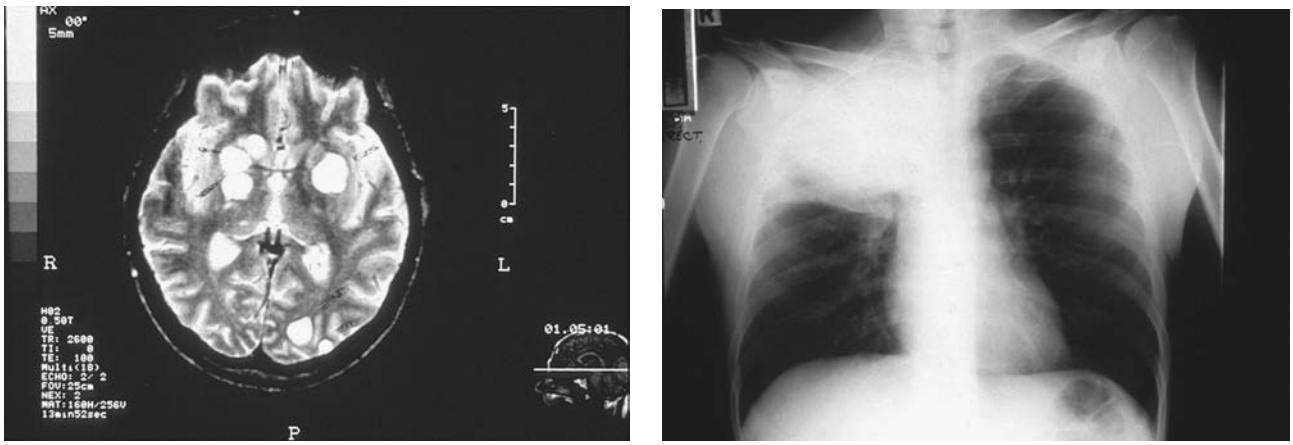
Cryptococcal infections

Cryptococcal infections are caused by *Cryptococcus neoformans* var. *neoformans*, an encapsulated yeast found in the excreta of pigeons and other birds in most parts of the world. The organism enters the body via the respiratory tract and is responsible for causing a mild, largely asymptomatic pneumonitis in humans. However, in immunosuppressed patients, the organism can become invasive and cause severe cryptococcal pneumonia. It may also spread to the brain, causing cryptococcal meningitis (see Figure 6.11). This occurs in about 80 per cent of AIDS patients. Cryptococcal infections are frequently seen in patients receiving steroids or in transplant patients on immunosuppressive drugs.

The variety *C. neoformans* var. *gattii* is found mainly in areas where the Australian river red gum (*Eucalyptus camaldulensis*) is growing. It occurs in certain parts of Australia and in other countries to which the tree has been exported. Infections seem to occur mainly during the flowering season. Aborigines who live in dry river beds in Northern and Central Australia are often infected with this variety. It is rarely seen in immunocompromised patients in urban areas.

Pneumocystis pneumonia

Pneumocystis pneumonia (**PCP**), is a major cause of illness and death in people with impaired immunity. It is caused by an unusual fungus, *Pneumocystis jiroveci*, which was originally classified as a protozoan and named *Pneumocystis carinii*. Nucleic acid testing in recent years has confirmed that the organism is a fungus. It is an obligate parasite, and has not been grown successfully in the laboratory. The usual diagnosis is by observation of clinical symptoms, X-ray of the lungs or examination of an induced sputum specimen (see Figure 6.12). *Pneumocystis* is thought to be present in the respiratory tract of many healthy individuals, but becomes invasive only in immunosuppressed patients. It is a primary cause of pneumonia in



(a) (b)

FIGURE 6.11

***Cryptococcus neoformans* infection**

(a) MRI scan showing multiple cryptococcomas (white masses) in the brain; (b) X-ray showing pulmonary cryptococcal infection in upper right lobe.

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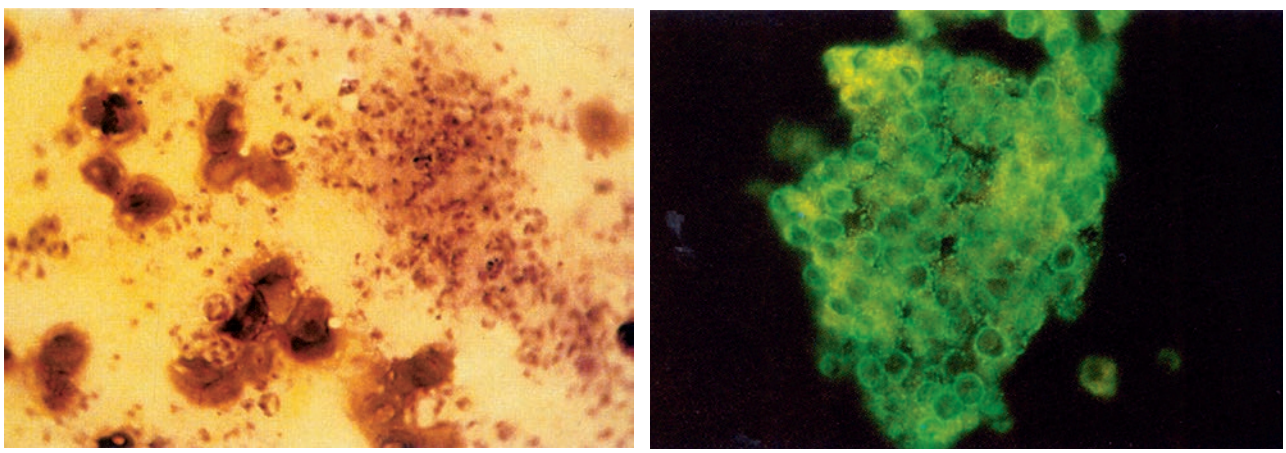

(a) (b)

FIGURE 6.12

Pneumocystis jiroveci

(a) Conventional stain where structures are difficult to identify; (b) fluorescent antibody stain clearly showing the cysts that block the alveoli.

Source: Courtesy of Dr A. Smithyman, Cellabs Pty Ltd, Sydney.

AIDS patients and is responsible for a large number of AIDS deaths.

Mould infections

Aspergillus infections are opportunistic infections caused predominantly by the mould *Aspergillus fumigatus*. Members of the *Aspergillus* genus are widely distributed in Nature and release spores that can be inhaled into the lungs (see Figures 6.13 and 6.14). If the fungus invades the lung tissue it is able to enter the bloodstream and be disseminated throughout the body, causing potentially fatal abscesses in various body organs. In health facilities, invasive aspergillosis is a major problem for immunosuppressed patients and transplant patients, especially bone marrow recipients. Infections are usually traced to contaminated air-conditioning ducts.

Fusarium species are common soil organisms and plant pathogens which can also cause superficial mycoses, especially of the skin and eyes. Disseminated *Fusarium* infections that occur in leukaemia or transplant patients are difficult to treat and have a high mortality rate. *Scedosporium* is another mould that sometimes causes infections in hospitalised patients.

Transmission of fungal infections

Most fungi live in the soil or on plants and are transmitted to humans by contact with the fungus or its spores. In health facilities fungal spores may be carried on air currents from contaminated air-conditioning units. They are frequently inhaled (e.g. *Aspergillus* spores and *Cryptococcus*), but they can also enter through broken skin. Some of the cutaneous mycoses are transmitted by contact with infected skin scales,

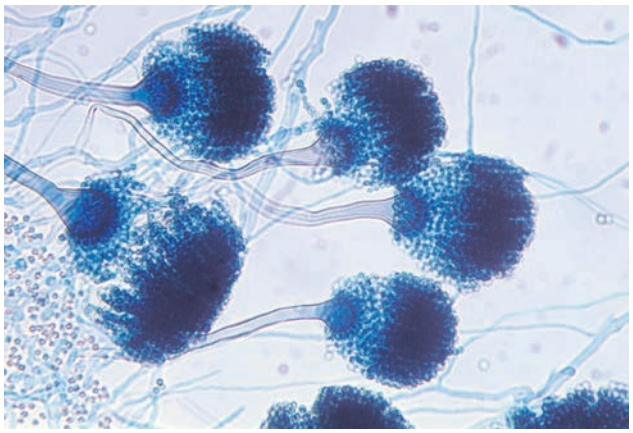

FIGURE 6.13

Microscopic morphology of *Aspergillus fumigatus* showing typical conidial heads

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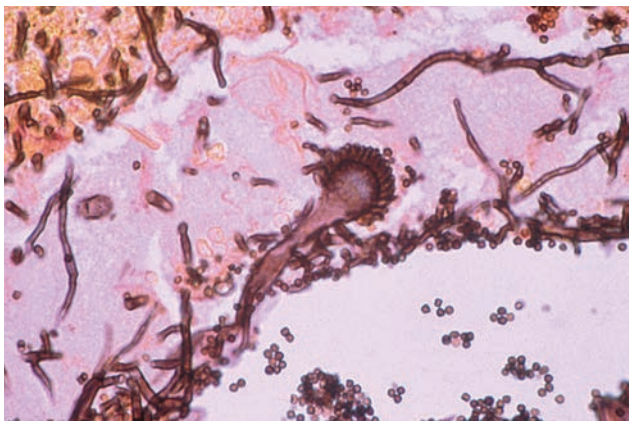


FIGURE 6.14

Silver stained section of lung tissue showing infection with *A. fumigatus*

Source: © The University of Adelaide. Reproduced with permission.

or by direct contact with a lesion on the skin of an infected human or animal (e.g. ringworm on cats).

A notable exception is *Candida*. Since *Candida* species are normal human commensals, they tend to cause infection when there is a lowering of the host resistance, as described above.

Factors contributing to fungal infections

It should be noted that most fungal infections are of only minor importance in healthy individuals. The overall integrity of the host immune system is of utmost importance in avoiding fungal infections.

A number of factors contribute to patient susceptibility. Neutropenia is a predisposing factor for many infections. Certain medical procedures also affect susceptibility, including:

- Transplant surgery with administration of immunosuppressive drugs.
- Prolonged broad spectrum antibiotic therapy which destroys the normal flora.
- Other invasive therapies such as intravenous total parenteral nutrition (TPN). This involves the use of solutions containing high concentrations of glucose which inhibit bacterial growth but favour the growth of fungi such as *Candida albicans*. The use of fat emulsion in TPN also encourages the growth of *Malassezia furfur*, an otherwise harmless superficial fungus.
- **CAPD** (**C**ontinuous **A**mbulatory **P**eritoneal **D**ialysis). This therapy is used as an alternative to haemodialysis for patients with renal failure. The main problem with this therapy is the risk of fungal infections as well as bacterial infections through the indwelling cannulae.
- Fungal infections may also occur in prosthetic devices. The organisms originate from contaminated equipment or materials.

Approximately 10 per cent of hospital-acquired infections are due to fungi.

Diagnosis of fungal infections

Dermatophyte infections may be identified by microscopic examination and staining of skin scrapings or by reaction with fluorescent antibodies (see Figures 6.15 and 6.16). The arrangement of their hyphae and the structure of their conidiospores are distinctive for each species. In the laboratory they can be identified by culture on Sabouraud's agar (see Figure 6.3, page 113).

Diagnosis of systemic fungal infections is difficult because the symptoms are often not definitive and laboratory growth of fungi is very slow. Fungal infections are usually suspected if the patient is immunocompromised and does not respond to empirical antibacterial therapy. Careful collection and processing of specimens is very important.

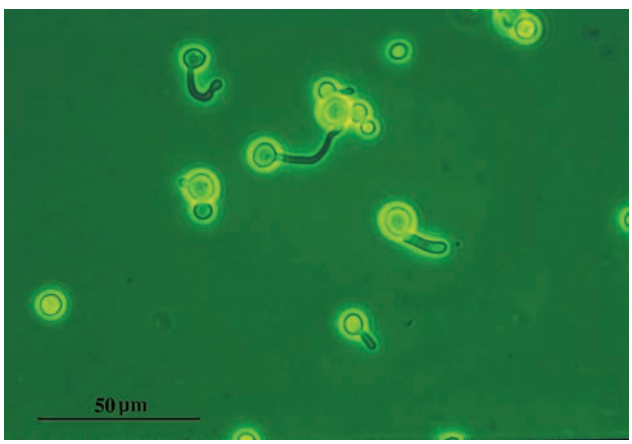


FIGURE 6.15

The presence of systemic *Candida albicans* can be detected using a fluorescent antibody stain that reveals characteristic germ tubes

Source: © The University of Adelaide. Reproduced with permission.

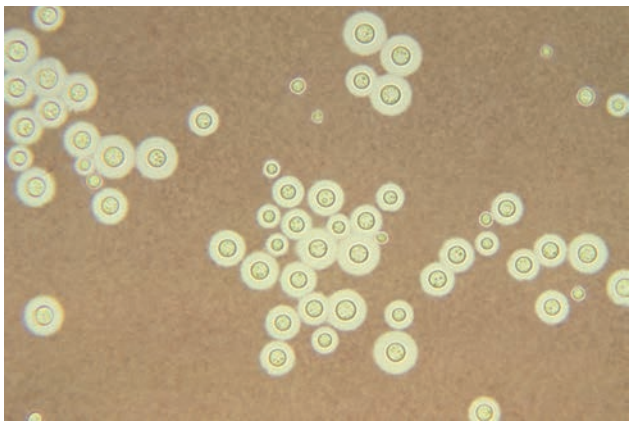


FIGURE 6.16

***Cryptococcus neoformans* is identified using an India ink stain**

Source: CDC/Dr Leanor Haley.

Treatment of fungal infections

Cutaneous fungal infections are treated with topical antifungal preparations. Systemic infections are more difficult to treat as there are few antifungal drugs that do not have serious side effects on the host. Amphotericin is the most effective drug for neutropenic patients. Candidiasis and cryptococcosis in AIDS patients (who are usually not neutropenic) are treated with fluconazole. Antifungal drugs are described in more detail in Chapter 12. No vaccines are available for any of the fungal infections.

Harmful effects of fungi

Apart from causing infections, fungi can have other harmful effects. These include the induction of allergic responses to fungal spores and the harmful effects associated with the ingestion of fungal toxins.

Mould-related health problems

It is becoming increasingly apparent that the growth of moulds in damp or poorly ventilated buildings can give rise to a number of minor or serious health problems. The release of spores, mould fragments or volatile products (toxins) into confined areas can have harmful effects on the people working in these buildings ('sick building' syndrome). Some of the symptoms produced include respiratory problems, difficulty breathing, sore throats, cough, nasal and sinus congestion, eye irritation, allergic reactions, skin irritation, headaches and central nervous system problems. Many of these symptoms are transient but, as with all fungal infections, they may cause serious problems in immunocompromised patients.

The release of dust and mould spores during building works and renovations also creates a potential hazard, especially in the hospital environment where mould and spores can be drawn into the ventilation system and reach susceptible patients.

Allergic reactions

Allergic bronchopulmonary reactions may result from the repeated inhalation of mould spores. Workers in farm industries are particularly at risk of occupational exposure due to inhalation of spores of fungi such as *Aspergillus* species, which are commonly found in grain storage bins, haystacks, silos and sugar cane piles (bagasse). The introduction of spores into sensitised lungs causes a hypersensitivity reaction, usually associated with severe breathing difficulties (farmers' lung).

Fungal toxins

Some fungi produce chemicals that are toxic to humans. The poisonous effects of eating some species of mushroom (commonly called toadstools) are well known. Symptoms such as nausea, severe diarrhoea, damage to body systems, muscle spasms and death may occur. Other fungi infect food and produce toxins that are ingested with the food. For example, **aflatoxin** is produced by the mould *Aspergillus flavus* when it grows on peanuts and some grains. It has been linked to the occurrence of liver cancer.

Ochratoxin A is produced by a number of fungi and, in Australia, has been isolated from *Aspergillus carbonarius*, a fungus found on grapes. It is suspected of being carcinogenic and teratogenic (affecting the foetus), and of having harmful effects on the immune system.

Another interesting toxin is **ergotamine**, produced by *Claviceps purpurea*, a mould that infects rye grain. It is a chemical similar to LSD (lysergic acid diethylamide) and produces hallucinogenic effects. It has been suggested that some of the witchcraft incidents of the Middle Ages were due to the ingestion of this mind-altering toxin in contaminated rye bread.

It is important to have strict health controls to prevent the contamination of food with fungal toxins. The presence and possible harmful effects of fungal toxins in animal feed is also of concern to farmers.

PARASITES

A **parasite** is defined as an organism that derives its nutrients from another living organism, its host. Parasitic diseases usually refer to those caused by protozoa, helminths and arthropods, which live at the expense of their host. As we explain in Chapter 7, parasitism encompasses a wide range of relationships, from those in which the host is only slightly harmed to those in which it is killed. The most successful parasites are those that maintain their own life processes without killing their host.

Parasitic infections are often given very little coverage in microbiology courses. However, each year they cause disease in more than a billion people throughout the world and lead to several million deaths, mostly in developing countries. Major diseases such as malaria, sleeping sickness, schistosomiasis and leishmaniasis are widespread in many areas, and cause severe debilitation and death among the populations they affect. Other parasitic infections that were once considered minor are becoming more important because of their

occurrence in immunocompromised individuals. These include the infections caused by the protozoa *Toxoplasma* and *Cryptosporidium*.

In Australia, serious parasitic diseases are still relatively uncommon. However, an increasing number of individuals in our community are immunocompromised and therefore potentially susceptible. These include AIDS patients, cancer patients and transplant patients on immunosuppressive therapy. There is also a significant number of people who are susceptible because of their lifestyle and other contributing factors. Many Indigenous Australians in remote communities have an increased susceptibility to parasitic infections because of pre-existing conditions such as malnutrition, alcoholism and diabetes. Their lifestyle (e.g. the custom of going barefoot) places them at risk of contracting the parasitic worms, hookworm and strongyloides, which gain entry to the body by burrowing through the skin.

Scabies is endemic in the Indigenous community, and the resulting irritation and scratching provides a portal of entry for more serious streptococcal infections (see Chapter 14).

Parasitic infections differ from those caused by bacteria or viruses in several ways.

- Parasites frequently have a complex life cycle involving the formation of resistant cyst forms of the organism. These cysts can survive for long periods outside the host.
- The life cycle may involve an insect vector (carrier) that is essential for the transmission of the disease. Theoretically, elimination of the vector would effectively control the spread of the disease, but this has proved impossible in most cases.
- Some parasites are capable of infecting both humans and other animals. In this case, both animals and humans can act as reservoirs for the infection. This is especially true for many of the protozoal infections carried by insects.
- Many of the helminths (worms) have two or more hosts in their life cycle. The **definitive host** harbours the mature adult form of the parasite, and the **intermediate host** has the immature or larval form. Frequently, humans are an 'accidental' host, obtaining the parasite by eating infected meat (e.g. the beef tapeworm).
- Many parasites have life cycles that require a finite time for the various stages to mature, either in soil or in an insect or host. In these cases, it is sometimes possible to control the disease by interrupting the cycle.

In the following sections we describe the properties of the protozoa, helminths and ectoparasites, and discuss the problems associated with the prevention and control of the infections they cause.

PROTOZOA

Characteristics of protozoa

Protozoa are single-celled eucaryotic organisms ranging from 20 to 50 μm in size. They are mostly found in water habitats, but a number exist as parasites in animals (including humans) and insects. They contain various subcellular organelles and are surrounded by an outer membrane rather than a cell wall. Some have flagella for movement (see Figure 6.17a). Protozoa obtain their nutrients by absorption of small molecules, or ingestion of food particles or even microorganisms from their environment. Some protozoa have a clearly defined gullet and anal pore (e.g. the paramecia, see Figure 6.17b). Others obtain food by phagocytosis. The cell surrounds the food particle and engulfs it, drawing the food inside the cell where it is contained in a vacuole, or sac, and then digested (e.g. amoeba, see Figure 6.17c). Intracellular parasites obtain their food by absorption of nutrients from the host cytoplasm through their outer layer.

Classification of protozoa

There are thousands of different species of protozoa, but relatively few cause serious disease in humans. Traditionally, protozoa have been classified into four main groups,

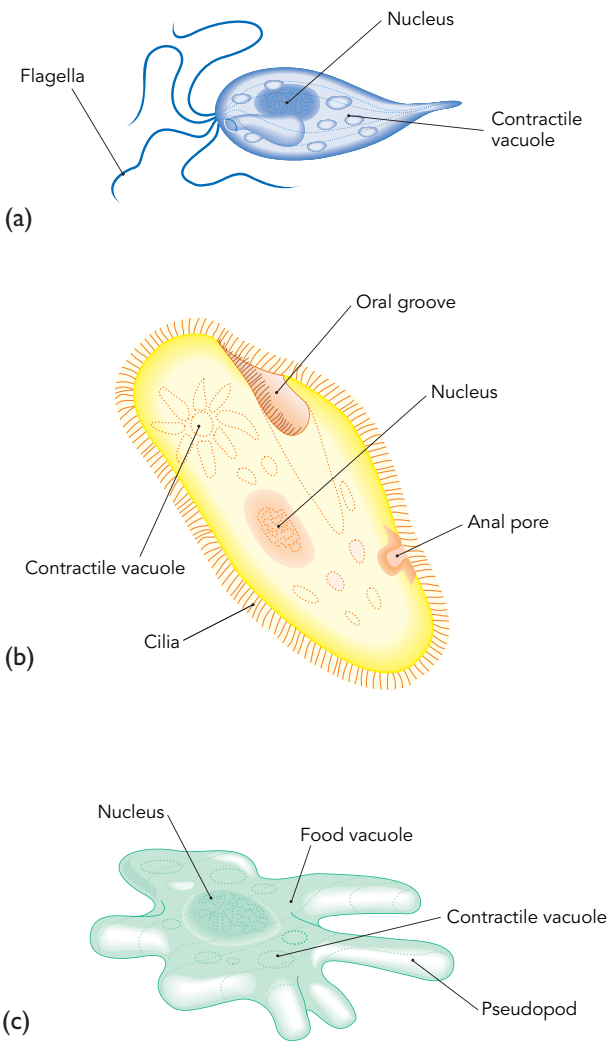


FIGURE 6.17

Protozoa

Diagrammatic representation of three different protozoa: (a) flagellate; (b) ciliate; (c) amoeba.

depending on their appearance and the way they move: the sarcodina, mastigophora, ciliates and sporozoa. Modern methods using ribosomal RNA are providing new understanding of protozoal classification, but for convenience in this text we will continue to refer to them in the traditional way.

Sarcodina (amoebae)

This group consists of the **amoebae**, large cells surrounded by a membrane and lacking a definitive shape. They move by extending the cell membrane and allowing the cytoplasm to flow into this extension, called a **pseudopod** (false foot). Amoebae absorb nutrients from their environment through their membrane, or engulf food particles by phagocytosis.

Mastigophora (flagellates)

These protozoa move by the action of flagella, whip-like tails that propel the organism through water environments. Mastigophora are usually oval in shape and reproduce asexually by longitudinal binary fission. Most of them are able to form cysts for survival. Several of the flagellates are important human pathogens.

Ciliates

Ciliates are single-celled creatures with a large number of small, hair-like appendages that move in a synchronised wave to propel the cell along. There are many ciliates found in the environment (e.g. paramecia) but the only human pathogen is *Balantidium coli*, a large protozoan that causes diarrhoea. Although it is widely distributed throughout the world, serious infections are rare.

Sporozoa

Sporozoa are non-motile protozoa; that is, the adult forms do not have any mechanism or appendages to enable them to move. This group contains several important human pathogens.

Reproduction

The simplest form of reproduction is asexual by a process of budding, binary fission or multiple fission (**schizogony**) of the infective stage (**trophozoite**). In schizogony, the nucleus undergoes multiple divisions before the cell divides. Cytoplasm forms around each new nucleus before division takes place, giving rise to multiple daughter cells. Sexual reproduction occurs mainly in protozoa that have an insect vector stage, although *Cryptosporidium*, which is a cause of diarrhoea in humans, can undergo sexual reproduction.

Most protozoa are capable of encystment—that is, the production of a resistant cyst form of the cell. **Cysts** are cells that are surrounded by a protective layer that is resistant to drying and enables them to survive for long periods (even years) in the soil. Some cysts are also resistant to the chlorine compounds used in water purification, and to stomach acid. This is an important characteristic that enables the survival and transmission of some medically important protozoa.

Many of the protozoa have a complex life cycle involving a number of different forms or stages—for example, the malaria parasite, *Plasmodium* (see Chapter 19, page 500).

Protozoal infections

Protozoa are responsible for causing some important human diseases. They may be found as intracellular or extracellular parasites in blood, intestines and other organs. Like opportunistic fungi, protozoal infections have a more serious outcome in immunocompromised patients (e.g. *Cryptosporidium* in AIDS patients).

Most protozoal infections are acquired by one of two main routes.

- The first route of transmission is by the ingestion of the parasite in contaminated food or water; or, in the case of *Toxoplasma*, by the inhalation of dried oocysts.
- The other route of transmission is via the bite of a blood-sucking insect. In some cases the insect acts purely as a vector; in others the parasite multiplies and undergoes a developmental stage in the insect (e.g. malaria).

The incidence of a protozoal disease depends on a number of factors, including climatic conditions, the distribution of the appropriate insect vector and the ease of transmission of the parasite.

Knowledge of the stages in the life cycles of the pathogenic protozoa is important for the prevention of transmission of the organisms and for the development of vaccines. For example, patients suffering from diseases that require an insect vector for transmission are not considered infectious to other patients or health workers, unless a suitable vector is present in the environment.

The protozoa that cause infections in humans are listed in Table 6.2. Not all these protozoa are found in Australia. Several of the more serious infections are transmitted by insect vectors, and so their incidence is limited to the geographical regions where these insects are found. A successful life cycle also depends on climatic conditions of temperature and humidity that favour the survival and replication of the insect vectors and the different stages of the parasitic life cycle. The references in the table are to the pages of this text where the diseases are discussed in more detail.

Pathogenic amoebae

Several different species of amoeba may inhabit the human intestine. They form cysts in the host that are excreted in the faeces and can then be ingested by another host (human) in contaminated food or water. The pathogenic amoeba *Entamoeba histolytica* is the major cause of amoebic dysentery worldwide, with most infections occurring in tropical and subtropical regions. The disease may range from mild diarrhoea to severe dysentery or ulcerative colitis, characterised by blood, pus and mucus in the faeces. It is only rarely seen in Australia, but travellers to endemic tropical areas may contract it there. Despite treatment, some individuals become asymptomatic carriers and continue to shed cysts

TABLE 6.2 **Protozoal infections in humans**

PHYLUM	ORGANISM	DISEASE	HABITAT SOURCE/VECTOR	REFERENCE
Sarcodina (amoebae)	*Entamoeba histolytica*	Amoebic dysentery	Contaminated water	Chapter 18, p. 455
Mastigophora (flagellates)	*Giardia intestinalis*	Giardiasis	Contaminated water	Chapter 18, p. 453
	Trichomonas vaginalis	Vaginitis	Vagina	Chapter 21, p. 553
	Trypanosoma brucei gambiense	Sleeping sickness	Tsetse fly	Chapter 6, p. 123
	Trypanosoma cruzi	Chagas disease	Kissing bug	Chapter 6, p. 124
	Leishmania spp.	Cutaneous leishmaniasis Visceral leishmaniasis (kala-azar)	Sandflies	Chapter 6, p. 124
Ciliata	*Balantidium coli*	Balantidial dysentery	Contaminated water	Chapter 6, p. 122
Sporozoa	*Plasmodium* spp.	Malaria	*Anopheles* mosquito	Chapter 19, p. 501
	Toxoplasma gondii	Toxoplasmosis	Cat faeces	Chapter 19, p. 504
	Cryptosporidium	Diarrhoea	Humans/animals	Chapter 18, p. 455
	Microsporidium	Diarrhoea	Humans/animals	Chapter 18, p. 445

of *E. histolytica* in the faeces, thus contributing to the spread of the disease.

Pathogenic flagellates

Giardia intestinalis (***Giardia lamblia***) is a common intestinal parasite found throughout the world, including Australia (see Spotlight box: Water quality and public health on page 126). It exists in two forms: the infective trophozoite stage, which has four pairs of flagella and a 'sucker' that allows it to attach to the wall of the intestine; and the resistant cyst stage, which is shed in the faeces and is able to survive for weeks in a moist environment (see Figure 6.18).

Contaminated drinking water is the main source of infection. Symptoms may take several weeks to appear, so it is often difficult to establish the true source of the infection. *Giardia* is notoriously difficult to diagnose. Symptoms may range from abdominal pain and prolonged bouts of diarrhoea to weight loss and general lack of energy. Examination of faeces is often inconclusive as the cysts and trophozoites tend to be shed intermittently. Physicians who are aware of the high incidence of *Giardia* infections in an area often treat patients who have prolonged gastrointestinal symptoms empirically with drugs such as metronidazole (Flagyl®) or tinidazole.

FIGURE 6.18

Scanning electron micrograph of the flagellate protozoan *Giardia lamblia*

Source: Professor Andrew Thompson, Murdoch University.

Drinking water should be filtered and chlorinated to remove *Giardia* cysts, as outbreaks frequently occur when drinking water supplies become contaminated with sewage. In urban areas, broken water pipes or an overflow of sewage during storms can cause problems. *Giardia* can be contracted from eating salad vegetables in areas where the water supply is contaminated. In rural areas, water supplies are often derived from natural creeks which may have become contaminated with sewage. There is some evidence that other animals can harbour the same species that infects humans. Bushwalkers should avoid drinking unboiled water from creeks and water courses.

Trichomonas is a flagellated protozoan that occurs as a commensal in a large percentage of the population. *Trichomonas hominis* is found in the large intestine and *Trichomonas vaginalis* is a frequent inhabitant of the female genital tract. Vaginitis due to *Trichomonas* is characterised by a smelly, greenish vaginal discharge. It may occur when the pH of the vagina becomes less acid, allowing *T. vaginalis* to multiply (see Chapter 21). The organism is sexually transmitted, so treatment should include both partners.

The **haemoflagellates** are a group of flagellated protozoa responsible for a number of serious diseases with a high morbidity and mortality rate. They are the cause of millions of deaths in some parts of the world. Although not endemic in Australia, travellers to regions where they occur are at risk of contracting these infections. A full history of overseas travel should always be taken when a patient presents with an infectious disease. *Trypanosoma brucei gambiense*, found in West Africa, and *Trypanosoma brucei rhodesiense* in East

Africa, are both carried by the tsetse fly and are the cause of African sleeping sickness. The protozoa affect the nervous system, leading to coma and death. *Trypanosoma cruzi*, the cause of **Chagas disease**, is also carried by an insect vector, the reduviid or kissing bug. This flightless insect is found in South America, usually in areas of poor-quality housing. The disease has a slow progression with increasing debilitation and eventual death, often due to myocarditis. None of these diseases occurs in Australia as the appropriate insect vectors are not found in this country.

The *Leishmania* are a group of haemoflagellates that are spread by sandflies and are responsible for the serious illness **leishmaniasis**. They are distributed over South and Central America, India, the Middle East and Africa. The disease takes two forms. Some species attack the skin, causing cutaneous leishmaniasis, which is not usually fatal (see Figures 6.19 and 6.20); others that affect the liver and spleen cause visceral leishmaniasis (also called **kala-azar**). Untreated kala-azar is invariably fatal. Several different drugs are used, but in many Third World countries access to treatment is difficult and the cost of drugs prohibitive. Prevention of these diseases depends on an effective campaign to control the sandfly vectors. Animals other than humans can serve as a reservoir for *Leishmania*, so it is very difficult to limit the incidence of the disease.

Leishmaniasis is only seen in Australia if the patient has contracted the disease while outside the country. However, cutaneous leishmaniasis has been reported in Australian kangaroos, which raises the possibility of its transmission to humans via sandfly bites.

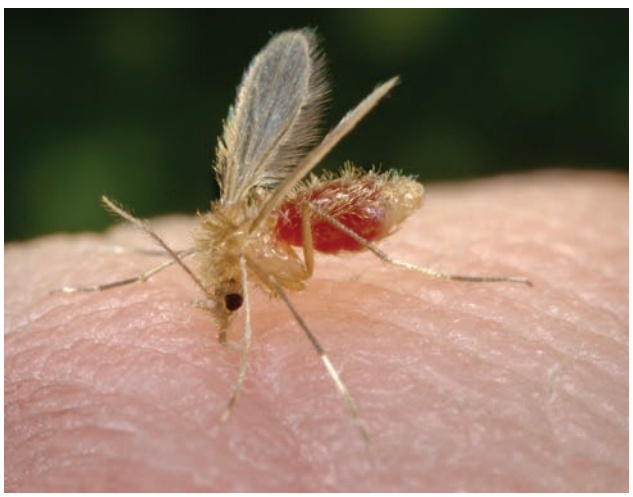


FIGURE 6.19

Sandfly feeding

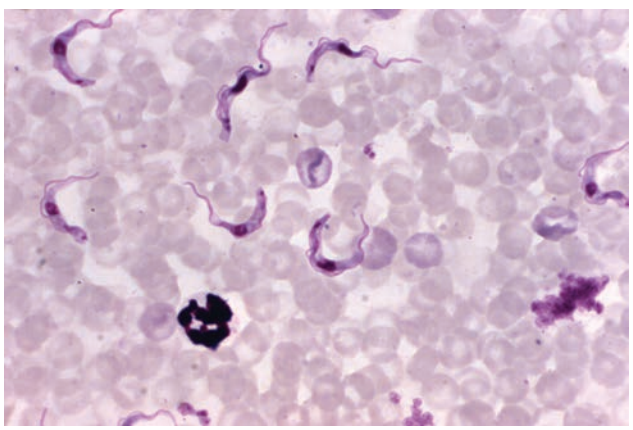
Source: James Gathany/CDC.

Pathogenic sporozoa

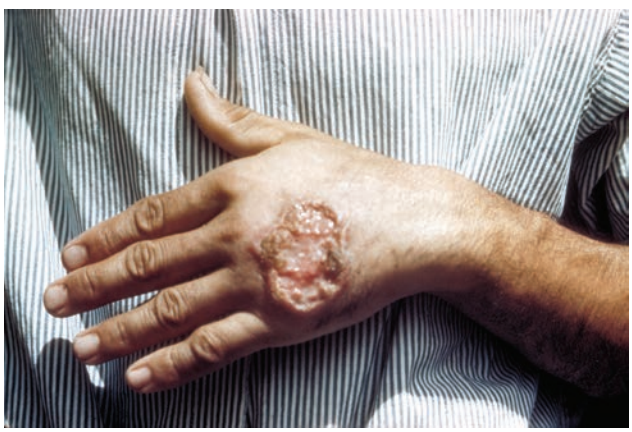
Sporozoa belonging to the genus *Plasmodium* are responsible for causing **malaria**. There are five species that infect humans—*Plasmodium falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. More recently, the simian (monkey) parasite *P. knowlesi* has been shown also to infect humans (see Case History 6.1). Plasmodia are non-motile protozoa and require an insect vector—in this case, the *Anopheles* mosquito—for transmission. It is estimated that worldwide there are over 200 million cases of malaria annually and 1–2 million deaths. The disease is widespread in tropical areas, but is not endemic in Australia at present. However, the north of Australia (above latitude 19°S) is a receptive area where *Anopheles* mosquitoes are found.

The malaria parasite has three distinct stages in its life cycle (see Chapter 19, page 502). The first involves the injection of the **sporozoite** into the human host via the bite of an infected female *Anopheles* mosquito. The sporozoite migrates to the liver where, over a period of several days or weeks, it replicates asexually, producing hundreds of **merozoites**.

The second asexual stage occurs in the red blood cells of the human host. The merozoites that were released from the liver into the bloodstream invade the red cells and multiply, causing haemolysis and releasing hundreds more merozoites into the bloodstream. It is this stage that produces the typical symptoms of malaria—headache, nausea, fever and



(a)



(b)

FIGURE 6.20

(a) Trypanosome; (b) Lesion of cutaneous leishmaniasis

Source: CDC/Dr D. S. Martin.

CASE HISTORY 6.1

Plasmodium knowlesi

A 39-year-old Australian man presented to a Sydney hospital with a history of morning fevers and headaches. His travel history revealed that during the previous 18 months he had been working adjacent to a forest region in Indonesian Borneo, most recently during the rainy season. He had not used any protection against malaria and had not travelled to other malarious areas. A Giemsa stained blood film revealed malaria parasites resembling *P. malariae*, but other parasites resembling *P. falciparum* were present. Molecular studies identified the parasite as *P. knowlesi.* He was treated with atavaquone/proguanil and recovered completely.

Comment

Until recently, human malaria was thought to be caused by only four plasmodium species: *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*. Previously, *P. knowlesi* was considered to infect only monkeys and not be readily transmitted to humans. More recently, molecular techniques have *identified P. knowlesi* as responsible for 70 per cent of infections in Sarawak, Borneo, and it is also present in other parts of South-East Asia. It is apparent that infections previously identified morphologically as *P. vivax* or *P. malariae* were probably due to *P. knowlesi*. Depending on the parasite level, *P. knowlesi* can have serious complications.

Source: Adapted from M. Figtree, R. Lee, L. Bain, T. Kennedy, S. Mackertich, M. Urban, Q. Cheng and B.J.I. Hudson 2010, *Plasmodium knowlesi* in human, Indonesian Borneo. *Emerging Infectious Diseases* 16(4), <www.cdc.gov/EID/content/16/4/672.htm>.

Questions

1. How did this patient acquire malaria?
2. What methods are used to avoid the disease?
3. Did the patient pose any risk to other patients in the hospital?
4. Why is it important to be able to differentiate between the different species of malaria? (*Hint:* See Chapter 19, page 500.)

chills. These symptoms occur at regular intervals, coinciding with the synchronised release of the merozoites from the red cells. As the life cycle continues, some merozoites produce **trophozoites** which develop into male and female gametes.

The gametes are ingested by the feeding *Anopheles* mosquito and undergo the third, and sexual, stage in the mosquito, which takes 7–10 days. Gametes fuse to form a zygote which matures into a sporozoite that is released through the salivary glands of the mosquito when it bites a new host, thus completing the cycle.

There is an interesting relationship between the occurrence of red blood cell disorders and susceptibility to malaria. Individuals who carry the genetic trait (i.e. are heterozygous) for sickle cell anaemia appear to have an increased level of immunity to malaria (see Spotlight box: Sickle cell anaemia, in Chapter 4, page 73). The red blood cells of these carriers are not as susceptible to *Plasmodium* as are normal blood cells. This also seems to apply to other genetically inherited blood disorders—thalassaemia and glucose-6-phosphate dehydrogenase deficiency (favism).

Programs for the prevention of malaria have been aimed at control or eradication of mosquitoes. These have not been very successful, partly because the mosquitoes have become resistant to pesticides. Spraying with DDT is very effective in controlling mosquitoes, but because of environmental concerns, its use is now restricted, and the mortality from malaria has increased again. Another serious problem is the appearance of strains of *Plasmodium* that are resistant to the antimalarial drugs currently in use.

Research into a vaccine for malaria has so far had only limited success. It is difficult to make a vaccine because the complex nature of the life cycle means that each stage of the parasite has a different antigenic structure. To eliminate the parasite successfully, a vaccine has to be active against more than one stage. Trials of a new vaccine for children in 2011 had moderate success, reducing the incidence of the disease by about 50 per cent. The sexual stage of the life cycle also allows for genetic variation to occur, so that there is a continual change in the antigenic structure of the parasite.

Toxoplasma gondii is a sporozoan responsible for a mild, flu-like illness in humans, **toxoplasmosis**. The life cycle of the parasite and characteristics of the disease are described in detail in Chapter 19. Humans can be infected by the accidental ingestion of oocysts that have been shed in cat faeces, or by the consumption of undercooked meat containing cysts.

Like many of the parasites discussed in this chapter, *Toxoplasma* has much more serious effects in patients whose immune system is deficient. These include the foetus during pregnancy, and immunocompromised patients such as those with AIDS.

Toxoplasma produces congenital defects when a non-immune mother is infected during pregnancy. The type of

congenital abnormality depends on whether infection occurs during the first, second or third trimester of pregnancy. The outcomes of infections during the first trimester are the most serious and include stillbirth and neurological defects such as blindness. If infection occurs later in pregnancy, the effects are not so severe and may not be clinically apparent until later in life. Neurological problems and learning difficulties are sometimes an outcome of late prenatal toxoplasmosis. *Toxoplasma* is widely distributed and many Australians carry antibodies to the protozoan. Women considering pregnancy can be tested to determine their level of immunity and, if not immune, should avoid contact with cat faeces and undercooked meat (see Chapter 19).

In patients who are immunodeficient (AIDS) or immunosuppressed (transplant), latent *Toxoplasma* can be reactivated to a fulminating fatal infection. Other serious consequences of reactivation include retinitis, encephalitis and pneumonia.

Cryptosporidium and ***Microsporidium*** are non-motile protozoa that commonly inhabit the intestines of some native animals and birds. They can enter the water supply and are then transmitted via the faecal–oral route. They are a probable cause of transient mild diarrhoea in humans, which can be quite serious in children and immunodeficient people. There have been reports of outbreaks of diarrhoea due to *Cryptosporidium* in daycare centres, and reports that were traced to public wading pools. In the United States a serious outbreak was linked to a contaminated water supply (see Spotlight box: Water quality and public health).

In individuals who are HIV-positive, *Cryptosporidium* and, more recently, *Microsporidium* have been found to be responsible for chronic diarrhoea with loose, watery stools. *Cryptosporidium* can be identified in faecal specimens using a special staining technique or by immunofluorescence (see Figure 6.21).

HELMINTHS

Helminths, or worms, are a common type of parasitic infection throughout the world. In general, their occurrence in humans is linked to low socioeconomic conditions and poor sanitation. Although infections can be widespread, especially in developing countries, they are rarely fatal on their own. Many individuals carry a significant worm 'load' without apparent ill effect, but in many cases the parasites contribute to significant morbidity.

Many helminths have a complex life cycle, often involving more than one host. Some require a vector for transmission.

SPOTLIGHT ON

Water quality and public health

A safe water supply is essential for public health, so there was alarm in July 1998 when part of Sydney's water supply was found to be contaminated with high levels of *Giardia* and *Cryptosporidium.* Initially, the contamination was thought to be in only one section of pipes serving a small section of the Sydney CBD, but over a period of days it was found to extend to most areas of Sydney supplied by the Sydney Water Corporation. Heavy rainfalls that filled the storage dams added to the problem. Residents were advised not to drink tap water unless it had been boiled for at least one minute.

Cryptosporidium and *Giardia* are protozoal parasites that are carried by many animals as well as humans and are excreted as oocysts in the faeces. Presumably, they enter the water supply in run-off from catchment areas. *Giardia* is often contracted by bushwalkers drinking from contaminated streams or by travellers in developing countries, and causes diarrhoea, stomach cramps and general malaise. It can be difficult to treat and may persist for months.

Cryptosporidium causes gastrointestinal infections with watery diarrhoea which can be serious and even fatal for immunocompromised people. Outbreaks are sometimes associated with children's wading pools. In Milwaukee, in the United States, in 1993 there was a breakdown in water treatment standards; over 405 000 people were infected with *Cryptosporidium* and a number of immunocompromised patients died.

Water treatment plants are designed to filter and disinfect the water before it is supplied to the public. However, there are problems associated with measurements of water quality. Protozoal cysts are much more resistant to the usual methods of water treatment than bacteria, and there is no accepted standard for the levels of contamination that may pose a health hazard. There are difficulties in measurement as the methods currently available do not distinguish between living (viable) and dead parasites.

The high levels of contamination were detected in Sydney's water for nearly two months and during this time people avoided drinking tap water. Sales of bottled water soared. Curiously, there was only a slight and probably not significant increase in reports of infections, despite the fact that surveys indicated less than 100 per cent compliance with the 'boil water' alert. The reasons for this are not clear. *Cryptosporidium* cannot be cultured in the laboratory and it may be that the cells detected were not viable or that the strain of *Cryptosporidium* was one that did not infect humans.

However, the events served as a timely reminder of the importance of a safe water supply and provided the incentive for a review of water treatment methods and standards in New South Wales.

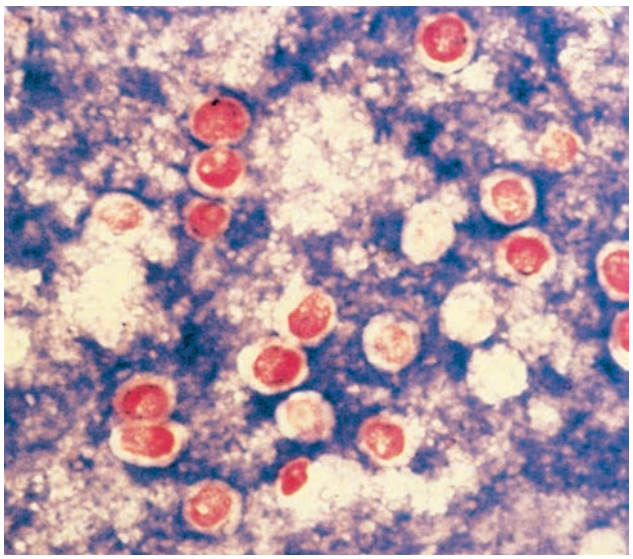


FIGURE 6.21

Cryptosporidium parvum

A Ziehl-Neelsen stain of oocysts.

Source: Stephen Neville, Department of Microbiology, South Western Area Pathology Service.

An understanding of the life cycle of each parasite is necessary in order to develop appropriate strategies for prevention and treatment of infection. The **definitive host** harbours the sexually mature adult form of the parasite, while the cyst or larval stages are found in the **intermediate host**. The human host is not necessarily an essential part of the life cycle of the parasite. Very often humans are an 'accidental' host—that is, they acquire the parasite by ingestion of contaminated food or water.

With rare exceptions, helminths do not replicate within their human hosts. However, the production of eggs in the human intestine, with subsequent shedding in the faeces, contributes to the continuation of the life cycle.

Classification of helminths

The parasitic helminths that occur in humans belong to two phyla—the **Platyhelminthes** (or **flatworms**), which include the **trematodes** (**flukes**) and **cestodes** (**tapeworms**); and the **Aschelminthes**, which contain the **nematodes** (roundworms). Helminths are usually large organisms with a complex body structure. However, the larval stages may be quite small, only 100–200 μm in size, and the eggs are microscopic.

Distribution

Table 6.3 lists the common species of worms that cause human infections in Australia. Other serious parasitic helminth infections—such as filariasis, onchocerciasis and schistosomiasis—occur in many parts of the world but are not usually seen in Australia. This is due to various factors, including the lack of the appropriate vector, lack of a suitable intermediate host for the parasite, or the absence of a reservoir of infection. Many of these infections are associated with tropical climates and poor sanitary practices. This section concentrates on helminth infections that are prevalent in Australia.

Flatworms

The **cestodes** (or **tapeworms**) live as intestinal parasites in their definitive host. They consist of a head, or **scolex**, and a long body made up of segments, or **proglottids** (Figure 6.22). The scolex has hooks or suckers, enabling the worm to attach to the mucosal cells lining the intestine. The worm obtains its food

TABLE 6.3 **Endemic helminth infections in Australia**

ORGANISM	DEFINITIVE HOST	INTERMEDIATE HOST	TRANSMISSION TO HUMANS	MEDICATION
Taenia saginata (beef tapeworm)	Humans	Cattle	Ingestion of undercooked beef	Praziquantel Niclosamide
Echinococcus granulosis (dog tapeworm)	Dogs	Humans (hydatid cysts)	Ingestion of eggs	Albendazole
Hymenolepis nana (dwarf tapeworm)	Humans (intestine)		Ingestion of eggs	Praziquantel
Ascaris lumbricoides (roundworm)	Humans (intestine)		Ingestion of eggs	Mebendazole
Enterobius vermicularis (pinworm)	Humans (intestine)		Ingestion of eggs	Albendazole
Ancylostoma duodenale Necator americanus (hookworm)	Humans (intestine)		Penetration of skin by larvae	Albendazole
Strongyloides stercoralis	Humans (intestine/other tissues)		Penetration of skin by larvae	Thiabendazole
Trichuris trichiura (whipworm)	Humans		Ingestion of eggs	Mebendazole

by absorption of predigested nutrients directly from the intestine of the host, through pores on the surface of the proglottids. Each segment contains both male and female sexual organs and is thus capable of egg production. Mature proglottids containing thousands of eggs are shed in the faeces. Other animals ingest vegetation or water contaminated with the eggs. In the new host, the eggs hatch into larvae which bore through the intestinal wall and establish cysts in the tissues of the infected animal (intermediate host). Ingestion of meat containing these cysts by the definitive host completes the cycle.

Humans act as the definitive host for the beef tapeworm, *Taenia saginata*, and the pork tapeworm, *Taenia solium*. Of these, only the beef tapeworm occurs in Australia, mainly in cattle-raising areas. Strict meat inspection procedures at abattoirs prevent contaminated meat from reaching the markets and, up to now, quarantine regulations have prevented the pork tapeworm from reaching Australia. Humans can often unknowingly harbour large tapeworms without any apparent symptoms.

The dwarf tapeworm *Hymenolepis nana* is sometimes found in Northern Australia. It is unusual in that it does not require an intermediate host. Ingested eggs can develop into mature worms in the human intestine.

Humans are the intermediate host for the dog tapeworm, *Echinococcus granulosis*, giving rise to a condition known as **hydatid cysts**. Dogs and cats are the definitive hosts for this tiny tapeworm, which is 2–8 mm in length. Eggs are shed in the faeces and may be transmitted to humans from faeces on the fur or tongue of the animal. The eggs hatch in the human intestine and migrate to various parts of the body, where they form large fluid-filled sacs of larvae, called hydatid cysts. Cysts may form in any tissue but those that form in the liver, lungs and brain are the most common and have the most serious consequences (see Chapter 18, page 463). Hydatids can be avoided by worming domestic animals regularly and not feeding them raw meat.

The **trematodes** (or **flukes**) have a flat, leaf-shaped body and a rudimentary digestive system consisting of a mouth and intestine but no anus. Most of the body is taken up by a complex reproductive system. On the outside surface of the fluke are muscular suckers that enable it to attach to host tissue (see Figure 6.23). Two main groups of flukes cause disease in humans:

- The tissue flukes attach to the lungs (e.g. *Paragonimus westermani*) or liver (e.g. *Clonorchis sinensis*, *Opis thorchis felineus*, *Opisthorchis viverrini* and *Fasciola hepatica*).
- The blood flukes (e.g. *Schistosoma*) reside in the vascular system of their human hosts.

The life cycle of all the flukes is very complex, often involving more than one intermediate host. They all require a species

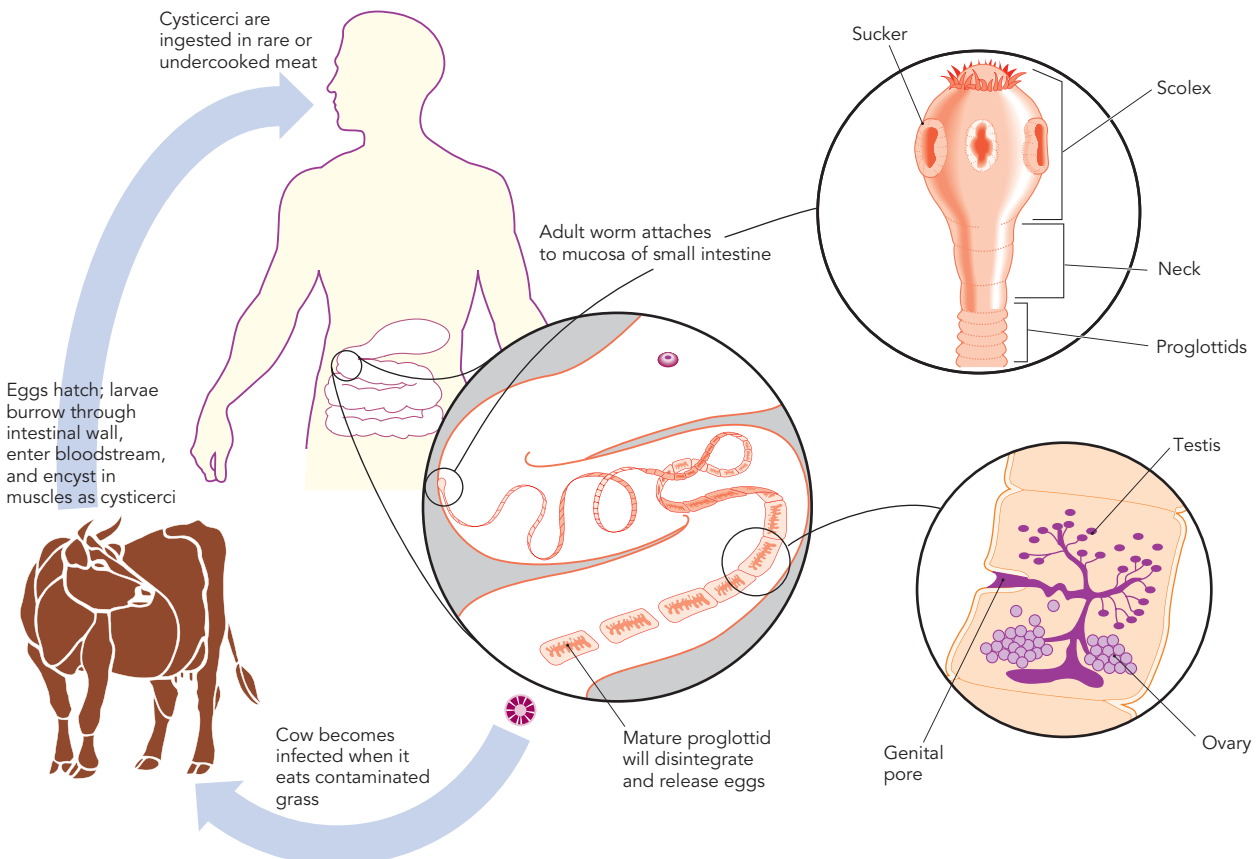


FIGURE 6.22

Beef tapeworm, *Taenia saginata*

The adult tapeworm has suckers to attach to the mucosa of the host intestine. Eggs are excreted in faeces and eaten by the cow, which is the intermediate host. Humans are infected by eating contaminated meat.

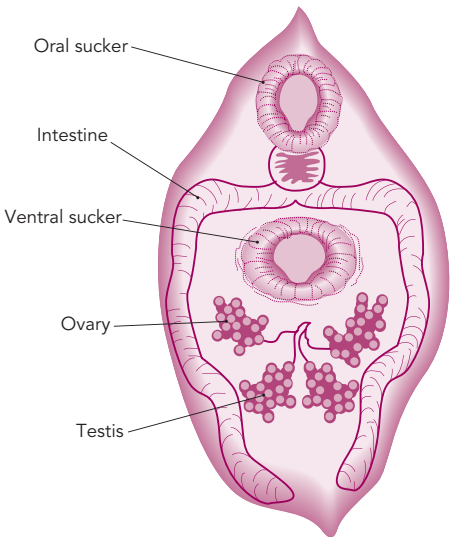


FIGURE 6.23

Flukes

This generalised diagram of the anatomy of an adult fluke shows the oral and ventral suckers. The suckers attach the fluke to the host. The mouth is located at the centre of the oral sucker. Flukes are hermaphroditic; each animal contains both testes and ovaries.

of freshwater snail which is usually specific for each of the fluke species and occurs only in certain habitats. Tissue flukes usually have a second intermediate host, a crustacean or fish, which acts as a source of infection for the definitive human host. The schistosomes (blood flukes) are released from the snail as the free-swimming fork-tailed infective form, **cercariae**, which invade humans by direct penetration through the skin (see Figure 6.24). **Schistosomiasis** is a major world health problem and is discussed further in Chapter 19.

With the exception of *Fasciola hepatica*, infections by flukes do not occur in Australia. Good sanitation, combined with the absence of the specific snail hosts, keep the parasite out of this country. However, cases of schistosomiasis, acquired from contaminated water or fish, have been seen recently in Australia in travellers returning from endemic tropical areas.

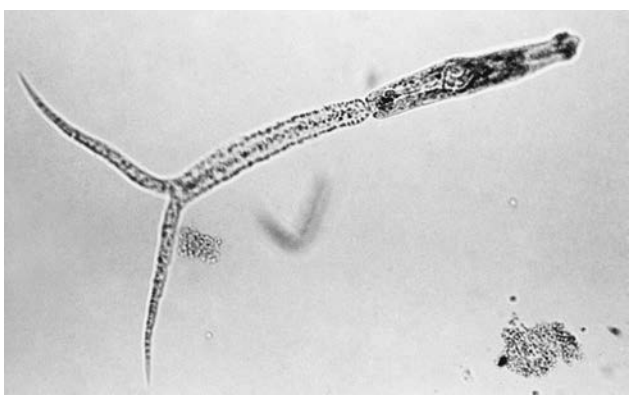


FIGURE 6.24

Cercariae of schistosoma showing typical forked tail

Source: CDC/Minnesota Department of Health, R.N. Barr Library.

Roundworms

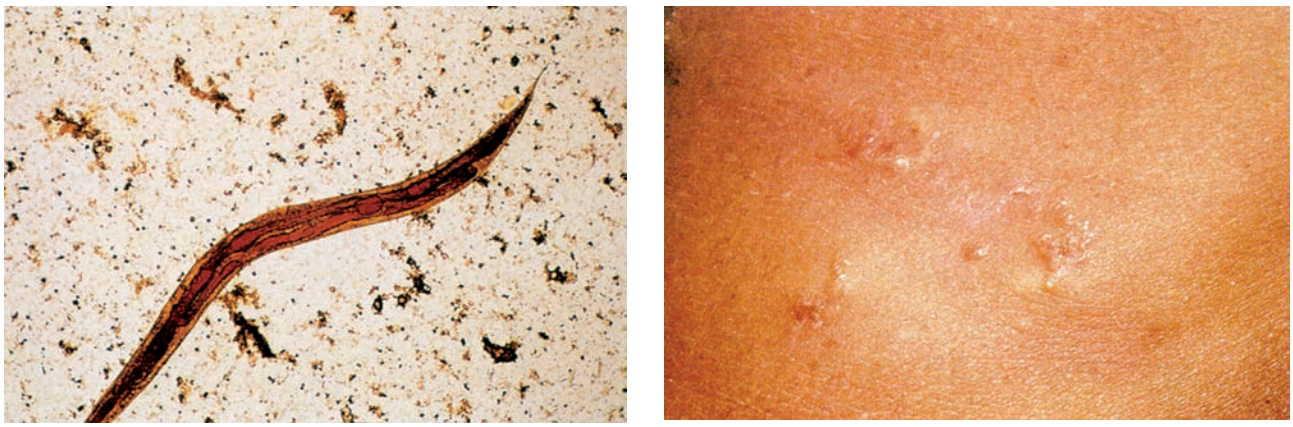
Nematodes (roundworms) are widely distributed throughout the world. They are long, cylindrical worms with a complete digestive system comprising a mouth, intestine and anus. Male and female sex organs are located on different organisms, the female worm often being significantly larger than the male. In general, nematodes have a less complex life cycle than flatworms. Several roundworms are able to parasitise humans and other animals, as well as insects and plants. For humans, the important parasites include the common roundworms, hookworms and threadworms. Many of these occur in Australia (Table 6.3, page 127).

The common **pinworm**, or **threadworm**, *Enterobius vermicularis*, is universally distributed. The tiny (0.5 cm) worm lives in the large intestine, the female emerging at night to lay eggs around the anus. The main symptom is anal pruritis (itching) due to irritation of the skin. Transmission of the worm occurs when the eggs are picked up on the fingers and ingested by the same host (re-infection) or another host. Eggs are frequently shed into clothing and bedding, which can also serve as a source of infection. Most children are infected at some time and the itching may cause disturbance to their sleep. Threadworm infections do not really have any major adverse effects on the host, and are usually easily treated.

Strongyloides stercoralis is another threadworm infection, commonly seen in tropical and subtropical regions, especially in Aboriginal people. It is acquired by larval penetration of the skin. The adult worms, which are only 2–3 mm long, are found lodged in grooves in the intestinal mucosa, but the larvae circulate constantly through the body. The worm is unusual in that it can replicate within the human host, so the infection may persist for long periods. If only a few worms are present, patients may be asymptomatic but, with heavy infestations, symptoms such as abdominal pain, rashes and pruritis occur. Immunocompromised or debilitated patients often have more severe symptoms and treatment may need to be repeated at regular intervals (Figure 6.25).

The roundworm *Ascaris lumbricoides* is a large worm that may reach 30 cm in length. It usually lives in the small intestine, producing mild symptoms of abdominal discomfort. Occasionally, a bolus of worms may cause intestinal obstruction in children. The female worm produces thousands of eggs that are passed in the faeces. They can survive for long periods in the soil. When ingested by another host, the eggs hatch in the intestine, mature in the lungs and then migrate back to the intestine. Occasionally, adult worms are excreted in the faeces.

Hookworm infections are caused by *Ancylostoma duodenale* and *Necator americanus*. Hookworms are about 1 cm in length and have a mouth with hooks or suckers which enable them to attach to the wall of the intestine and suck blood. Depending on the level of infection (worm load), symptoms may range from slight discomfort to anaemia and abdominal pain. Eggs are shed in the faeces and develop into larvae in the soil. Infection takes place when the larvae burrow through the skin, enter the blood vessels of the host and are carried to the lungs. The larvae are coughed up in



(a) (b)

FIGURE 6.25

Strongyloides infection

(a) Worm; (b) a patient with disseminated Strongyloides *infection (trails under the skin indicate the migrations of the worms).*

Source: Stephen Neville, Department of Microbiology, Southwestern Area Pathology Service.

sputum, swallowed and finally arrive in the intestine where they attach to the mucosal cells and establish an infection.

Hookworm occurs mainly in remote areas of Australia, when sanitation is poor. The Aboriginal population is particularly susceptible because of chronic malnutrition and the practice of walking barefoot (see Figure 6.26).

Trichinosis is a serious infection caused by the roundworm *Trichinella spiralis*. It is acquired by eating raw or undercooked pork that is contaminated with cysts of the worm. Although widespread in North America, trichinosis is not a problem in Australia; the pork sold in Australia is currently free of this parasite.

Several other roundworms that do not occur in Australia are responsible for severe infections in other parts of the world. Among these are the **microfilariae**, tiny worms that are carried by insects. **Filariasis**, or **elephantiasis**, is caused by *Wuchereria bancrofti* and transmitted by mosquitoes (see Chapter 19). The worm blocks the vessels of the lymphatic system, preventing drainage of fluid from the tissues and causing gross distortion of parts of the body—for example, legs, arms, breasts, vulva and scrotum. Filariasis is common in the Pacific Islands.

Onchocerca volvulus is a tiny worm carried by the black fly and is responsible for river blindness, a debilitating blindness that affects many thousands of people in developing countries. Guinea worm (*Dracunculus medinensis*) is another nematode acquired from contaminated drinking water; it produces large painful abscesses when the worm emerges from the body.

The cat and dog roundworms, *Toxocara cati* and *Toxocara canis*, are transmitted to humans, mainly children, by the ingestion of eggs in dog or cat faeces. After ingestion, the eggs hatch and the larvae migrate to various body organs, including the eye, when blindness may result. The worm occurs in Australia although it is more widespread in other parts of the world. Regular worming of pets is an effective control.

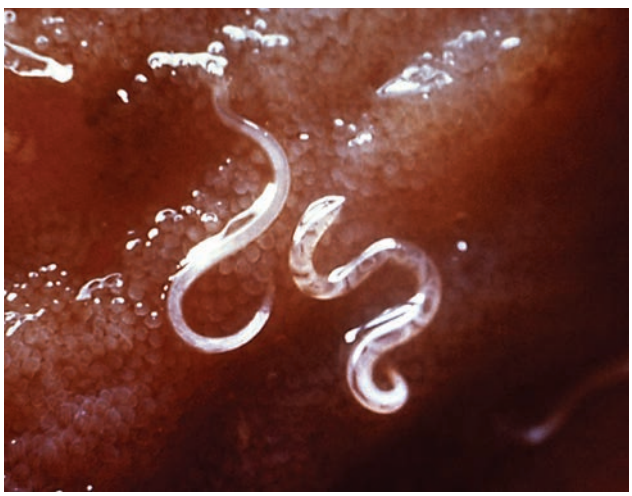


FIGURE 6.26

Hookworms attached to the intestinal mucosa

Barely visible larvae penetrate the skin (often through bare feet), are carried to the lungs, go through the respiratory tract to the mouth, are swallowed and eventually reach the intestine. The journey takes about a week.

Source: Centers for Disease Control (CDC).

Treatment and prevention of helminth infections

In recent years the attitude of the medical profession to the treatment of helminth infections has altered significantly. This is due to a number of factors.

- We now know that most helminths do not replicate inside their definitive human host.
- It is also recognised that worms cause significant damage to their host only when the infestation is very heavy and that, in general, most infected individuals do not carry a sufficient population of worms to cause disease.

- More importantly, in recent years a number of effective antihelminthic drugs have become available. These are listed in Table 6.3.

For Australia, this means that there are safe, effective drugs available to treat any worm infestations that may occur. These drugs, combined with education about proper sanitation and improved awareness of the importance of nutrition, should improve the health of people in remote areas.

In developing countries, there has been a shift in the strategies employed for the control of helminth diseases. It should be apparent from this discussion that the life cycles of the various helminths are very complex. Attempts to prevent worm infections by destruction of intermediate hosts, elimination of vectors, or alteration of environmental conditions to destroy larvae are all very costly and have not proved effective. The availability of inexpensive, effective drugs means that public health measures aimed at prevention and treatment now concentrate on the administration of these drugs at regular intervals.

Worldwide policy for the treatment of worm infestations in children in developing countries now involves regular administration of antihelminthic drugs at yearly intervals. Since 1993 this has been implemented for school children in developing countries, along with the administration of nutritional supplements. The program has seen a marked reduction in worm load, accompanied by significant improvements in general health and development.

ECTOPARASITES

A number of arthropods (insects) are capable of existing in a parasitic relationship on the outside surface of the human body. These are called **ectoparasites**. The most common are fleas, ticks, lice, and mites such as scabies These insects usually cause skin irritations and allergic reactions. However, in some cases they act as vectors of infectious diseases (see below).

Scabies

Scabies is an itchy skin infection caused by a tiny mite about 0.4 mm in diameter, called *Sarcoptes scabei* (Figure 6.27). The female mite burrows under the surface of the skin, forming a tunnel in which she lays two or three eggs each day. The infection is visible as a red, inflamed line, caused by an allergic reaction to the faeces left in the tunnel by the mite. Infection may occur anywhere on the body but is most common on the hands and forearms, or in warm, moist areas such as the armpits or groin (see Figure 6.28). The mites are transmitted directly from person to person, but they are also found on bedding and clothing and can survive for 2–3 days away from a human host.

Treatment with benzyl benzoate or a cream containing 5 per cent permethrin is effective, but eradication requires that all clothing and bed linen is washed and household contacts are also treated.

Scabies is endemic within Aboriginal communities in Northern Australia, and also occurs in urban areas where there is close human contact, such as in nursing homes. Prevalence rates in Northern Territory Aboriginal school children are 30–65 per cent. One of the major problems associated with scabies infection is that the itching and subsequent scratching allow secondary bacterial infections to occur.

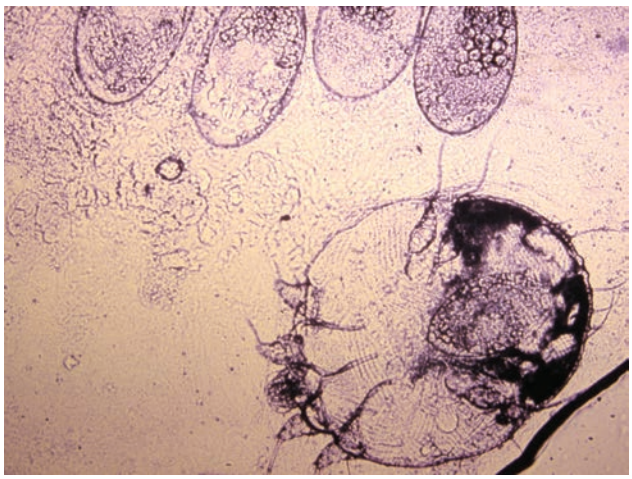


FIGURE 6.27

Scabies

Adult scabies mite with an egg and newly hatched mite.

Source: Reproduced with permission of New Zealand Dermatological Society Incorporated at DermNetNZ.org.

Staphylococcal and group A streptococcal infections are commonly associated with scabies. They can be readily treated with antibiotics, but if the scabies infection is not eliminated the bacterial infection may recur and antibiotic-resistant strains of bacteria may arise. If untreated, group A streptococci can give rise to bacteraemia, rheumatic fever and kidney diseases such as acute post-streptococcal glomerulonephritis (see Chapter 14).

Fleas

Fleas are blood-sucking insects that live parasitically on any warm-blooded animal. Occasionally, they will move from

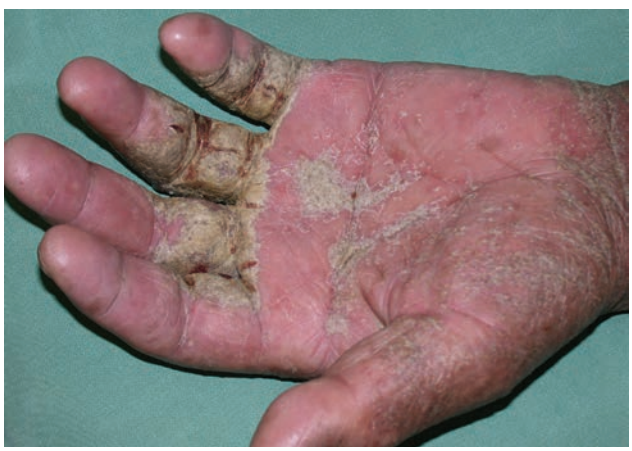


FIGURE 6.28

Scabies

Crusted scabies.

Source: Reproduced with permission of New Zealand Dermatological Society Incorporated at DermNetNZ.org.

their usual animal host (cats, dogs, rats, wild animals) to humans. Usually, they do not create a problem, other than a brief irritation, and can easily be removed by improved hygiene or, if necessary, treating houses with insecticides.

Lice

The louse, *Pediculus humanus*, is a small insect that lives on the outside of the body, particularly on the scalp and in the hair. The bite causes redness, itching and some lymph exudate which can provide a medium for secondary bacterial infections. Lice infestation is easily diagnosed by the presence of small white eggs (nits) attached to the hair shaft. The lice are transmitted directly by person-to-person contact. Outbreaks of lice are common in school children and are difficult to treat as they are resistant to many of the chemicals that were previously effective. Unless there is a coordinated approach to treatment in a school, re-infestation may occur. Bedding and clothing should also be washed thoroughly.

The crab louse, *Phthirus pubis*, is found mainly in the pubic region and is transmitted by sexual contact (see Figure 6.30).

Bed bugs

The common bed bug, *Cimex lectularius* (see Figure 6.31), is found worldwide. Infestations are common in the developing world, occurring in settings of unsanitary living conditions and severe crowding. Bed-bug infestations used to be viewed as a condition that occurred in travellers returning from developing countries. However, anecdotal reports suggest that bed bugs are increasingly common in Western countries. The bugs inject saliva into the bloodstream of their host, and this causes the intense itching. The delay in the onset of itching gives the feeding bed bug time to escape into cracks and crevices. In some cases, the itchy bites can develop into painful welts that last several days. Bed bugs are responsible for loss of sleep, discomfort, and disfiguring from numerous bites; occasionally, bites may become infected. They are not known to carry any disease.

FIGURE 6.29

Human body louse, *Pediculus humanus*

Source: CDC/Frank Collins, PhD.

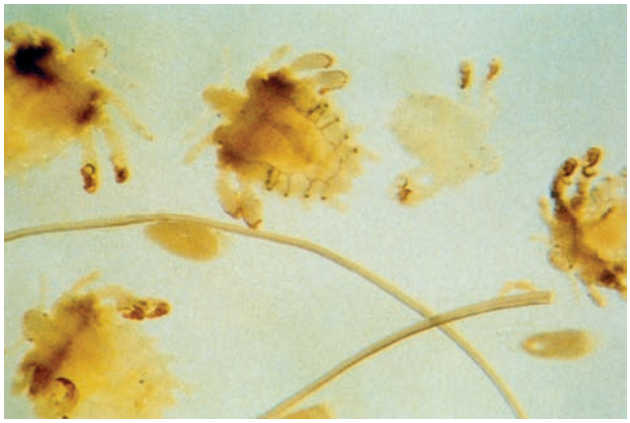


FIGURE 6.30

Pubic louse

False-colour SEM of the pubic louse, Phthirus pubis, *also known as a crab louse, clinging to a pubic hair. The lice suck blood, feeding about five times a day.*

Source: Stephen Neville, Department of Microbiology, Southwestern Area Pathology Service.

FIGURE 6.31

The common bed bug having a blood meal

Bed bugs are 4–5 mm in length and lurk in cracks in bedding.

Source: CDC/Harvard University, Dr Gary Alpert; Dr Harold Harlan; Richard Pollack.

ARTHROPOD VECTORS

Some ectoparasites—ticks, mites and lice—can harbour disease-causing organisms in the cells of the alimentary canal. The insect acquires the organism by biting an infected animal or human and transmits the infection to another host by biting and injecting the organism into the bloodstream or excreting infectious faeces on to the skin. Scratching the bite allows the organism to penetrate the skin and enter the new host. Of these infections, the most serious are bubonic plague and epidemic typhus.

Fleas are the carrier (vector) of the bacterium that causes bubonic plague (*Yersinia pestis*). Usually, the infection occurs in rodents (rats, squirrels), but when the animal dies the infected fleas may move to a human host. Plague is not endemic in Australia, although there have been outbreaks in the past (see Chapter 19).

Epidemic typhus is caused by the rickettsia (*Rickettsia prowazekii*), which is carried by the human body louse *Pediculus humanus*. Typhus occurs in crowded conditions often associated with wars, refugee camps, prisons or conditions of poor hygiene, especially in developing countries.

Ticks carry other rickettsias which cause spotted fevers such as Flinders Island spotted fever (*Rickettsia honei*) and Queensland tick typhus (*Rickettsia australis*). Spotted fever is characterised by fever, headache, myalgias, arthralgias, a maculopapular rash and focal skin lesions. The bacterium responsible for Lyme disease (*Borrelia burgdorferi*) is also carried by ticks.

Mites are responsible for carrying scrub typhus (*Orientia tsutsugamushi*), which occurs in Northern Queensland and the Northern Territory. A number of viral diseases are carried by insects and other arthropods (see Chapter 5, Table 5.4, page 102 and Chapter 8, Table 8.4, page 174).

SUMMARY

- A number of infectious diseases are caused by eucaryotic microorganisms.
- Patients who are immunocompromised are very susceptible to opportunistic infections caused by protozoa and fungi.

FUNGI

- Fungi comprise two groups: yeasts and moulds. Fungal cells are surrounded by a cell wall; they lack chlorophyll and cannot carry out photosynthesis.
- Fungi can grow at extremes of pH, temperature and osmotic pressure and are important for the decomposition of organic material.
- Yeasts are unicellular organisms that reproduce by budding. Some pathogenic yeasts (e.g. *Candida*) exhibit dimorphism.
- Moulds are filamentous fungi; they grow by producing a mat of hyphae called a mycelium as well as aerial hyphae that extend up above the mycelium.
- Asexual reproduction takes place by the formation of spores on the tips of the aerial hyphae.

Fungal infections

- A fungal infection (mycosis) may be superficial, cutaneous, subcutaneous or systemic.
- Cutaneous infections (tinea) are caused by fungi called dermatophytes.
- Subcutaneous infections occur when the fungus is inoculated beneath the skin.
- Systemic mycoses are caused by pathogenic fungi, or fungi that have invaded an immunocompromised patient.
- Most serious fungal infections are opportunistic.
- *Pneumocystis jiroveci* is an opportunistic fungus that causes atypical lung infections in immunocompromised patients.

Transmission of fungal infections

- Fungi can be transmitted by direct contact with an infected lesion or skin scales.
- Fungal spores are spread throughout the atmosphere on wind currents.
- Patient susceptibility to fungal infection is affected by use of immunosuppressive drugs, invasive procedures, IV therapy and prolonged antibiotic therapy.

Treatment of fungal infections

- Topical antifungal preparations are useful for cutaneous infections.
- Systemic infections are difficult to treat as most drugs have side effects.

Harmful effects of fungi

- Allergic bronchopulmonary reactions occur from repeated inhalation of fungal spores.
- Some fungi produce toxins that are poisonous to humans.

PARASITES

- Parasites are organisms that derive their nutrients from another living organism.
- Parasites often have a complex life cycle, involving two or more hosts. The definitive host harbours the mature adult form of the parasite. The intermediate host has the immature or larval form.
- Serious parasitic diseases are relatively uncommon in Australia.
- Lifestyle factors and deficiencies in the immune defences increase susceptibility to parasitic infections.

PROTOZOA

- Protozoa are single-celled animals surrounded by a cell membrane. They are found mainly in water habitats.
- Reproduction is mainly asexual by binary or multiple fission.
- Protozoa form cysts that are able to survive for long periods under adverse conditions.
- Protozoa can exist as intracellular or extracellular parasites in humans and animals.
- Protozoal infections can be acquired by ingestion of the parasite or via the bite of a blood-sucking insect.
- Protozoa are classified by the way they move.
- Sarcodina (amoebae) move by extending the cell membrane to form a pseudopod and allowing the cytoplasm to flow into it.
- Protozoa that move by means of flagella include *Giardia*, which is responsible for gastrointestinal infections, and *Trichomonas vaginalis*, a sexually transmitted pathogen.
- Ciliates move by hair-like appendages called cilia.
- Sporozoa are non-motile protozoa.
- The genus *Plasmodium* is the cause of malaria and requires the *Anopheles* mosquito for transmission.
- *Toxoplasma gondii* is a non-motile sporozoan carried by cats. It has serious effects on immunosuppressed patients and can cause congenital defects.
- *Cryptosporidium* and *Microsporidium* cause chronic diarrhoea in immunodeficient patients.

HELMINTHS

- Helminths (worms) include the phylum Platyhelminthes (flatworms, i.e. flukes and tapeworms) and the phylum Aschelminthes (roundworms).
- Many helminths have a complex life cycle involving an intermediate and a definitive host.
- Cestodes (tapeworms) consist of a head (scolex) and a body made of proglottids (segments) with both male and female organs on each segment. Eggs are produced and excreted in the faeces.
- Trematodes (flukes) have a flat body with muscular suckers for attachment.
- Nematodes (roundworms) are cylindrical worms with male and female organs located on different worms.

Treatment and prevention of helminth infections

- Several antihelminthic drugs are available.
- Adequate nutrition and good sanitation are effective control measures.

ECTOPARASITES

- Arthropods such as scabies mites, fleas and lice can exist on the surface of the human body, causing skin irritation.

ARTHROPOD VECTORS

- Some ectoparasites harbour disease-causing organisms and transmit infection by biting and injecting the organism into the bloodstream or excreting infectious faeces on to the skin.

STUDY QUESTIONS

1. How do opportunistic infections differ from other infections?
2. Name the two main groups of fungi.
3. Describe the structure and method of replication of yeasts.
4. Describe the structure of moulds and their method of asexual reproduction.
5. What is a mycosis?
6. Differentiate between superficial, cutaneous and subcutaneous mycoses.
7. What is tinea?
8. Under what conditions is *Candida albicans* likely to cause infections?
9. How are fungal infections transmitted?
10. List two harmful effects of fungi other than mycoses.
11. What is a parasite?
12. What are some of the predisposing factors that allow parasitic infections to occur?
13. How do diseases caused by protozoa and helminths differ from those caused by bacteria or viruses?
14. What is the difference between an intermediate host and a definitive host?
15. What are protozoa?
16. How do protozoa reproduce?
17. What is the importance of the cyst form of protozoa?
18. Describe two important flagellate diseases present in Australia.
19. What is the most important sporozoal infection worldwide?
20. What role does the domestic cat play in the transmission of *Toxoplasma*?
21. What is a common cause of diarrhoea in AIDS patients?
22. How are worms classified?
23. How do tapeworms reproduce?
24. Why are infections due to flukes not commonly seen in Australia?
25. What is the most common roundworm infection of children?
26. Why are worm infestations more common in people of lower socioeconomic status?
27. How are helminth infections (a) prevented, (b) treated, and (c) controlled?
28. What is scabies? Describe the major problems associated with scabies infections.
29. What is an ectoparasite? Give three examples.
30. Which diseases can be carried by ectoparasites?

TEST YOUR UNDERSTANDING

1. What is the difference between a cutaneous and a systemic mycosis?
2. Why are many hospitalised patients at risk of acquiring systemic fungal infections?
3. Why is it difficult to find an effective vaccine for malaria?
4. What other health problems would you suspect in a patient who is diagnosed with atypical pneumonia due to *Pneumocystis jiroveci*? Why?
5. Why should pregnant women avoid soiled kitty litter, sandpits and undercooked meat?
6. How do climate and environmental conditions influence the occurrence of parasitic infections? Explain your answer.
7. Discuss why effective drug therapy for diseases caused by eucaryotic organisms is difficult to achieve.

FURTHER READING

Mycology on Line, <www.mycology.adelaide.edu.au/>. (A comprehensive website with lecture, pictures and information about medically important fungi.)

WHO initiatives on tropical diseases. Available at: <www.who.int/neglected_diseases/en/>.

TDR research on drug development for treatment of helminth infections. Available at: <http://apps.who.int/tdr/svc/research/drug-development-helminths-ntds>.

UNIT
2

Host–microbe interactions

CHAPTER

7

Host–microbe interactions and principles of disease

CHAPTER FOCUS

- What kinds of relationships exist between living organisms?
- What are the properties of the microorganisms that inhabit the human body?
- What is an infectious disease?
- What factors determine whether a microorganism will cause an infection?
- What are the signs and symptoms of infectious diseases?
- What are the stages in the progress of a disease?

INTRODUCTION

In previous chapters we have described in some detail the properties of the major types of microorganisms. However, in order to understand the nature of infectious diseases we need to answer the following questions:

- What is the definition of disease?
- How do we know that microorganisms cause disease?
- What determines whether an organism will cause disease?
- Why do some organisms cause disease and others do not?
- There are microorganisms all around us in the environment and on the human body, so:
  —Why do some people get sick and others do not?
  —How does disease spread from one person to another?

The chapters in this unit examine the interactions between microorganisms and their human hosts that determine whether the host will exist in a state of health or disease; whether the microorganism will cause significant morbidity (illness) or mortality (death); whether the disease will spread and cause an outbreak or an epidemic. This interaction is complex and depends on a number of factors, including the likelihood of exposure to disease-causing microorganisms, the susceptibility of the human host, and the method of transmission of the organism. The virulence of the pathogen and the ability of the body's defences to withstand invasion by the microorganism also influence the outcome.

In this chapter we focus on what is meant by infection and infectious disease, pathogenicity and virulence, and discuss the ways in which the delicate balance in the relationship between microorganisms and humans can be altered, thus giving rise to a state of disease. Other chapters in this unit discuss how diseases are spread, the properties of microorganisms that enable them to overcome human host defences and produce damage and disease, and the way in which the immune system protects the host against disease.

SYMBIOSIS

The biological term used to describe two organisms living together is **symbiosis**. All living organisms exist in some kind of relationship with each other. Sometimes organisms do not interact unless they are physically brought together—for example, humans and animals. Sometimes the relationship is very close, as with members of a family. The study of how organisms interact in an environment is called **ecology**, and each defined environment is termed an **ecosystem**.

The human body can be regarded as a special type of ecosystem. At any moment in time, the human host may be interacting with numerous microorganisms and is subjected to physical, nutritional and traumatic alterations in environmental conditions that may have a marked influence on this interaction. Within this ecosystem, microorganisms live either permanently or transiently and, depending on various other factors, they may or may not cause an infection.

Not all symbiotic relationships are equally beneficial to both organisms. There are three broad categories of symbiotic association, based on the degree of benefit each organism receives—mutualism, commensalism and parasitism. In practice, the categories overlap to some extent, especially when applied to the human body, as the type of interaction may vary depending on physiological circumstances.

Mutualism

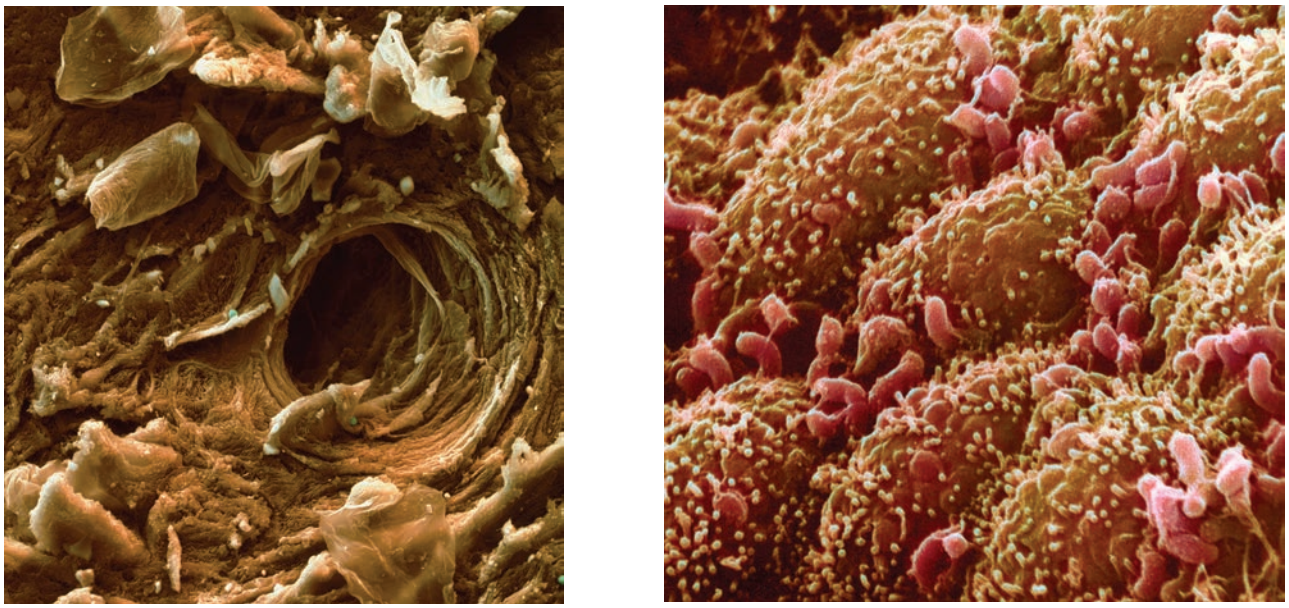
Mutualism usually refers to a situation where two independent organisms live together to their mutual benefit. The most commonly cited example is that of bacteria living in the stomach of ruminant cattle, where they aid in the digestion of cellulose in the animal's diet and in return receive a constant supply of nutrients. In humans, the bacteria living in the large intestine (colon) are of benefit to the host by producing vitamin K and some B vitamins; these are absorbed from the large intestine into the bloodstream and contribute to the host's requirements for those vitamins. The bacteria also help in the breakdown of waste material and the formation of faeces. In return, the bacteria benefit because they have a sheltered environment and an assured food supply.

Commensalism

Commensalism is an association between two organisms where one benefits while not causing any harm to the other. This is the term normally used to describe the relationship between the human host and many of the microorganisms that reside in or on the human body (see 'Normal flora' below). For the most part, these organisms are harmless, living on the body surfaces and making use of the waste products (oils and fatty acids) that are excreted through pores on the skin surfaces (see Figure 7.1). Indeed, by preventing colonisation by harmful bacteria, they may also be regarded as being of indirect benefit to the host. However, when environmental conditions alter, these commensals sometimes gain entry to other parts of the host and set up an infection. This situation is called **opportunism** and is discussed later in this chapter. The definition of commensalism is therefore not clear-cut, as the relationship between host and resident microbes may vary with conditions.

Parasitism

Parasitism is defined as a situation where one organism benefits at the expense of the other, usually larger,



(a) (b)

FIGURE 7.1

Normal flora

(a) Scanning electron micrograph of human skin showing uneven surface, skin scales and bacteria; (b) scanning electron micrograph of human intestine showing bacteria on mucosal surfaces. Most of the bacteria are commensals which indirectly benefit the host by competing with harmful organisms for nutrients and preventing them from finding a site to attach and invade the tissue.

Sources: (a) Eye of Science/Science Photo Library; (b) Biomedical Imaging Unit, Southampton General Hospital/Science Photo Library.

organism—the host. This usually means that the host is damaged or disadvantaged in some way. Most infections caused by microorganisms result in some degree of damage to the host cells. Parasitism, therefore, encompasses a wide range of relationships, from those in which the host is only slightly harmed to those in which it is killed. The most 'successful' parasites are those that maintain their own life processes without killing their host, which is their source of nourishment. Many bacteria are commensals and are usually only considered to be in a parasitic relationship when they cause an infection. However, other microorganisms, such as the viruses and some protozoa and helminths, are true parasites in that they are unable to live outside their host and so always cause an infection and produce some degree of damage (see Chapter 6).

As will become apparent in the following sections, the relationship between the microorganisms and their human host is complex and continually changing, due to alterations in the physiological and nutritional states of both. This relationship determines whether a clinically identifiable infection or disease will occur.

MICROORGANISMS OF THE HUMAN BODY: NORMAL FLORA (MICROBIOTA)

Before discussing the process of infection and disease, we should examine the human body as a habitat for microorganisms. Microorganisms are found everywhere in the environment and a rich population of microbes, especially bacteria, resides on the human body. These are called the **normal flora** of the body.

The human foetus develops in a sterile environment in the uterus and at birth the neonate is free of microorganisms. However, within days, even hours of birth, a population of microorganisms, mainly bacteria, takes up residence on the various body surfaces. They are derived from the baby's immediate environment, usually the birth canal of the mother, the hands of the people who care for the infant, the surfaces in the hospital nursery and other parts of the hospital environment. Because of the different organisms to which they are exposed, babies who are bottle-fed tend to have different bacteria in the large intestine from those who are breast-fed.

For the rest of their lives, humans are constantly exposed to a changing population of microorganisms. Some of these take up permanent residence on the skin and on the mucosal surfaces of the upper respiratory tract, mouth, gastrointestinal system and genital tract. They are called **normal** or **resident flora**. Others (some of which may be pathogens) establish themselves briefly in suitable areas (e.g. nose and throat) and are said to **colonise** these surfaces. They are not permanent residents and, after some time, are excluded from the site by competition with the normal flora or by the host's immune defence mechanisms. Other microorganisms are **transient** and may be carried for a brief time on hands or other skin surfaces (see Figure 7.3, page 140); they are usually considered to be **contaminants** and can be removed by physical means such as handwashing.

The human body provides an excellent shelter and source of nourishment for microbes. The various areas of the body have different conditions of temperature, moisture, pH, nutrients and oxygen so that different species can select

the most suitable environment in which to reside. Most body surfaces (internal and external) that are exposed to the environment can, and do, harbour microorganisms. The bloodstream and the internal organs, however, are usually maintained in a sterile state by the various body defence mechanisms. When microorganisms enter these sites and persist, a state of disease exists.

The normal flora have a number of useful and protective effects. They occupy sites in the body and create an environment which inhibits colonisation by other organisms (e.g. lowered pH on the skin) so that harmful organisms cannot become established and cause an infection. Once established in a suitable area, they can modify their environment—they may alter the pH (e.g. lactobacilli in the vagina lower the pH to 5, which inhibits the growth of other bacteria and fungi), excrete chemicals with antibacterial activity (e.g. bacteriocins and organic acids in the large intestine) or produce vitamins that are of benefit to the host.

The value of the normal flora to the human host is apparent when their numbers are reduced by long-term, broad spectrum antibiotic therapy. A frequent side effect of such therapy is an overgrowth of undesirable organisms; for example, *Clostridium difficile* may flourish in the large intestine, causing diarrhoea and pseudomembranous colitis. **Vaginitis**, or **thrush**, is caused by growth of *Candida albicans* in the vagina if the lactobacilli are destroyed. Cessation of antibiotic therapy usually allows the normal flora to become re-established.

The microbes that make up the normal flora are relatively stable but can be influenced by factors such as age, nutritional status, exposure to antibiotic therapy or a change in environment, such as a prolonged stay in hospital. Studies have shown that the composition of the microbial population alters very rapidly after a patient enters hospital, the normal flora being replaced with hospital strains that are generally resistant to antibiotics. Infection with these hospital strains has serious implications for the patient as they are usually more difficult to treat. Normal body flora can also be the cause of infections if the delicate balance between host and microbe is upset (see 'Opportunistic infections' on page 143).

Normal flora: types of organisms

By far the most predominant microorganisms found on the human body are bacteria (see Figure 7.2). They can be easily identified by standard laboratory techniques and their role in maintaining the human ecosystem has been accepted for some time. The yeast *Candida* is the major fungal inhabitant, and there are a few protozoa that appear to live in harmony with the human host (e.g. *Trichomonas intestinalis, Entamoeba coli*).

Because viruses are always thought of as disease-producing, they are not generally considered part of the normal flora. However, it may be that some viruses are able to reside in the human body without producing disease symptoms unless there is a change in the immune status of the host. An

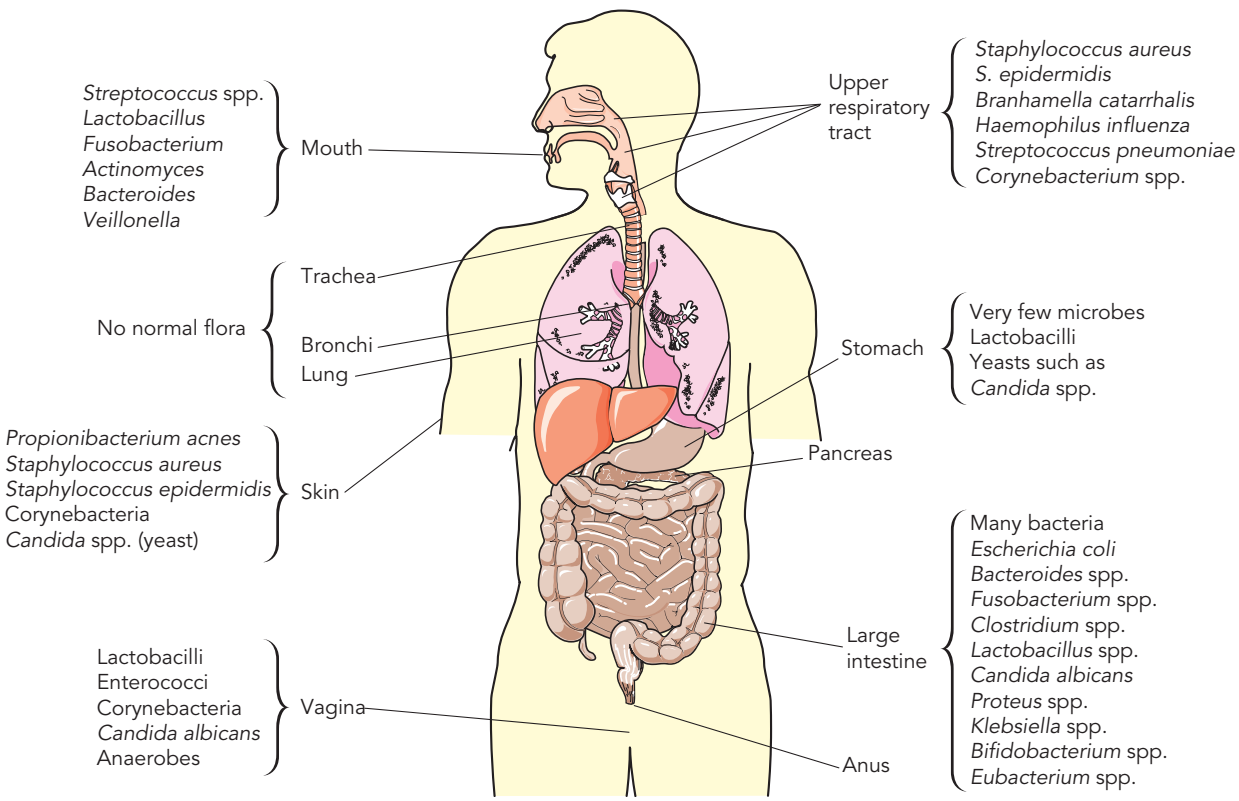


FIGURE 7.2

Location of normal flora on the human body

example of these would be latent viruses, such as members of the herpes family—herpes simplex virus, Epstein-Barr virus (EBV) and cytomegalovirus (CMV)—but there may be others not yet identified (see Chapter 5).

Normal flora of the skin

The skin harbours a diverse population of microorganisms. Bacteria reside in or on the dead layers of skin, obtaining nutrients from the secretions of the sebaceous glands and hair follicles. There are two distinct cutaneous populations: contaminants and resident flora. The **contaminants**, or **transient** organisms, cling to the skin surfaces but do not usually replicate there. They include any organisms that the body (especially the hands) may have come into contact with during normal daily activities. Contaminants are greatly influenced by the personal hygiene of the individual. For health workers, the organisms carried on the hands are important in the transmission of disease.

The **resident flora** of the skin usually live in warm, moist areas of the body such as the axillae (armpits) and groin, in deep crevices in the skin layers, in hair follicles and in sweat glands. There are relatively few organisms present on the exposed, dry areas of the skin surface. The organisms usually present include species of *Staphylococcus*, *Corynebacterium*, *Propionibacterium* and some fungi. *Staphylococcus epidermidis* is found mainly on drier areas of the skin, whereas *S. aureus* prefers a moist environment such as the nasal passages. The organisms that populate the axillae metabolise the chemicals in sweat, producing breakdown products with a distinctive odour (body odour). Most commercial deodorants act by inhibiting the metabolic action of the bacteria or by drying the skin surface so that the bacteria cannot multiply.

Normal flora of the respiratory tract

The upper respiratory tract has a rich population of resident flora, including streptococci, staphylococci, diphtheroids and Gram-negative cocci. *Staphylococcus aureus* is found mainly in the nasal passages. Alpha- and beta-haemolytic streptococci and *Haemophilus influenzae* are frequently found in the throat. *Streptococcus pneumoniae* may be present and is often responsible for middle ear infections (otitis media). Other organisms that may be present include *Neisseria meningitidis*, *Moraxella (Branhamella) catarrhalis* and *Candida albicans*.

The bronchial tubes and lungs are relatively free of organisms in healthy individuals.

Normal flora of the gastrointestinal system

The gastrointestinal system consists essentially of a long tube extending from the mouth to the anus. Varying conditions of oxygen tension, availability of nutrients and pH occur along this system, and this is reflected in the different microbial populations that exist in each region.

The mouth contains a distinctive population of oral bacteria, the mucous membranes of the mouth supporting as many as 10^{11} bacteria per gram wet weight of tissue. These bacteria are usually present as commensals, but can also be responsible for diseases in the mouth. The viridans group of streptococci are common members of the flora of the upper respiratory tract. They include the aerobes *Streptococcus mutans* and *Streptococcus sanguis* which can contribute to dental decay (**caries**) by secreting glucan, a sticky polysaccharide that attaches to the enamel of the tooth surface. **Plaque** consists of a film of bacterial cells anchored in a matrix of glycoproteins and polysaccharides that the organisms have secreted. This matrix provides a stable environment in which other oral bacteria thrive and metabolise sugars, producing acid that contributes to dental decay (see Figure 7.4).

Other bacteria, including *Lactobacillus*, *Fusobacterium* and *Actinomyces*, which prefer an anaerobic environment, thrive on the gums or in crevices between the teeth. Sometimes these organisms are responsible for an infection of the gums known as **gingivitis**.

The pH of the stomach is acid due to the secretion of hydrochloric acid, and inhibits the growth of most

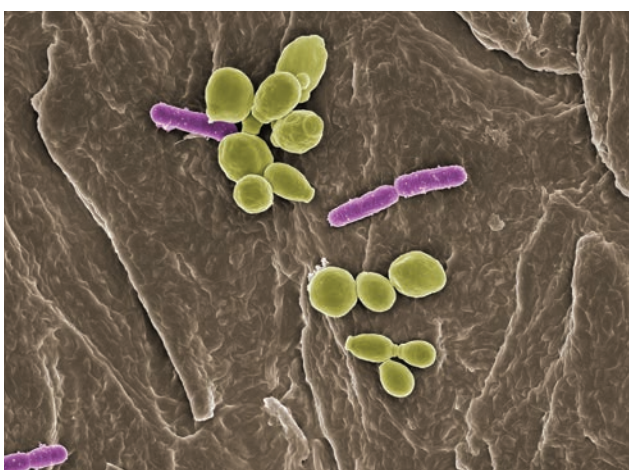


FIGURE 7.3

Coloured SEM showing *E. coli* (purple) and *Candida albicans* (green), contaminants on human finger

Source: Visuals Unlimited, Inc./Dr Stanley Flegler/Getty Images Australia Pty Ltd.

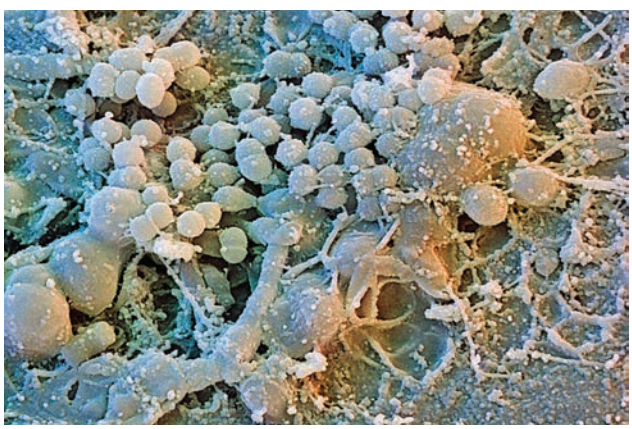


FIGURE 7.4

Dental plaque

Source: David Scharf/Science Photo Library.

microorganisms. A few acid-tolerant lactobacilli may be present in the gastric mucosa. *Helicobacter pylori*, a bacterium that has been shown to be involved in the occurrence of gastric ulcers, can also tolerate this environment, but is not considered part of the normal flora. The upper part of the ileum (duodenum) is slightly alkaline and is also relatively free of bacteria. The lower intestine, the caecum and colon, harbours a large population of bacteria (approximately 10^{12} bacteria per gram of intestinal contents) which can comprise up to 30 per cent of the faecal volume.

The predominant flora are anaerobes or facultative anaerobes such as *Escherichia coli*, *Klebsiella* and *Enterobacter*, but strict anaerobes such as *Bacteroides* are also present in large numbers (see Table 7.1). The presence of *E. coli* in water samples is often used as a test for faecal contamination. Bacteria in the colon are of benefit to the host by digesting waste material and converting it to faeces, as well as the production of vitamins K and B and short-chain fatty acids (SCFA) which are important in maintaining the health of the colon. Anaerobic conditions in the colon result in the formation of gas (flatus) and various breakdown products with a strong, distinctive odour.

Normal flora of the genitourinary tract

The major components of the urinary system—the kidneys, bladder and upper part of the urethra—are usually sterile. The lower portion of the urethra, especially in females, may harbour microorganisms that are representative of those present on the skin, vagina or colon. They are usually flushed out regularly by the flow of urine but if they persist can cause urinary tract infections.

The composition of the normal flora of the vagina illustrates how the flora change depending on age and physiological conditions. Before puberty, the pH of the vagina is about 7 and the main inhabitants are staphylococci, streptococci, diphtheroids and some coliforms. After puberty, the hormone oestrogen stimulates the secretion of glycogen by the vagina. Fermentation of glycogen is carried out by lactobacilli with the production of acid, which lowers the pH to about 5. This acidic pH is protective, inhibiting the growth of many other organisms and establishing conditions suitable for fertilisation. If the lactobacilli of the vagina are destroyed by prolonged antibiotic therapy, or the pH is altered by hormonal or physiological disturbances, an overgrowth of other organisms frequently ensues (e.g. *Candida albicans*, causing thrush).

INFECTION AND DISEASE

Up to now we have been considering the microorganisms that live on the human body as a normal part of our environment. However, a delicate balance exists between a state of health and disease. Under certain conditions, many of the normal flora, as well as organisms from the external environment, have the potential to cause disease.

The first step is for potential pathogens to become established or colonise the human body. They then have an opportunity to penetrate the host defences, invade the tissues, multiply and set up an **infection**. When the results of the infection produce clinical symptoms, a disease results.

Disease is defined as any harmful alteration to the physiological or metabolic state of the host. An **infectious disease** occurs when this alteration is caused by a microorganism or its products, such as **toxins** (see Chapter 10). Any organism capable of causing disease is called a **pathogen**. The microorganisms responsible for the infection may come from anywhere in the environment or from the human body itself.

Endogenous and exogenous infections

Depending on the source of the causative organism, an infectious disease can be classified as endogenous or exogenous.

Endogenous infections occur when the source of the pathogen is the human host itself. Thus, diseases caused by

TABLE 7.1 Representative examples of normal human body flora[a]

Skin	*Propionibacterium acnes*, *Staphylococcus aureus*, *S. epidermidis*, *Corynebacterium xerosis*, *Candida* spp. (yeast)
Eyes	*Staphylococcus aureus*, *S. epidermidis*, diphtheroids
Upper respiratory tract	*Staphylococcus aureus*, *S. epidermidis*, diphtheroids (mainly in nose)
Nose/throat	*Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria* in throat, *Moraxella* spp., *Micrococcus* spp.
Gastrointestinal system	
Mouth	*Streptococcus* spp., *Lactobacillus*, *Fusobacterium*, *Actinomyces*, *Bacteroides*, *Veillonella*, *Candida* (yeast)
Stomach	*Lactobacillus*
Large intestine	More than 300 species, including *Bacteroides*, *Enterococcus*, *Enterobacter*, *Escherichia coli*, *Proteus*, *Klebsiella*, *Bifidobacterium*, *Candida* (yeast), *Clostridium perfringens*
Genital tract	*Lactobacillus*, *Streptococcus*, Corynebacterium, diphtheroids, *Staphylococcus*, *Candida* (yeast), *Trichomonas vaginalis* (protozoan)
Urinary tract[b]	*Staphylococcus*, *Streptococcus*, *Proteus*, *Enterococcus*, *Lactobacillus*, *Klebsiella*

[a] Unless otherwise indicated, these organisms are bacteria.
[b] In females, flora exist in the lower portion of the urethra. The population is similar to that found in the large intestine. In males, the urethra has a much smaller population of organisms.

normal flora are endogenous. These organisms usually cause the infection when they are displaced from their normal habitat to a susceptible body site. For example, skin bacteria such as *Staphylococcus epidermidis* are a major cause of infection in surgical wounds and intravenous lines. Nasal carriers of *S. aureus* can transfer bacteria from the nose to an open wound or sore. Cells of *Escherichia coli* may be carried from the colon to the bladder and cause a urinary tract infection. Latent viral infections may be reactivated when the host immune defences are reduced.

Congenital infections are a type of endogenous disease that occur in the foetus or neonate when the source of the pathogen is the mother. Sometimes, the pathogen crosses the placenta during pregnancy and produces congenital defects such as occur with rubella, syphilis, cytomegalovirus and toxoplasmosis. If the mother is a carrier of a blood-borne virus, the baby may become infected late in pregnancy or at the time of birth, as occurs with hepatitis B and HIV. Sometimes, the foetus is infected *in utero* by organisms from the vagina (e.g. group B streptococci or *Mycoplasma*). These latter infections have been implicated as a cause of premature labour.

Exogenous infections are caused by organisms from the external environment. They may be acquired by cross-infection from patients in the hospital environment, or from people in the wider community. Most infectious diseases are acquired in the community and are transferred from person to person by various routes (see Chapter 8). If the infectious disease is treated at home, the pathogen remains in the community. Sometimes the disease requires hospitalisation and so the pathogen is introduced into the hospital environment, where it may spread.

Exogenous infections acquired during a patient's stay in hospital are called **healthcare-associated infections** (**HCAIs**). They were previously called **hospital-associated infections** (**HAIs**) or **nosocomial infections** (see Chapter 13). It is not always possible to distinguish between a HCAI and one that is acquired in the community. Infections due to pathogens acquired in hospital are often life-threatening, as the hospital environment harbours a large population of antibiotic-resistant organisms and hospitalised patients are frequently very susceptible. The major risks to patients are from medical or surgical procedures, or from contact with contaminated surfaces or the hands or clothing of health workers. When such infections can be largely attributed to invasive medical procedures they are termed **iatrogenic**.

THE DISEASE PROCESS

Pathogenicity and virulence

Pathogenicity is defined as the capacity to produce disease. This capacity depends on certain characteristics of the organism, including the ability to:

- gain entry to the host
- attach to the host tissues and multiply
- evade the host defences
- damage tissue and produce disease symptoms.

The degree of pathogenicity or **virulence** of different types of microorganisms ranges from mild to serious or even fatal. Some diseases are not as serious as others, and this depends on the type of pathogen involved. For example, the cold virus is a pathogen, but the disease it causes is usually only mild, whereas infection with the influenza A virus is much more serious. An infection with the rabies virus is nearly always fatal without treatment.

Differences in pathogenicity and virulence are also related to the strain of the organism and its relative ability to infect and cause disease.

Virulence depends on a number of factors, such as how easily the organism invades the host cell and produces damage. Some organisms are able to carry this out more effectively than others and so are described as being very virulent. For example, encapsulated strains of *Streptococcus pneumoniae* cause pneumonia, whereas unencapsulated strains are not pathogenic. The presence of the capsule allows the bacterium to attach to and invade the host tissues, and to evade phagocytosis by the host's white blood cells.

Other virulence factors include the enzymes and toxins that damage the host cell. Different strains of the same organism may show a variation in virulence. For example, some strains of *Streptococcus pyogenes* are very invasive and cause rapid, severe, sometimes fatal infections (see Case History 7.1). Other strains are not as virulent and produce milder symptoms (e.g. 'strep throat'). Some strains of *E. coli* produce toxins which cause serious diseases such as 'travellers' diarrhoea' and haemolytic uraemic syndrome (see Chapter 18). The difference in virulence between strains is related to the virulence factors (and toxins) they produce. Some strains of influenza are more virulent than others, causing serious epidemics throughout the world.

The mechanisms used by pathogens to produce disease are discussed in detail in Chapter 10.

The virulence of a particular organism may be altered by its passage through a susceptible animal host. The mutational changes that give rise to new strains of influenza each winter tend to occur when the influenza virus enters the pig or duck population in Asian countries, allowing mixing of strains that can give rise to a more virulent strain. Sometimes this change occurs spontaneously, when influenza spreads through the human population. The strain of the virus isolated at the end of an epidemic may be more or less virulent than the original pathogen. The virulence of a particular strain of influenza A is determined by the characteristics of the haemaglutinen (H) and neuraminidase (N) spikes on the capsule (see Spotlight box: Influenza A, Chapter 5, page 96).

In the laboratory, the virulence of a particular organism can often be decreased or *attenuated* by repeated passage through cell culture, or prolonged growth in artificial media. This technique has been used successfully to produce weakened strains of organisms which can then be used as vaccines (e.g. the MMR vaccine contains attenuated strains of measles, mumps and rubella viruses).

CASE HISTORY 7.1

Life-threatening cellulitis after tattooing

A 23-year-old man presented to the emergency department of a hospital in the North Island of New Zealand with an infection associated with a tattoo on his lower back and legs. He had undergone a traditional Samoan tattoo and become progressively unwell after the procedure. By day 7 he was suffering from fever, rigor and breathlessness. On admission he was hypotensive, and there was pain, inflammation, skin breakdown and a purulent discharge from the tattooed area. He was diagnosed with severe acute cellulitis, septic shock and acute renal failure. *Staphylococcus aureus* and *Streptococcus pyogenes* were isolated from the infected site. He was treated with intravenous fluids, a prolonged course of intravenous antibiotics, and required surgical debridement of the wound, plastic surgery and skin grafts. He was discharged after six weeks but required ongoing outpatient treatment.

Comment

This was one of three cases that presented to the same hospital over a period of six weeks. Another 26-year-old man also presented with severe cellulitis and multi-organ failure. The third case required intravenous antibiotics for cellulitis of his arm.

A public health investigation found that all three men had visited the same tattooist, who practised traditional Samoan tattooing from his garage. Although very experienced, he did not follow any sterilisation or infection control procedures, there were no handwashing facilities, and it appeared that the tattooist did not recognise the infection risks involved. Further investigation revealed eight other cases of infection linked to traditional tattooing in the same area within the previous six months.

These cases highlight the risks associated with tattooing, especially by traditional practitioners who are not aware of appropriate infection control procedures. There are legal, cultural and ethical issues surrounding the rights of traditional practitioners while ensuring that health standards are maintained.

Source: Adapted from M. McLean and A. D'Souza 2011, Life-threatening cellulitis after traditional Samoan tattooing. *Australian and New Zealand Journal of Public Health* 35(1): 27–29.

Questions

1. Discuss the ways in which these men could have become infected.
2. What is the likely source of the infection?
3. What other diseases have been shown to be transmitted by unsafe tattooing practices?
4. How can the risks be avoided?

Infective dose

Another important factor in determining whether a disease will occur is the **infective dose**, or the number of cells of the organism that are able to enter the human body and become established. If only a few cells are present, then the host defences may be able to destroy them. However, if the numbers are large they may overwhelm the defences. For example, stomach acid may be sufficient to neutralise the few bacteria normally contained in food, but is not able to inactivate all the microbes in a heavily contaminated food sample. In general, the more virulent the organism, the fewer the number of cells that will be needed to establish an infection and produce disease symptoms. For example, a gastrointestinal infection due to *Shigella* may require as few as 100 organisms to be present in contaminated food or on the hands, whereas a million *Salmonella* cells might be needed under similar circumstances.

Opportunistic infections

So far we have been describing the characteristics of true pathogens—that is, microorganisms that always overcome the natural host defences and cause disease in an otherwise healthy host. However, a significant number of diseases are caused by microorganisms that set up an infection because they have been moved from their normal habitat, or because the host defences are lowered or compromised in some way. For this reason, we define another type of relationship that may occur.

Opportunism refers to a situation where microorganisms that *would not normally cause an infection* do so because of an alteration in physiological conditions in the host, or a change in the environment where the organism is located. These organisms are called **opportunistic pathogens**. They are able to gain entry and set up an infection in a host when the host defences are lowered or compromised in some way. The seriousness of the infection and the ease of treatment depends on various factors, including the immune status of the host, the site of infection and the properties of the particular organism causing the infection.

These infections may be caused by normal flora of the host that have been displaced from their usual habitat. For example, many of the infections in surgical wounds or those associated with intravenous catheters or central lines are due to *Staphylococcus epidermidis*, derived from the patient's own skin. Urinary tract infections are frequently caused by bacteria that are normally resident in the large intestine but which have been transported from the anus to the opening of the urethra, from where they can gain entry to the bladder.

An alteration in the physiological or hormonal state of a patient may encourage the overgrowth of one of the normal flora. For example, as mentioned before, the yeast *Candida albicans*, a normal inhabitant of the vagina, is often the cause of vaginitis, or thrush. Prolonged use of antibiotics destroys the normal flora of the bowel and allows overgrowth of organisms such as *Clostridium difficile*, which causes severe diarrhoea and pseudomembranous colitis.

The most serious opportunistic infections occur when the normal defences of the host are lowered. This is probably of greatest importance in hospitals where many of the patients have a real or induced deficiency of their immune system, placing them at great risk of acquiring infection. Transplant patients on immunosuppressive drugs, cancer patients undergoing chemotherapy and AIDS patients are particularly vulnerable to opportunistic infections as their own immune system cannot function to fight the invading pathogens. Some of the common opportunistic infections seen in these patients are fungal infections such as systemic candidiasis and cryptococcal meningitis, reactivation of latent viral infections such as cytomegalovirus and herpes, and the atypical pneumonia due to *Pneumocystis jiroveci* (previously called *Pneumocystis carinii*).

Host resistance or susceptibility

One of the most important factors to consider when discussing an infectious disease is the ability of the host to resist invasion by the pathogen. The human body has a complex system of specific and non-specific mechanisms to deal with foreign particles and microorganisms. These are discussed fully in Chapter 9. One of the most important of these is the mechanical barrier provided by an intact skin. Once this barrier is broken (e.g. in wounds, surgery or burns), microorganisms are able to gain easy access to the tissues. Table 7.2 summarises the risk factors that increase susceptibility to infection.

TABLE 7.2 Factors that increase susceptibility to infection

FACTOR	SUSCEPTIBILITY
Age	Neonates have immature immune system. Elderly have decreased immune system. Some pathogens have increased virulence in different age groups.
Pregnancy	More susceptible to some diseases, e.g. hepatitis E, malaria.
Nutrition	Malnutrition and alcoholism lower immune response.
Illness	Underlying illnesses—e.g. diabetes, cancer, liver disease—contribute to susceptibility.
Immunosuppressive drugs	Transplant patients have lowered immune response.
Chemotherapy	Cancer patients susceptible, due to effects of drugs.
Atmospheric pollution	Lung damage increases susceptibility to respiratory infections.
Surgery/Trauma	Provides portal of entry for pathogens.
Physical defects	Provide site for infection.
Stress	Lowers immune response.
Immune diseases, acquired or genetic	Lower immune response.
Gender/Genetic predisposition	Variable effect on susceptibility to different diseases.

The general health of the host is important in determining the outcome of exposure to a pathogen. A healthy person is often able to ward off an infection, whereas someone with an additional, often unrelated, illness may succumb. One of the most difficult problems for health workers is when a patient presents with an infection or infectious disease which is really an expression of a number of underlying diseases. This is especially true of patients in some remote areas of Australia, and among Indigenous Australians and people in lower socioeconomic groups. Underlying illnesses such as diabetes or anaemia may contribute to patient susceptibility. Those with a lowered white blood cell count (**neutropenia**) or suffering from leukaemia are at greater risk of infection.

Some treatments for other diseases affect the functioning of the immune system—for example, cancer patients receiving chemotherapy and transplant patients receiving immunosuppressive drugs to prevent rejection. Most of these drugs also interfere with the body's ability to fight foreign microorganisms, rendering these patients at risk from infectious diseases. Patients with immune disorders are obviously more susceptible to all types of infection.

General lifestyle and nutritional status affect the susceptibility of the host. Malnutrition and alcoholism both contribute to lowered body defence mechanisms. Injecting drug users are at risk of acquiring blood-borne pathogens such as hepatitis B, hepatitis C and HIV. Cigarette smokers tend to be at greater risk of lung infections and bronchitis. Many people who die from Legionnaires' disease have a previous history of lung problems associated with smoking.

Age is an important factor, as it often reflects the status of the immune system. Neonates and infants up to about one year of age have a poorly developed immune system and so are particularly susceptible to infection, even though they have some protection from maternal antibodies. As their immune system matures they are able to deal with invading pathogens and develop their own immunity. The immune system appears to decline in old age, so the elderly are at greater risk of infection. Males and females show a different pattern of susceptibility. For example, females suffer more urinary tract infections because of the comparative shortness of the female urethra and the proximity of the anus to the urethra. During pregnancy, the immune system is slightly compromised and so pregnant women tend to be more susceptible to some infections. Hepatitis E and malaria are two diseases with an increased risk of a fatal outcome during pregnancy.

There is considerable evidence that the genetic makeup of an individual plays a part in susceptibility to infection. The reason for this is not well understood at present, but there are documented cases of differences in the ability of various individuals to resist disease that can only be explained on the basis of genetic differences in their immune system.

Predisposing factors

As well as individual susceptibility, a number of external factors contribute to the likelihood of someone acquiring an infectious disease. High on the list is the environment in

CASE HISTORY 7.2

Respiratory syncytial virus in a nursing home

In early 2011 the deaths of five elderly residents in a nursing home in Western Sydney were reported to NSW Health. The residents were found to have contracted respiratory syncytial virus. The virus is a common cause of bronchiolitis in children under 2 years, but is usually not serious in older people. The nursing home had to be closed temporarily, but no breaches of infection control protocol were found. It was thought that the virus had been brought in by a visitor.

Questions

1. Why was NSW Health informed of the deaths?
2. Why was the nursing home closed temporarily?
3. What factors contributed to the fatal outcome?
4. Is there any way these deaths could have been prevented?

which they live and the general hygiene of their surroundings. The climate often has an effect, as it may favour the persistence of a pathogen or its vector. The weather, winds, temperature or natural disasters such as floods will facilitate the spread of microorganisms. Air pollution affects the lungs and lowers resistance to respiratory pathogens. A viral infection such as a cold or influenza may predispose a patient to a secondary bacterial infection such as pneumonia. Many infectious diseases have a seasonal occurrence; for example, influenza occurs more often in the winter months, chickenpox usually in spring.

Patients who are hospitalised are often at greater risk of acquiring an infection than they would be outside the hospital environment. This is largely due to their lowered resistance because of illness, but is also related to the invasive procedures they may have undergone. In the hospital environment, they are likely to be exposed to large numbers of microorganisms, many of which are resistant to antibiotics. Hospital-acquired infections are discussed in Chapter 13.

KOCH'S POSTULATES

How do we know that microorganisms cause disease? Today, we take it for granted that microorganisms are responsible for the clinical signs and symptoms of an infection. This was not always the case—in 1876, when the German microbiologist Robert Koch (see Figure 7.5) proposed his **germ theory of disease**, he had to overcome significant opposition to his ideas. Koch investigated the cause of a disease, anthrax, that was killing the cattle in Europe and discovered that a bacterium he named *Bacillus anthracis* was responsible (see Chapter 1, Figure 1.4, page 8). He subsequently went on to isolate other bacteria that were responsible for causing

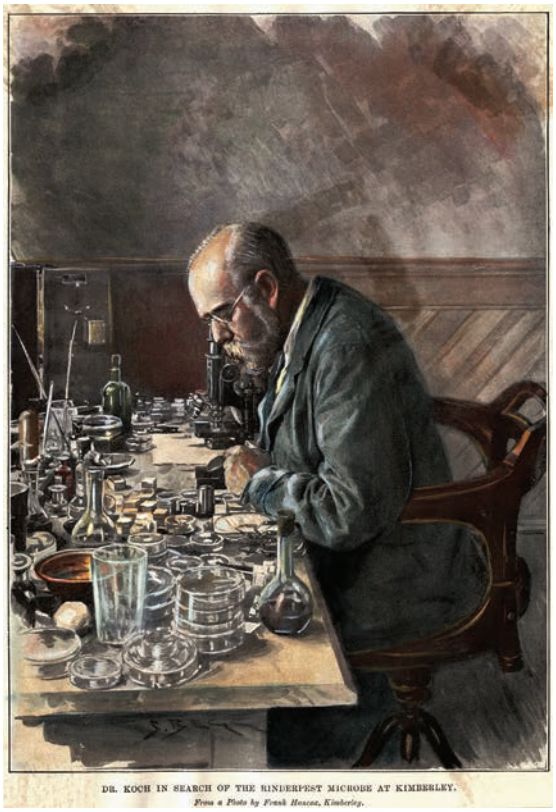


FIGURE 7.5

Nobel Laureate: Robert Koch

Source: © Bettmann/Corbis.

tuberculosis and cholera. Based on his research, Koch put forward a set of criteria that should be met when deciding whether an organism is responsible for causing a particular disease. They are called **Koch's Postulates** and can be summarised as follows:

- The organism must always be present in every case of the disease.
- It must be possible to isolate the organism from the diseased host and grow it in pure culture.
- The pure culture of the organism, when inoculated into a new susceptible host, must produce the same symptoms of disease.
- It must be possible to recover the organism from the experimental host.

Koch's work was important in developing the concept of 'one germ, one disease'—that is, that a single type of organism is responsible for causing a disease with a characteristic group of symptoms. These postulates provided a sound scientific approach to the problem of identification of the causative agents of most bacterial diseases and, in the 30 years following the publication of his postulates, scientists around the world adopted his methods and were successful in isolating and identifying the bacteria responsible for many of the then known diseases (see Chapter 1, Table 1.3, page 9).

These methods were very useful for the identification of bacteria that could be easily grown in the laboratory but, during the 20th century, diseases caused by viruses were identified. Although the basic principles of Koch's Postulates still stand, they need some modification in the light of our current understanding of the nature of infectious diseases. Other pathogens have been described in recent years and there are new technologies being used for identification of microorganisms. For example:

- Some organisms are difficult to grow in pure culture away from the host. *Mycobacterium leprae*, the causative organism of leprosy, has never been grown on artificial media, nor have the hepatitis C or human papillomaviruses. Viruses and the intracellular bacteria chlamydiae and rickettsiae can be grown only in cell culture.
- Some of the newer viruses have only been able to be identified by molecular techniques (e.g. hepatitis C).
- In 1976 a previously unknown organism, *Legionella pneumophila*, was identified as the bacterium responsible for Legionnaires' disease. Although it is now possible to grow it in the laboratory on special medium, the bacterium was originally identified by an indirect method. Samples of lung tissue from infected patients were injected into guinea pigs to produce antibodies to the infectious agent. Patients who had had Legionnaires' disease were tested and found to have antibodies to the same bacteria that had produced the disease symptoms in the guinea pigs.
- Some diseases can be caused by more than one type of pathogen. For example, pneumonia, which is really an infection of the lungs, can be caused by any one of a number of organisms, some of which are listed in Table 7.3.
- Some organisms apparently cause more than one disease. For example, streptococci, which are responsible for 'strep throat', may also release bacterial antigens into the bloodstream and affect the heart, causing a post-streptococcal hypersensitivity response known as rheumatic fever.
- Sometimes a pathogen may enter the body, giving rise to a **subclinical infection** without any clinically identifiable disease symptoms. This is often the case with diseases such as rubella (German measles) and glandular fever. Other pathogens, especially viruses belonging to the herpes family, can remain in the body in a *latent* form and be reactivated when the physiological state of the host changes.

None of these situations fulfils the criteria used by Koch. However, even with these modifications, Koch provided a set of scientific principles that formed the basis for a sound experimental approach to the investigation of the **aetiology** (cause) of infectious diseases.

TABLE 7.3 Some microbial causes of pneumonia

Bacteria	*Streptococcus pneumoniae*
	Staphylococcus aureus
	Haemophilus influenzae
	Escherichia coli
	Klebsiella pneumoniae
	Pseudomonas aeruginosa
	Legionella pneumophila
	Mycobacterium tuberculosis
	Mycoplasma pneumoniae
Chlamydia	*Chlamydia* spp.
Rickettsia	*Coxiella burnetii*
Viruses	Influenza virus
	Respiratory syncytial virus
	SARS (severe acute respiratory syndrome)
Fungi	*Pneumocystis jiroveci**
	*Histoplasma capsulatum**

Note: *In immunosuppressed patients.

SIGNS AND SYMPTOMS OF DISEASE

In the previous sections we distinguished between situations where microorganisms reside normally in or on the human body and those cases in which an infection or disease is present. To decide whether a state of infection or disease exists, it is usual to observe the patient for the presence of tell-tale signs or symptoms. These represent pathological changes or damage to the host cells and tissues; they are often characteristic of the type of infection and so are useful for diagnosis.

Signs are measurable changes in the patient that can be observed by examination of the patient or their body fluids. They include occurrences such as fever, swelling (oedema), rashes, vomiting and diarrhoea, as well as data derived from laboratory tests.

Symptoms are changes that are felt and reported by the patient, such as pain, headache, nausea, or a general feeling of illness (**malaise**). Sometimes these categories overlap. The combination of signs and symptoms that characterises a particular disease state is called a **syndrome** (e.g. AIDS stands for **a**cquired **i**mmune **d**eficiency **s**yndrome).

Fever

Fever (**pyrexia**) almost invariably accompanies serious infections and is one of the most useful early warning signs. Monitoring of a patient's temperature will often indicate that an infection is present, long before other symptoms appear. Human body temperature is maintained within narrow limits by the temperature-regulating centre in the hypothalamus in the brain. An increase in body temperature (fever) is produced when chemicals called **pyrogens** act on the hypothalamus and reset the thermostat to a higher temperature.

Endogenous pyrogens are produced within the human body as part of the immune response by the phagocytic white blood cells, monocytes and macrophages (see

Chapter 9). The main ones are **interleukin-1** (**IL-1**) and **tumour necrosis factor** (**TNF**).

Exogenous pyrogens are products of infectious organisms and act by stimulating the release of endogenous pyrogens such as IL-1, which in turn act on the hypothalamus as described above. One of the most important is an endotoxin called lipid A, which forms part of the lipopolysaccharide found in the cell wall of some Gram-negative bacteria (see Chapter 3).

Fluids to be administered to patients (such as IV solutions) are frequently labelled 'pyrogen-free'. This means they do not contain any fever-producing substances such as fragments of dead microorganisms. Endotoxins can also cause other serious effects in the human host (see Chapter 10).

The body's immediate response to an increase in the temperature set point in the hypothalamus is to try to raise the body temperature by involuntary muscle contraction (shivering) and the narrowing of surface blood vessels to prevent heat loss (**vasoconstriction**). The effect is that the patient becomes pale and feels cold or chilled. When further fluctuations occur in the set point control, returning it to the normal lower temperature, the body responds by trying to increase heat loss to lower the body temperature. This involves sweating, and dilation of the surface blood vessels, causing reddening of the skin or flushing. The patient feels hot and has a fever.

Nearly all fever is caused by an infection. **Pyrexia of unknown origin** (**PUO**) is a term used to describe a situation where the patient has an unexplained fever. In more than 50 per cent of these cases, an infection is present. There are a few non-infective causes of sustained fever, including some malignancies and some vascular diseases.

Inflammation

Inflammation is a common non-specific response that occurs at the site of injury or infection. The syndrome, which consists of heat, pain, redness, vasodilation and swelling, is described in Chapter 9. When the host tissue is damaged, one of the first reactions is the activation of the inflammatory response, which is mediated by a number of chemicals released by the damaged cells and other cells of the immune system.

Important among these chemicals is **histamine**, which causes vasodilation and an increase in permeability of the blood capillaries. Fluids and white blood cells (phagocytes) move into the tissues, resulting in redness, swelling and pain. A localised increase in temperature at the site of inflammation is due to this vasodilation. Other chemicals released by the damaged cells attract phagocytic white blood cells (monocytes and macrophages) to the site. They may release pyrogens such as interleukin-1 (IL-1) which act on the hypothalamus (as described above) and produce a rise in temperature throughout the body (fever). The pus that forms at the site of an infection is a mixture of dead microbial cells, dead or damaged host cells and white blood cells.

Many of the signs and symptoms observed are actually a result of the host's own defence system attempting to counter the infection.

Cellulitis

Cellulitis is a common skin condition caused by bacterial infection, usually staphylococci or streptococci. When the skin is broken due to cracking or peeling, or through trauma, wounds, surgery, insect bites, ulcers or abscesses, bacteria are able to gain access to the tissues, setting up an infection and damaging the cells. Symptoms include fever, pain and inflammation in the affected area and a skin rash that progresses to a tight, glossy 'stretched skin' appearance with cracking and peeling (see Figure 7.6). Diagnosis is usually by blood culture to identify the causative bacteria and determine correct antibiotic treatment. In severe cases of disseminated infection the patient may suffer septic shock, infection of other body sites and organ failure (see Case History 7.1, page 143).

Immune reactions

The reaction of the body to infection involves an immune response that is discussed in detail in Chapter 9. Some of the signs of this immune response can be detected and serve as an indication of the presence of infection. Stimulation of the immune system results in increased activity in the lymph nodes or glands, causing **lymphadenopathy** (swollen glands) see Figure 7.7. Changes in the white blood cell population are typical of the body's response to infection and are indicative of certain kinds of disease. **Leukocytosis** is an increase in the white cell count, while **leukopenia** is a decrease in the number of white cells circulating in the bloodstream.

The specific immune response also results in the formation of **antibodies** to the organism responsible for the infection. These can be detected in the blood and their number and type are indicative of the progress of the disease in the patient. IgM antibodies indicate a recent infection, whereas IgG antibodies are indicative of an old infection.

Skin signs

Skin rashes are often non-specific, but some are quite distinctive and play a major role in diagnosis (see Figures 7.8–7.14). For example, the pustular lesions of chickenpox

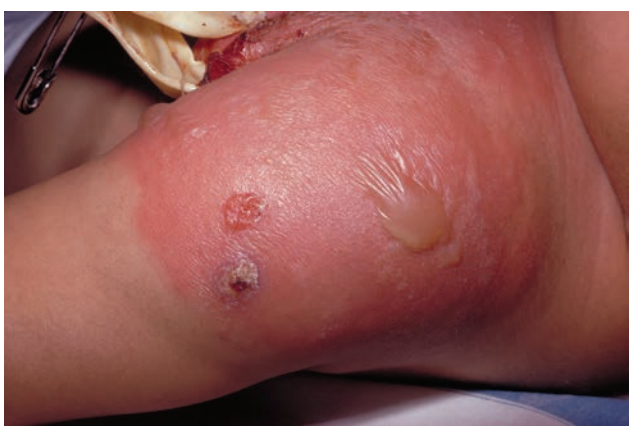


FIGURE 7.6

Cellulitis on the leg of a child

Source: CDC/Allen W. Mathies MD/California Emergency Preparedness Office (Calif/EPO), Immunization Branch.

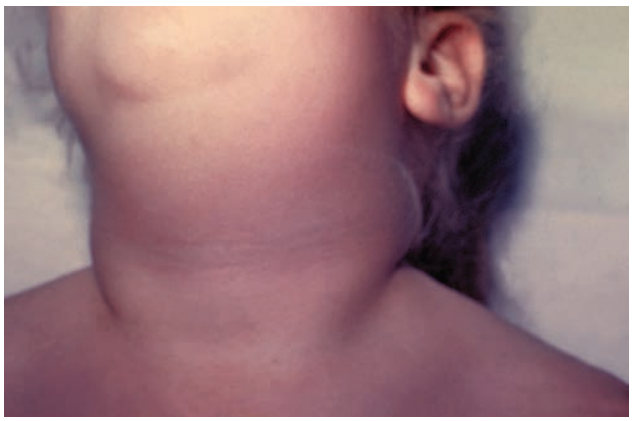


FIGURE 7.7

Child with typical swollen glands associated with mumps

Source: CDC/Dr Heinz F. Eichenwald.

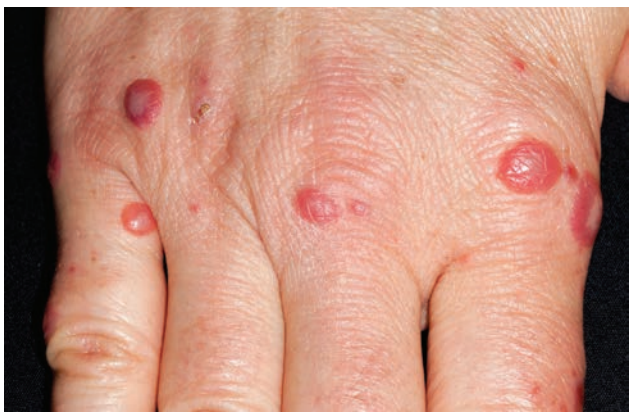


FIGURE 7.8

Distinctive blisters are a symptom of hand, foot and mouth disease, a viral infection caused by coxsackie virus and common in childcare centres

Source: Dr P. Marazzi/Science Photo Library.

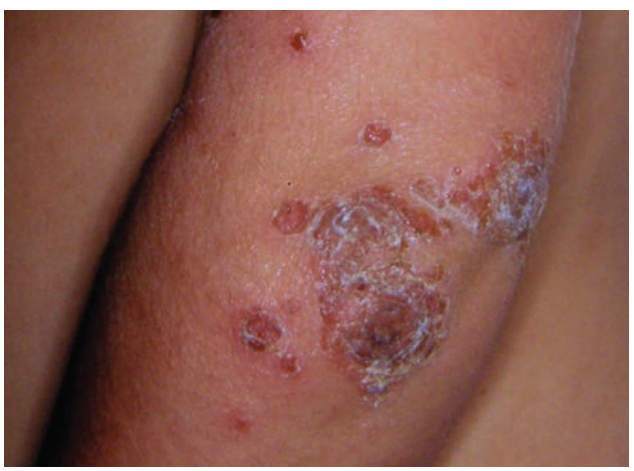


FIGURE 7.9

Impetigo, a skin condition usually associated with staphylococcal or streptococcal infection

Source: Reproduced with permission of New Zealand Dermatological Society Incorporated at DermNetNZ.org.

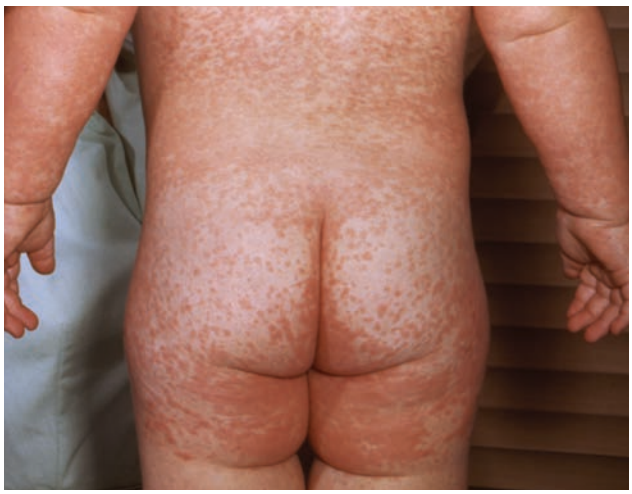


FIGURE 7.10

Maculopapular rash of measles

Source: Centers for Disease Control (CDC).

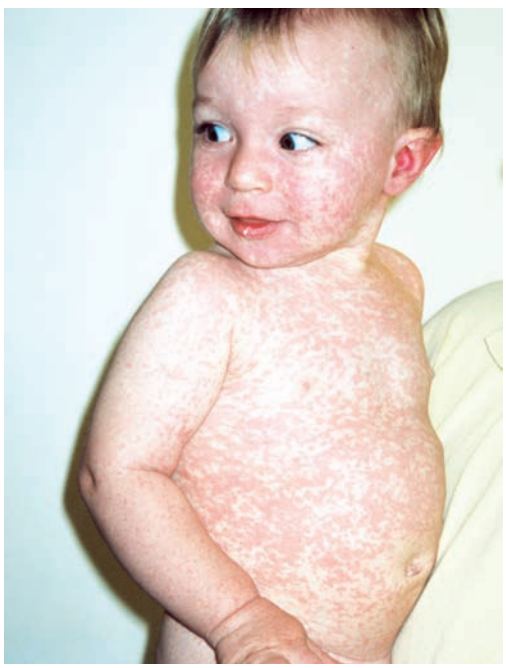


FIGURE 7.11

Non-specific viral rash on 12-month-old child

Children often present with this type of rash, which is difficult to diagnose without other symptoms being present.

Source: Dr Penny Bishop.

are easy to identify. The reaction of the skin during infection can be described in various ways (see Table 7.4).

The signs and symptoms of disease are usually indicative of the body system that is affected. Infections of the gastrointestinal system typically cause nausea, stomach cramps, vomiting and diarrhoea. Headache, stiff neck and confusion indicate infection of the nervous system. Some symptoms are non-specific. For example, headache, nausea and fever may occur in a number of diseases. Frequently, the presence of a high fever will induce vomiting that may be unrelated to

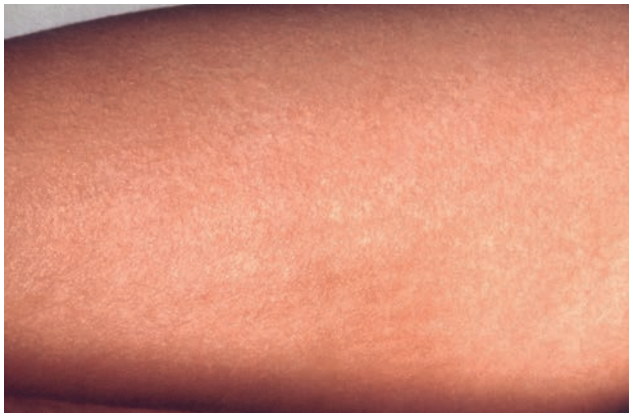


FIGURE 7.12

Rash associated with scarlet fever, caused by *Streptococcus pyogenes*

Source: Centers for Disease Control (CDC).

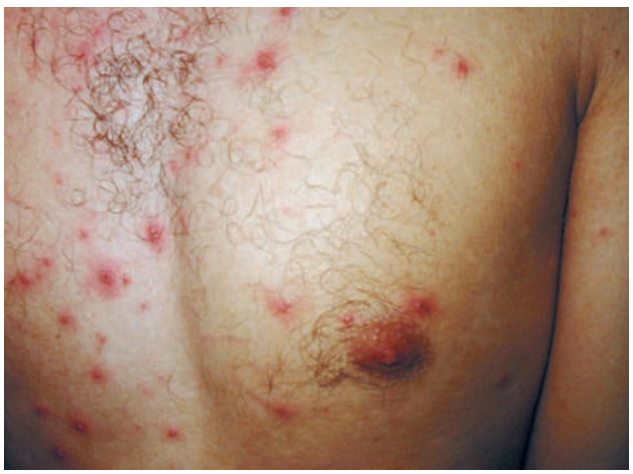


FIGURE 7.13

Chickenpox skin rash

Source: Dr Norma Scott.

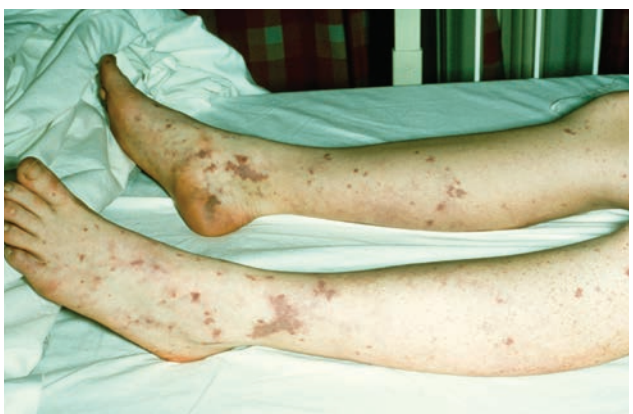


FIGURE 7.14

Meningococcal disease due to infection by *Neisseria meningitidis*

Rash on leg showing extensive damage.

Source: John Radcliffe Hospital/Science Photo Library.

TABLE 7.4 Typical skin signs

Lesion	Any area of damaged tissue.
Erythema	Reddening of the skin, due to dilation of the surface capillaries.
Macule	A small, flat, reddish patch.
Papule	A slightly raised red spot, such as a pimple.
Vesicles	Small blisters, often filled with clear, yellowish fluid (serum).
Pustule	A lesion containing creamy, opaque material (pus).
Ulcer/Erosion	A skin lesion that is deep and reaches subcutaneous tissue.
Abscess	A localised collection of pus.

the true cause of the illness. To diagnose a disease correctly, it is usually necessary to carry out laboratory tests.

Laboratory tests

Laboratory examination of swabs or specimens plays an essential role in diagnosing the causative agents of an infection (see Chapter 15). Tests can be carried out on specimens of blood, sputum, urine, cerebrospinal fluid (CSF) and other tissue samples in order to detect the presence of a pathogen. The presence of microorganisms in a specimen or swab from a normally sterile body site usually indicates that an infection is present. Blood cultures are used to detect systemic infections that are not visible on the body surface, since microorganisms responsible for infections in internal tissues can enter the bloodstream through breaks in the capillary walls.

The detection of bacteria (**bacteraemia**) or viruses (**viraemia**) in the blood is not necessarily significant unless other symptoms are also present.

Sepsis that occurs when the organisms multiply in the bloodstream may result in a **systemic inflammatory response** (**SIR**), which can be fatal.

DEVELOPMENT OF DISEASE

After exposure to the pathogen the course of infection and disease in the human body generally follows a defined pattern or sequence of events (see Figure 7.15). The initial incubation period is followed by the prodromal phase. Then comes the invasive or acute phase of disease (which sometimes involves a peak or crisis). Finally, a period of convalescence follows, while the body recovers. The outcome of the disease may be complete recovery, death, or the establishment of a latent or chronic infection (see below).

Incubation period

After successfully gaining entry to the body, the pathogen has to overcome the host defences in order to establish itself, multiply and cause disease. The **incubation period** is the time interval that occurs between the exposure of the host to

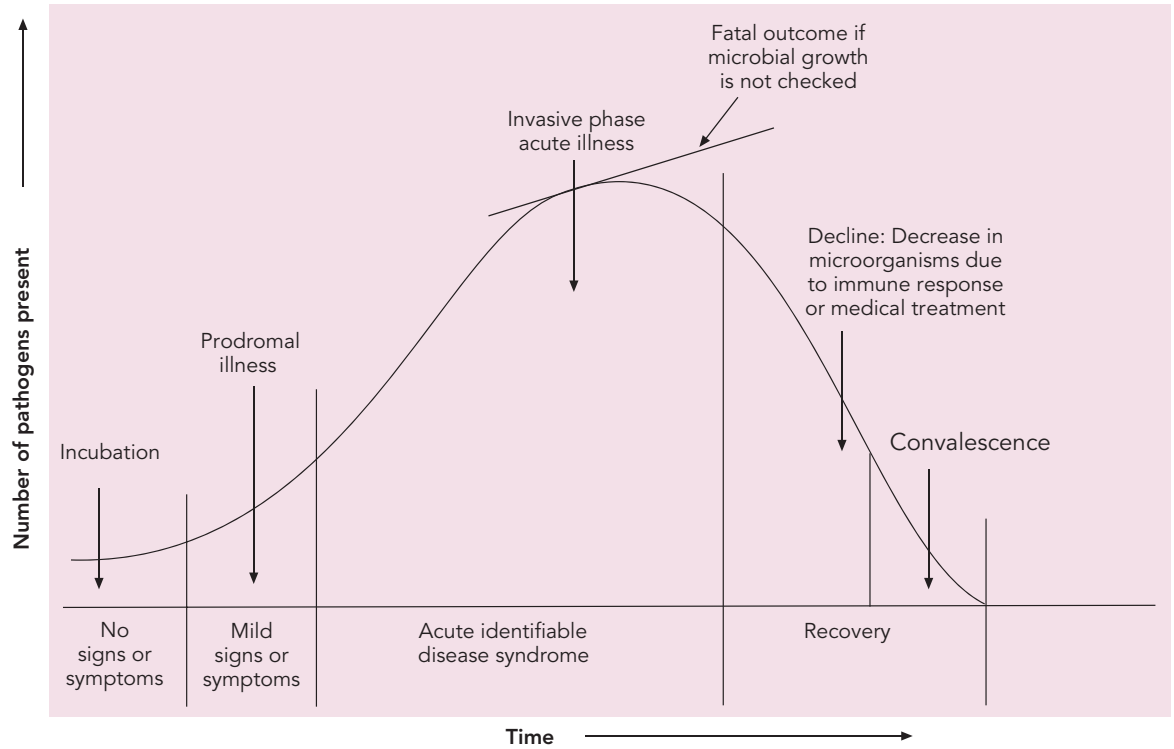


FIGURE 7.15

Stages in the progression of disease

the pathogen and the appearance of any signs or symptoms of disease. The incubation time is affected by a number of factors, including the properties of the pathogen, its virulence, the infective dose, the place of entry of the pathogen into the host relative to its target organ, and the resistance of the host. For example, in gastrointestinal infections, the length of time before the onset of symptoms depends on the nature of the ingested pathogen (bacteria, bacterial endospores, viruses or preformed toxins).

Table 7.5 shows typical incubation times for some infectious diseases. During the incubation period, patients are often unaware that they have been exposed to the pathogen. However, during this period as well as the following prodromal period, they are usually infectious and thus able to spread the disease to others.

Prodromal period

As the pathogen continues to multiply, non-specific disease symptoms are produced such as headache, nausea and general malaise. This is termed the prodromal period and signals the beginning of disease. Sometimes the host defences overcome the pathogen at this stage and the symptoms disappear before a recognisable disease syndrome develops. The host may or may not be left with an acquired resistance (antibodies and memory cells) to the pathogen but, if antibodies are produced, the host is said to have experienced a subclinical infection (see page 154). Not all diseases have a prodromal phase. Many start

TABLE 7.5 **Incubation times for some common infectious diseases**

DISEASE	TIME
Chickenpox	2–3 weeks
Measles	8–14 days
Mumps	12–25 days
Diphtheria	2–6 days
Tuberculosis	4–12 weeks
Rubella (German measles)	14–21 days
Influenza	2–3 days
Food poisoning	12–36 hours
Gangrene	24–48 hours
Tetanus	24–48 hours after injury
Whooping cough	7–10 days
Glandular fever	4–6 weeks
Hepatitis A	3–5 weeks
Hepatitis B	2–6 months
Hepatitis C	6–8 weeks
HIV (appearance of antibodies)	Minimum 3 weeks; may be months or years
Gonorrhoea	2–5 days
Syphilis	2–3 weeks

suddenly with acute symptoms of pain and fever, reflecting the nature of the infection.

Acute or invasive phase

If the early host defences do not overcome the pathogen, the prodromal stage is followed by a period of acute disease, the **invasive phase**, during which the pathogen invades and damages the host tissue. Fever and chills, caused by the presence of pyrogens, are typical symptoms of the invasive phase of the disease.

Some diseases, such as malaria, have repeated episodes of fever and chills, mediated by the synchronised release of merozoites from the red blood cells (see Chapters 6, 19). Others have a longer period of sustained elevated temperature before a sudden drop or a slow decline. There is some evidence that a moderate increase in temperature (up to 40°C) is protective. The multiplication of some microorganisms is inhibited or slowed by higher temperatures. In addition, the higher body temperature may stimulate the rate of the inflammatory immune response.

Some invasive phases reach a crisis or peak over a short period of time, after which symptoms (especially fever) quickly subside. Other diseases are characterised by a longer invasive phase during which additional signs and symptoms occur, such as cough, diarrhoea, jaundice, loss of muscle control, swelling, pain and discharge. These additional symptoms may be due to the action of toxins produced by the pathogen (see Chapter 10). For example, the cough associated with whooping cough develops as the disease progresses and is due to inflammation and damage to the trachea caused by the toxins produced by the causative bacterium (*Bordetella pertussis*). The cough persists for about six weeks while the body's immune system eliminates the toxin—long after the invasive phase has ended and the patient is no longer infectious.

The ability of the host to overcome the invasive phase determines the outcome of the disease—that is, recovery or death (terminal outcome). A **fulminating disease** is an acute infection that progresses very rapidly, often with a fatal outcome. The symptoms appear suddenly and develop quickly into a serious infection (e.g. cerebral malaria, meningococcal meningitis or pneumonic plague). In this situation, the pathogen overcomes the host before protective immunity can develop.

Decline phase

If the immune system responds to the infection and overcomes the effects of the pathogen, the symptoms subside, the disease enters the decline phase and the patient begins to recover. In some cases, however, the host defences are unable to eliminate the pathogen completely from the body and the disease progresses to a latent or chronic state (see 'Types of infection', below).

Effects of drug therapy

The above description of the progress of a disease needs to be modified when drug therapy (antimicrobials, antipyretics or analgesics) is used. In most cases, the use of an appropriate antimicrobial drug will speed up the destruction of the pathogen, dramatically shorten the invasive phase, and prevent a fatal outcome. However, in the case of meningococcal meningitis, one of the undesirable outcomes of antibiotic use is that endotoxins are released from the breakdown of the dead Gram-negative bacteria. These endotoxins can produce serious, sometimes fatal, effects such as fever and septic shock, leading to haemorrhage, collapse of the circulatory system and sequential failure of essential body organs (see Chapter 10).

There is considerable discussion about the merits of using antipyretics, especially aspirin, to reduce fever in the early stages of infection. As mentioned above, fever may sometimes be beneficial as the higher temperatures may slow the rate of bacterial growth, provided the body temperature does not exceed 40°C, when organ damage may occur. There is evidence that the overuse of aspirin in children is associated with the development of **Reye's syndrome**, a sometimes fatal disease characterised by brain damage and degeneration of some internal organs. For this reason, the use of paracetamol is considered preferable, although excess use can cause liver damage.

Convalescence

Convalescence is the time when the body repairs itself and regains strength. The energy that has been used to activate and support the immune response is directed back to the normal processes of cell regeneration and tissue repair. In today's busy society, many people fail to allow sufficient time for convalescence and so are frequently exposed to another pathogen before regaining their full strength. The result is a series of illnesses because the immune system is too weak to protect the body effectively.

TYPES OF INFECTION

When microorganisms invade host tissue, an infection occurs. The type of infection depends on the nature of the invading organism, the site of entry and the susceptibility of the host. The definitions of many of the terms commonly used to describe infection and disease are listed in Table 7.6.

Localised infections

Infections that are confined to the site where the pathogen enters the body are said to be **localised**. Some examples are an infected wound (usually due to bacteria), cutaneous fungal infections, and warts (papillomavirus). In the case of cutaneous infections (warts and fungi), the infection remains localised because the pathogen preferentially attacks epidermal tissue. Other organisms remain on body surfaces because they prefer the lower temperature on the skin (33°C). *Mycobacterium leprae*, the causative organism of leprosy, is one such example, usually occurring only on the nasal mucosa, skin and superficial nerve cells of the limbs (see Chapter 20, Figure 20.11, page 527).

Wound infections, ulcers and abscesses remain localised because they are contained by the inflammatory reactions

TABLE 7.6 Definitions of infection and disease

TERM	DEFINITION
Infection	Situation where microorganisms invade the host tissue and multiply.
Disease	Harmful alteration to the physiological or metabolic state of the host.
Infectious disease	A disease caused by a microorganism.
Pathogen	A microorganism capable of causing disease.
Infective dose	The number of cells of a pathogen that are required to produce disease symptoms.
Virulence	The severity or intensity of the disease symptoms produced by a pathogen.
Signs	Measurable changes in the patient as the result of an infection.
Symptoms	Changes felt by the patient as a result of an infection.
Syndrome	The combination of signs and symptoms.
Bacteraemia	Presence of bacteria in the blood.
Viraemia	Presence of viruses in the blood.
Septicaemia	Multiplication of bacteria in the blood.
Toxaemia	Presence of toxins in the blood.
Types of disease	
Acute	Characteristic symptoms appear and the disease runs its course quickly.
Fulminating	The symptoms appear suddenly and the disease proceeds rapidly, often to a fatal outcome.
Chronic	The disease progresses slowly and persists for long periods with continuous shedding of the pathogen.
Latent	The pathogen remains dormant in the host and may be reactivated under certain conditions.
Subclinical	The infection produces an immune response without recognisable symptoms.
Types of infection	
Localised	Confined to one area of the body.
Systemic	The pathogen progressively affects more than one organ.
Primary	The first sign of infection in a healthy host.
Secondary	Develops when the defences are lowered by the primary infection.
Superinfection	Results from overgrowth of opportunistic organisms, following destruction of normal flora.

described above. Inflammation involves fibrin deposition and causes swelling; this effectively walls off the site of the infection and prevents the escape of the infectious agent (usually bacteria) into the surrounding tissue and blood vessels. Large numbers of phagocytic cells are attracted to the infected site by chemotaxis and attack the pathogen.

Superficial localised infections can be treated with topical antiseptic or antimicrobial ointment. A combination of antiseptic treatment and the non-specific immune response often limits the pathogen to the original site of infection. More serious infections require **debridement** (removal of necrotic tissue and debris) and/or wound cleansing, which may be accompanied by the administration of oral or intravenous antibiotics (see Figure 7.16).

Disseminated infections

A **disseminated infection** occurs when the pathogen escapes from its primary site of infection into the bloodstream or lymphatic system and spreads to other parts of the body where it establishes another infection. Disseminated

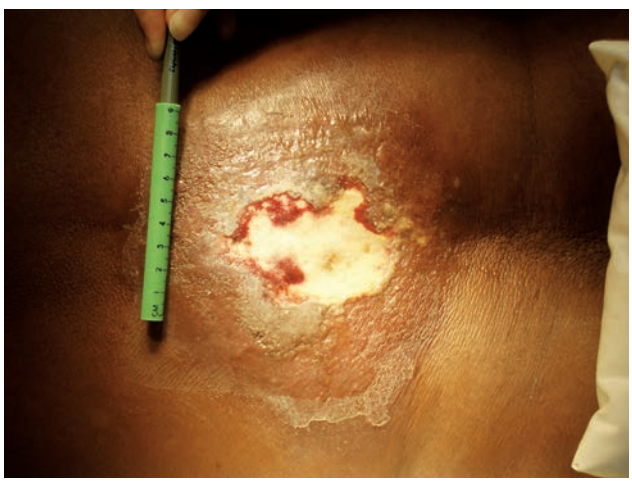


FIGURE 7.16

Staphylococcal abscess requiring surgical debridement

Source: Dr Genevieve Bishop.

infections include situations such as the spread of *Streptococcus pneumoniae* from the throat (pharyngitis) to the ears (otitis media) or lungs (pneumonia), or when opportunistic infections spread in immunocompromised patients (e.g. cytomegalovirus or tuberculosis in AIDS patients).

Minor skin infections due to streptococci can also be disseminated throughout the body (see Case History 7.3). Usually, the bacteria gain entry to the lymphatic system via a break in the skin and are carried to the lymph nodes. The inflammation that occurs in the lymph capillary may appear as a red line running from the site of the infection towards the lymph node. The stimulation of the lymphocytes of the specific defence system in the lymph nodes causes swelling, producing 'swollen glands', which are an indication of the immune system being activated to fight the invading pathogen.

The presence of bacteria or viruses in the lymphatic system or bloodstream does not always lead to an infection or disease. Sometimes, microorganisms gain entry and circulate, producing transient viraemia or bacteraemia without any disturbance to body functions. In these cases the pathogens are destroyed by circulating white blood cells. However, serious infections may result when the bacteria lodge in susceptible locations such as the ends of growing bones, causing osteomyelitis, or on defective heart valves, causing endocarditis. Sometimes, infections from the ear or the lungs can spread to the brain and cause meningitis. *Streptococcus pyogenes* can spread from a localised infection on the skin to the kidneys and establish a serious condition known as acute post-streptococcal glomerulonephritis (APSGN), or to the heart and initiate a hypersensitivity reaction known as rheumatic fever. This type of spread is a major health problem among the Indigenous population in the Northern Territory (see Chapter 14).

Systemic diseases

A **systemic disease** occurs when the pathogen affects several different organs or tissues in the body during the normal course of the infection (e.g. typhoid, measles). The pathogens gain entry to the body via the mucosal or skin surfaces of the respiratory or gastrointestinal tract and are then spread to other body organs via the blood or lymph. This may involve a stepwise invasion of various tissues before the final site of replication and disease production is reached.

For example, typhoid enters via the gastrointestinal system, then spreads via the blood and lymphatic system to the liver, spleen and bone marrow, causing fever, septicaemia, haemorrhage and diarrhoea. The measles virus enters via the upper respiratory system and spreads slowly via the lymphatic system to the lymph glands and then to the bloodstream. It finally invades various epithelial cells, causing cold-like symptoms in the upper respiratory tract, distinctive white patches (Koplik's spots) in the oral mucosa and a distinctive maculopapular skin rash (see Figure 7.10, page 148).

Some infections remain localised, but the pathogen produces toxins that have a systemic effect. For example, *Clostridium tetani*, the cause of tetanus, sets up an anaerobic infection at the wound site, and produces a powerful neurotoxin that circulates through the bloodstream and causes muscular spasms.

CASE HISTORY 7.3

Systemic disease related to tinea

In October 1999 a 34-year-old businessman who had recently arrived from London via Bangkok was admitted to a Sydney teaching hospital suffering from leg pain. On examination the leg was found to be swollen with a red blistered area on the left ankle, and the lymph nodes in the groin were enlarged. The patient had chronic tinea infection on both feet. Swabs and blood samples for bacterial culture were taken and the patient was started on IV antibiotics.

Over the next few days the patient remained febrile (40°C) and suffered headache, delirium, rigor and peripheral shutdown. *Streptococcus pyogenes* was identified from the swabs. The condition of the patient deteriorated, deep cellulitis was present, and the ulcerated area affected 30 cm on the lower left leg. Antibiotic medication was increased but there was a real possibility that amputation would be necessary. Because he was a businessman who travelled extensively, the loss of the leg would have had serious implications. Fortunately, the treatment was successful; the man recovered completely but required extensive rehabilitation.

Source: S. Greig, personal communication.

Questions

1. Where are Group A streptococci, GAS (*Streptococcus pyogenes*), found?
2. What is the most likely way that the bacteria entered the patient?
3. What properties of *S. pyogenes* caused such serious symptoms? (See Chapter 10, page 229.)
4. What advice should be given to the patient in order to avoid further infections?

Mixed infections

Some diseases are due to the effects of more than one pathogen; that is, they are mixed infections. In such cases the pathogens act *synergistically* (they help each other) by breaking down tissue or providing a favourable environment for another microorganism to multiply. For example, gas gangrene is often associated with the presence of more than one organism. The initial wound infection may involve a mixture of organisms but, once the tissue is infected and damaged, **necrosis** (death of tissue) occurs. This produces an anaerobic environment that favours the growth of *Clostridium perfringens*, the cause of gangrene.

Primary and secondary infections

Sometimes, the disease symptoms change or develop over a period of time. The first or **primary infection** may lower the host's resistance, allowing another or **secondary infection** to occur. Often, an upper respiratory tract infection (URTI), caused by one of the cold viruses, creates a favourable environment for a secondary bacterial infection such as bronchitis or a middle ear infection (otitis media) to develop. Bacterial pneumonia may occur following influenza, often with serious consequences. Diseases that involve itchy skin lesions (e.g. chickenpox, scabies) encourage scratching, which allows easy access for secondary streptococcal or staphylococcal infections.

A **superinfection** is a type of secondary infection that occurs as a result of the destruction of the normal flora, usually by the prolonged use of broad spectrum antimicrobials in the treatment of a primary infection. It involves the overgrowth of an opportunistic pathogen, which occurs when the normal flora are suppressed (e.g. *Candida albicans* in vaginal thrush, or *Clostridium difficile* in pseudomembranous colitis; see Chapter 18, page 451).

Subclinical infections

Although many infections are obvious and produce identifiable symptoms, it is sometimes possible for a pathogen to cause a **subclinical infection**. When this occurs, the patient/host does not exhibit any symptoms of the disease, but the pathogen multiplies in the host and elicits an immune response that can be detected by the presence of antibodies in the bloodstream. Many people in the community have antibodies in their blood to diseases such as hepatitis C, cytomegalovirus or glandular fever, without any previous history of disease symptoms. Before widespread vaccination programs were introduced, many people had subclinical infections of rubella (German measles) or polio.

CASE HISTORY 7.4

Pneumonia outbreak in a boarding school

In August 2006, five students from the same secondary boarding school were admitted to hospital suffering with pneumonia. *Streptococcus pneumonia* was isolated from blood cultures. Community-acquired pneumonia (CAP) is not common in healthy adolescents. Further surveillance revealed another 20 students from the school who were suffering from pneumonia. The pneumonia cases occurred during a period of widespread influenza infections. All the hospitalised cases responded to antibiotic therapy and the students recovered. The school was closed for a period and there were no further cases.

Questions

1. What is the significance of the fact that there was an epidemic of influenza at the same time as the students contracted pneumonia?
2. What factors increased the likelihood of students acquiring influenza and/or pneumonia?
3. Why did the school closure have an effect on the outbreak?

Source: Adapted from P. Cashman et al. 2007, Pneumonia cluster in a boarding school: Implications for influenza control. *Communicable Diseases Intelligence* 31(3): 296–98.

Persistent infections

A number of infectious agents are able to persist in the human body for long periods after the original disease symptoms have disappeared. These agents are classified as *slow*, *latent* or *chronic*, depending on the activity of the pathogen over time. Many of them are viral and are described in more detail in Chapter 5.

Slow infections

An unusual persistent infection is the 'slow' infection called subacute sclerosing panencephalitis (SSPE). This is a fatal, degenerative nerve disease which occasionally occurs after infection with the measles virus. The virus stays in the brain and produces symptoms several years later. Creutzfeldt-Jakob disease (CJD), which was originally thought to be due to a slow viral infection, is now known to be caused by a prion (see Spotlight box: Prions, Chapter 11, page 247).

Latent infections

Latent infections occur where the pathogen (usually a virus) remains in the body after disease symptoms have subsided (see Chapter 5). The viral DNA replicates inside the cell with the host cell DNA, but mature viral particles are not formed or shed; in other words, the host is not infectious. Reactivation of the infection may occur when the host defences are lowered. During reactivation, new viruses are formed and shed and the host again becomes capable of transmitting the disease. Many of the viruses belonging to the herpes family are capable of latency, the most common ones being herpes simplex types I and II, herpes zoster (the cause of chickenpox, which reactivates as shingles), Epstein-Barr (glandular fever) and cytomegalovirus.

Chronic infections

Chronic infections occur when multiplication and shedding of the pathogen continue at a low level without any symptoms of disease. The host is still capable of transmitting infection and in this situation is said to be a **carrier**. Typical examples are pathogens that lodge in the liver, such as typhoid, hepatitis B (HBV) and hepatitis C (HCV). In chronic hepatitis, the patient may have had a subclinical infection and be unaware of their carrier (infectious) status. In some patients, HBV and HCV persist and after many years can cause permanent liver damage (**cirrhosis**), which may develop into liver cancer. HIV is also considered a chronic infection as the virus is always present and the patient is infectious even when no symptoms are apparent.

Oncogenic infections

Some viruses persist and cause transformation of the cells they infect into cancer cells. They include hepatitis B and C viruses (liver cancer), human papillomavirus (cervical cancer), Epstein-Barr virus (Burkitts lymphoma) and human herpes virus 8 (Kaposi's sarcoma). There is still much to be learnt about the way viral infections affect the host (see Chapter 5).

Chronic diseases

There is growing evidence for the concept that some non-communicable chronic diseases can stem from exposure to infectious agents. A patient can develop a chronic disease in one of three ways. First, the infection causes tissue damage or an immune response that results in long term disability—for example, the development of chronic liver diseases, cirrhosis or hepatocellular carcinoma as a result of infection with hepatitis B or C, or rheumatic heart disease following a streptococcal infection. Second, the infection may cause permanent disability, such as infection with the polio virus that gives rise to chronic paralysis. Third, infection indirectly predisposes a person to chronic disease—for example, infection with the bacterium *Helicobacter pylori* was found to be responsible for chronic gastric ulcers that were previously thought to be due to stress. Other examples include the chronic arthritis of Lyme disease or Ross River fever, cervical cancer as a result of infection with the human papillomavirus, and chronic disabilities in babies that can be traced to maternal infections *in utero*.

SPREAD OF INFECTIOUS DISEASES

In this chapter we have examined the characteristics of different types of infection and the factors that lead to the development of a state of disease. The spread of disease depends on the ability of the microorganism to move from one host to another, to gain entry and to cause damage to the host. This topic is discussed in the next chapter.

SUMMARY

SYMBIOSIS

- The interaction between host and microorganisms determines whether an infectious disease will occur.
- The biological term used to describe two organisms living together is symbiosis.
- Mutualism occurs when two organisms live together to their mutual benefit.
- Commensalism is an association where one organism benefits without harm to the other.
- Parasitism is when one organism benefits at the expense of the other (host) organism.

MICROORGANISMS OF THE HUMAN BODY: NORMAL FLORA

- Normal flora are the microorganisms found on the human body. They may be present on the skin, or in the gastrointestinal, respiratory or genitourinary tract.
- Normal flora may protect the host from invasion by pathogens.
- Bacteria are the most common kind of organism found on the human body. Other organisms are the yeast *Candida*, some protozoa and latent viruses.
- Some microorganisms reside permanently. Others colonise surfaces. Some are transient or contaminants.
- Contaminant microorganisms cling to skin surfaces, but do not replicate there. Resident flora live in skin crevices, and comprise species of staphylococci, streptococci, corynebacteria and some fungi.
- Oral bacteria (e.g. *Streptococcus* spp.) are found in mucous membranes and in plaque attached to the teeth. The stomach, which has an acid pH, inhibits the growth of most bacteria; the lower intestine harbours a large population of bacteria.
- The upper respiratory tract has a large population of resident microorganisms, but the bronchial tubes and lungs are free of microorganisms in healthy individuals.
- The major components of the urinary system are sterile, but the lower region of the urethra may harbour organisms representative of those found in the genital tract and colon.
- The normal flora of the vagina may vary depending on factors such as hormonal influences, stress, puberty and antibiotic therapy.
- When normal flora are destroyed by long-term antibiotic therapy, opportunistic pathogens often flourish.

INFECTION AND DISEASE

- It is important to be able to define these terms: infection, disease, infectious disease, pathogen, pathogenicity, virulence, infective dose.
- Endogenous infections occur when the source of the pathogen is the human host (e.g. normal flora, congenital infections).
- Exogenous infections are those where the pathogen is derived from the external environment.
- Nosocomial infections (now known as healthcare-associated infections) are acquired in hospital.
- Iatrogenic infections are due to medical procedures.

THE DISEASE PROCESS

- Pathogenicity is the capacity of an organism to cause disease.
- The degree of pathogenicity depends on the presence of virulence factors in the microorganism.
- The likelihood of the occurrence of an infection is also dependent on the size of the infective dose.
- Opportunistic pathogens are able to gain entry to the host and set up an infection when the normal host defences are lowered or compromised.
- The ability of the host to resist invasion by a pathogen determines whether a disease will occur.

- Factors that contribute to patient susceptibility include: underlying diseases such as diabetes or anaemia; immunosuppression; chemotherapy and diseases of the immune system; poor nutritional status; alcoholism and IV drug use; age; genetic makeup.
- Other factors that influence the occurrence of an infection include: general environment and hygiene; climatic conditions; air pollution; seasonal variations.

KOCH'S POSTULATES

- In the germ theory of disease, German microbiologist Robert Koch proposed a set of criteria to determine whether an organism was responsible for a particular disease. They are known as Koch's Postulates.

SIGNS AND SYMPTOMS OF DISEASE

- Signs are measurable changes in the patient.
- Symptoms are changes that are felt and reported by the patient.
- The combination of signs and symptoms is called a syndrome.
- Signs of infection include: fever; inflammation; lymphadenopathy; changes in white blood cell count; formation of antibodies; skin rashes.
- Signs and symptoms of a disease are usually related to the body system affected.

DEVELOPMENT OF DISEASE

- The pattern of disease consists of the incubation period, the prodromal phase, the invasive or acute phase, and convalescence.
- A fulminating disease develops very rapidly, often with a fatal outcome.
- Drug therapy modifies the progress of disease.

TYPES OF INFECTION

- Infections may be localised, disseminated or systemic.
- Some pathogens produce toxins that have systemic effects.
- Some diseases are due to the presence of more than one pathogen.
- Primary infections may lower the host's resistance, allowing a different or secondary infection to develop.
- Superinfections result from an overgrowth of opportunistic pathogens.
- Infections that do not have symptoms may be subclinical, latent or chronic.
- Some chronic non-communicable diseases may be related to an infection.

STUDY QUESTIONS

1. What is meant by the terms: symbiosis, commensalism, mutualism, parasitism?
2. Define 'normal flora'.
3. How does the presence of the normal flora benefit the human host?
4. List two bacterial species that may be found as normal flora of (a) the skin, (b) the gastrointestinal system, (c) the upper respiratory tract, and (d) the vagina.
5. What is a pathogen?
6. What characteristics are required for pathogenicity?
7. What is meant by 'virulence'?
8. What is meant by the 'infective dose' of a pathogen?
9. What is the difference between endogenous and exogenous infections? Give examples of each.
10. Define opportunism.
11. Give two examples of opportunistic infections.
12. Who was the microbiologist responsible for the germ theory of disease?
13. What criteria are used to determine whether an organism is the cause of a disease?
14. What is the difference between signs and symptoms of disease?
15. List four of the common signs of an infection or infectious disease.
16. Why is fever sometimes considered to be beneficial?
17. What is cellulitis?
18. Describe each of these skin signs: erythema, vesicle, lesion, pustule, ulcer.
19. Describe the stages in the development of an infectious disease.
20. What is pyrexia of unknown origin (PUO)?
21. What is the difference between an acute and a fulminating disease?
22. What is meant by a 'systemic disease'?
23. What is the difference between primary and secondary infections?
24. What is meant by the terms: superinfection, subclinical infection, latent infection, chronic infection?

TEST YOUR UNDERSTANDING

1. What external factors affect the composition of the normal flora?
2. Why are some organisms more virulent than others?
3. Are opportunistic infections more serious than those caused by other pathogens?
4. Describe the factors that contribute to a host's susceptibility to infection.
5. Discuss how the criteria in Koch's Postulates need to be modified in the light of current knowledge of infectious diseases.
6. How is fever produced in the course of an infectious disease?
7. Under what circumstances can the administration of antibiotic therapy have a negative effect for the patient?

CHAPTER

8

Epidemiology: how diseases are spread

CHAPTER FOCUS

- What is the difference between communicable and non-communicable diseases?
- What are the major reservoirs of infection?
- How do organisms gain entry to the human body?
- What are the major portals of exit from the human body?
- How are diseases transmitted from one person to another?
- How is the science of epidemiology used to control the spread of infectious diseases?

INTRODUCTION

The science of **epidemiology** is the study of the origins and patterns of all types of disease and plays an important part in the provision of effective healthcare. The epidemiology of infectious diseases involves the study of the occurrence, causes, transmission and control of infectious diseases.

An infectious disease is one that can be transmitted (spread) from an infected patient to a susceptible host. Its spread depends on the ability of the pathogen to move from one host to another, to gain entry and to cause damage to the host. Some diseases are easily spread; others require a carrier or intermediate host. In this chapter we describe the difference between communicable and non-communicable diseases and identify reservoirs and sources of pathogenic microorganisms. We also describe the ways in which different microorganisms gain access to the human body, how they are transmitted from one host to another, and how outbreaks of disease occur.

Epidemiological investigations are an important part of disease control. They involve the surveillance of events and outcomes, and the collection and analysis of data related to outbreaks of disease. The results of these studies can be used to trace the source of the infection and to develop policy guidelines and strategies aimed at improvements in the prevention and control of infectious diseases.

COMMUNICABLE AND NON-COMMUNICABLE DISEASES

Communicable diseases are infectious diseases that can spread from one host to another. Some spread more easily than others and are described as very infectious or **contagious**. The term 'contagious' is used to describe the *ease* of spread, not whether the disease can be spread or not. Communicable diseases include colds, influenza, measles, chickenpox, rubella, diphtheria and polio, as well as gastrointestinal diseases and sexually transmitted diseases. These diseases are transmitted in various ways:

- by contact with respiratory aerosols from an infected host
- via body substances such as blood, sputum, urine, semen and faeces
- by infected skin cells that have been shed into the environment
- from open lesions or wounds
- by contact with contaminated surfaces.

Non-communicable diseases are those that would not normally be spread from an infected host to a healthy person during the course of usual contact and normal daily activities. They include diseases that are acquired by the mechanical transfer of a pathogen from the environment into the host tissue, such as occurs with tetanus. Tetanus spores are usually carried on an implement into a puncture wound where they germinate, multiply and produce the toxin responsible for disease symptoms. Diseases such as cystitis (urinary tract infection), which are caused by the displacement of normal body flora from one site to another, are also considered to be non-communicable.

Diseases transmitted by insects are unlikely to be communicable unless all the conditions for spread are ideal; that is, there is a large reservoir of infected hosts and an unlimited number of vectors. Parasitic diseases that require an intermediate host are not directly communicable from one definitive host to another.

Some diseases are caused by the ingestion of a preformed toxin produced by a microorganism. Strictly speaking, this should be defined as **intoxication** rather than infection. For example, the symptoms of botulism are caused by the ingestion of a preformed neurotoxin produced by the bacterium *Clostridium botulinum*, which grows anaerobically in inadequately sterilised canned or bottled food. The bacterium does not multiply in the human body but is shed in the faeces. For transmission to occur, the spores have to find their way into unsterilised canned or bottled food.

In practice, there is some overlap between the strict definitions of 'communicable' and 'non-communicable', but it is a useful grouping that enables health workers to assess the risk of transmission of infection from a person with a disease to another individual—non-communicable diseases have essentially a low or negligible risk of transmission, communicable diseases have a high risk of transmission.

RESERVOIRS OF INFECTION

In order to acquire an infectious disease, an individual must be exposed to a reservoir or source of the pathogen and a situation must exist whereby the pathogen can be transmitted to the human body and gain entry in sufficient numbers to cause an infection.

By now it should be apparent to the reader that there are thousands of different types of microbes living in our environment and that humans are in constant contact with these organisms during the course of their normal daily activities. Some of these organisms are able to cause disease in healthy individuals (natural pathogens), while others cause disease only in people whose immune system is compromised in some way (opportunistic pathogens). Some are easily spread from one person to another, while others are only introduced into the human body by mechanical means. Many of the organisms that cause disease in humans survive for only a limited period outside the human body unless they are provided with a suitable alternative environment.

For a disease to be **endemic** (always present) in a community, there has to be a permanent reservoir of the infectious agent. This **reservoir** is a habitat where the microorganisms can persist for long periods. It may be a favourable environment where they are able to survive or even increase in numbers, or it may be an unfavourable environment where the organism survives in a resistant form—for example, protozoal cysts or bacterial endospores. It may be a human or other animal host, or a non-living environment such as soil or water. For some infectious diseases, humans are the only reservoir (e.g. syphilis, gonorrhoea, the hepatitis viruses, smallpox and measles). Other pathogens (e.g. many kinds of bacteria) can survive in the external environment, in soil and water. Some microorganisms infect animals as well as humans, in which case the animal acts as the reservoir of the pathogen.

We can distinguish between a reservoir of infection and a source of infection. The **source** is the individual or object from which the infectious agent is actually acquired. The reservoir may also be a source if an opportunity exists for the microorganisms to pass directly from the reservoir to a susceptible host. For example, infected humans are both the reservoir and source for diseases such as measles and syphilis. However, sometimes the reservoir and source are different. For example, if a person's nose or skin is colonised by staphylococci, it can be considered a reservoir of staphylococcal infection, but when the staphylococci from the human reservoir contaminate food, the food is the *source* of the infection.

A source may also be contaminated object, or a fomite. A **fomite** is an inanimate object that can carry microorganisms (usually bacteria) on its surface. In the hospital environment, fomites are important sources of infection. They include such items as medical equipment and instruments, computer keyboards, door handles, identity badges, soiled linen and dressings, keys and other utensils. The *reservoir* of diseases such as polio or hepatitis A is humans, but the *source* of the infectious agent (virus) is usually food or water contaminated by infected human faeces. This applies to most diseases transmitted by the faecal–oral route.

Human reservoirs

The most important reservoir of pathogenic microorganisms is the human body itself. Patients who are suffering from an infectious disease are constantly producing and shedding pathogens that can readily be spread to others during the incubation period and the early prodromal phase, as well as when the disease is fully established.

An individual with an obvious symptomatic infection is usually regarded as a reservoir for the pathogen and a potential source of infection. However, as we saw in Chapter 7, there are a number of situations where a person is infected without showing any apparent signs of illness. These include patients who have subclinical infections and people who are chronic asymptomatic carriers of a disease. Humans with latent viral infections are also important reservoirs for viral pathogens.

The normal flora of the human body also represent a significant reservoir of potential pathogens if they are displaced from their normal habitat or if their host is compromised in some way. For example, most urinary tract infections are caused by normal flora of the colon such as *Escherichia coli.* A large number of the infections that occur in surgical or other wounds are caused by normal skin flora such as *Staphylococcus epidermidis*. *E. coli* may also be the cause of diarrhoea when toxin-producing strains of the bacteria are ingested in contaminated food or water (see Case History 8.4: Tracing the outbreak of haemolytic uraemic syndrome in Germany, 2011, page 176, and Spotlight box: Outbreak of haemolytic uraemic syndrome, Chapter 18, page 449).

Animal reservoirs

Many animals, both wild and domestic, harbour microorganisms that can cause disease in humans. About 200 of these diseases—termed **zoonoses** (singular: zoonosis)—are known. In some cases (e.g. rabies), both animals and humans suffer from a similar disease. In others, the animal does not appear to suffer from the disease but the organism is pathogenic for humans.

Three new zoonotic viruses were identified in Australia in the 1990s. They are the Hendra virus (HeV, previously called equine morbillivirus), Menangle virus (MeV) and Australian bat lyssavirus (ABL). Flying foxes, commonly called fruit bats, are the natural reservoirs for these viruses (see Spotlight box: Viral zoonoses linked to flying foxes).

Fruit bats or flying foxes are also the natural reservoir for the SARS coronavirus from China, and the Nipah virus from Malaysia. We now know that SARS is transmitted to humans when the virus passes into an animal such as the civet cat, which has contact with humans. Nipah virus infects domestic animals such as pigs and is transferred to humans by contact with farm animals.

The avian flu virus, H5N1, which causes disease in domestic and wild birds, has also been shown to infect humans who have close contact with diseased birds. Other influenza viruses infect domestic pigs and ducks and can be transferred to humans.

Transmission of many of these diseases from an animal reservoir to humans requires a vector in the form of a blood-sucking insect (e.g. plague is carried by fleas). For other diseases (e.g. Q fever), transmission requires close contact with the animal, its fur, feathers or body secretions. Some types of tinea (e.g. ringworm) are acquired from exposure to infected domestic animals or pets.

A particular group of zoonoses are the **arboviral** diseases. These viruses infect native animals or birds, apparently causing minimal effects, and are transmitted to humans by the bite of a mosquito. They include dengue fever, Ross River fever, Barmah Forest virus, Murray Valley encephalitis (see Spotlight box: Arboviruses endemic to Australia, Chapter 14, page 355).

Some of the protozoal and helminthic parasites have complex life cycles involving both animals and humans. Humans may be either an accidental or a definitive host,

SPOTLIGHT ON

Viral zoonoses linked to flying foxes

Recently, flying foxes (fruit bats) have been implicated as the reservoir for a number of new zoonotic viruses.

Hendra virus was first recognised in September 1994 when a racehorse in Brisbane, Queensland, died from a severe respiratory disease. Within days of the horse's death, a stable hand and the trainer who had cared for the horse became ill with an influenza-like illness. Over a period of two weeks, a total of 14 horses in the stable died. The stable hand recovered but the trainer died about two weeks after becoming ill.

A previously unidentified virus with characteristics of the Paramyxoviridae family was isolated from the lungs of six of the dead horses and from the kidneys of the trainer. It was at first named equine morbillivirus (EMV) but has since been renamed Hendra virus (HeV).

In October 1995, another death was reported of a man from Mackay in Queensland. He died from encephalitis, but had assisted at the autopsy of a horse in August 1994 and blood tests showed the presence of antibodies to Hendra virus. Two more deaths of veterinarians who had treated infected horses occurred in 2008 and 2009. Of the seven people infected to date, there have been four deaths. The virus appears to be transmitted by close contact with blood or secretions from the horse's nose or mouth.

A number of animals were tested for the presence of antibodies to the virus, and flying foxes (fruit bats) were identified as a natural reservoir of the virus. Serological studies and testing of urine samples have established that as many as half the fruit bats in colonies throughout Queensland and northern NSW regions are infected with the virus. It is thought the virus is transmitted to horses when they graze on grass contaminated with bat droppings or urine. Since 1994 there have been sporadic outbreaks of Hendra virus in horses.

In 2011 there were outbreaks of Hendra virus on a number of properties in northern NSW. More than 20 horses died and many more tested positive for the virus and had to be destroyed. About 50 humans were exposed to infected horses in the 2011 outbreak, but no one tested positive for Hendra virus. A dog was found to be infected with the virus, raising the possibility of transmission to and from other domestic animals. Horse owners have been advised to keep their animals away from paddocks containing flowering trees which attract the fruit bats, and veterinarians are advised to use protective clothing when examining a horse suspected of being infected. CSIRO is currently working on a vaccine against Hendra virus.

The **Australian bat lyssavirus** (**ABL**), which causes neurological symptoms, was identified in 1995 as the cause of death of two people who had been bitten by flying foxes. It belongs to the Rhabdoviridae (rabies-like) family of viruses. Rabies vaccine is an effective treatment for exposure to the virus. Lyssavirus is the only virus shown to be transmitted directly from bats to humans.

Menangle virus (**MeV**) was isolated in 1997 from stillborn piglets in New South Wales. Two piggery workers exposed to animal secretions suffered severe fever and rash and were found to have antibodies to Menangle virus.

Nipah virus, from Malaysia, is closely related to Hendra virus, and also has its reservoir in flying foxes. It was identified as the cause of encephalitis in pigs and pig farmers. **SARS-CoV**, which originated in China, also has flying foxes as its reservoir.

The appearance of these viruses is an example of the effect of human interaction with the environment, leading to the emergence of new diseases by the transfer of the viruses from their natural hosts to animals and then humans. Many species of bats, including flying foxes, are widespread throughout Australia and play important roles in ecosystems by dispersing rainforest seeds, pollination or control of insects.

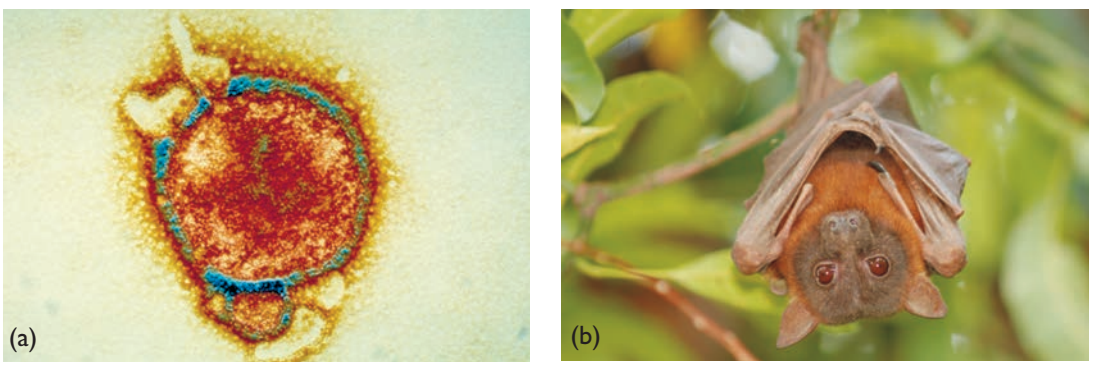


FIGURE 8.1

(a) Electron micrograph of Hendra virus; (b) *Pteropus scapulatus* (red flying fox).

Sources: (a) © CSIRO scienceimage.csiro.au; (b) Raina Plowright.

while the animal may be an intermediate or definitive host for the parasite (see Chapter 6). For example, the beef tapeworm exists in the larval or cyst form in the muscle tissue (meat) of cattle, from where it is ingested by humans who are the definitive host. *Toxoplasma gondii* is harboured in the intestine of domestic cats and other animals and excreted as oocysts in their faeces. From there it may be ingested by animals, lodging in the muscle tissue which is then eaten by humans. An alternative route is the direct inhalation or ingestion of oocysts from soil or kitty litter. Most zoonoses are impossible to eradicate, as to do so would require the destruction of all animal reservoirs. Table 8.1 lists some of the common zoonoses found in Australia.

Non-living reservoirs

Many microorganisms thrive in the external environment, especially in soil and water.

Soil

Bacteria may be present in soil as vegetative cells or, in some cases, as resistant endospores. Species of Gram-negative pseudomonads are commonly found in soil and on plants, and can be introduced into the hospital environment on flowers and plants. They are important opportunistic pathogens that are naturally resistant to a number of antimicrobial drugs and are a common cause of hospital infections, especially in burns patients and patients suffering from cystic fibrosis.

TABLE 8.1 Selected zoonoses occurring in Australia

ORGANISM	DISEASE	ANIMAL RESERVOIR	TRANSMISSION
Bacteria			
Bacillus anthracis	Anthrax	Cattle	Contact with cattle or endospores
Brucella spp.	Brucellosis	Cattle	Direct contact
Borrelia spp.	Lyme disease	? Native animals	Tick bites
Chlamydia psittaci	Psittacosis (ornithosis)	Birds/parrots	Direct contact
Coxiella burnetii	Q fever	Cattle	Direct contact
Leptospira	Leptospirosis	Wild mammals, cats/dogs	Contact with urine/water
Listeria monocytogenes	Listeriosis	Domestic animals	Unpasteurised milk
Salmonella spp.	Salmonellosis	Poultry	Ingestion of contaminated food, water
Campylobacter	Gastroenteritis	Domestic livestock	
Bartonella henselae	Cat scratch fever	Cats	Scratching, contact
Rickettsia tsutsugamushi	Scrub typhus	Mites/ticks	Bites
Viruses			
Influenza virus	Influenza (some types)	Pigs, ducks	Direct contact
Flavivirus	Murray Valley encephalitis	Fowl/birds	Mosquitoes
Orf virus (parapoxvirus)	Orf	Sheep, goats	Direct contact
Alphavirus	Ross River fever	Native animals	Mosquitoes
Alphavirus	Barmah Forest polyarthritis	Native animals	Mosquitoes
Hendra virus	Influenza-like illness	Flying fox	Contact with infected animals (horses)
Menangle virus	Fever/rash	Flying fox	Contact with animals (pigs)
Bat lyssavirus	Rabies-like illness	Flying fox	Bite, scratch by flying fox
Protozoa			
Toxoplasma gondii	Toxoplasmosis	Cats/other mammals	Ingestion of contaminated meat, contact with faeces in soil or kitty litter
Fungi			
Trichophyton, *Microsporum*	Ringworm (tinea)	Domestic animals	Direct contact
Helminths			
Echinococcus granulosis	Hydatid cysts	Dogs	Contact with faeces
Taenia saginata	Tapeworm	Cattle	Ingestion of contaminated meat
Toxocara spp.	Worm infestation	Dogs/cats	Ingestion of eggs of body organs

Some of the spore-forming bacteria are important pathogens, especially species of the genus *Clostridium*, which are commonly found in the soil. For example, spores of the tetanus bacillus (*Clostridium tetani*) are excreted in faeces and remain viable for long periods in the soil. They are transferred to humans when a person sustains an injury from an object contaminated with the tetanus spores. Gas gangrene (*Clostridium perfringens*) spores are also very resistant and widely distributed in the environment. If they gain entry to the body at the site of tissue injury or via surgical wounds they can cause gangrene; however, if the spores are ingested in food, gastroenteritis may result. Botulism is caused by the ingestion of poorly preserved food in which spores of *Clostridium botulinum*, commonly found in the soil, have germinated and grown under anaerobic conditions, producing the powerful neurotoxin responsible for botulism. Anthrax spores are able to survive for many years in soil and infect animals or humans who come in contact with the contaminated soil.

Many protozoa form cysts as a stage in their life cycle and these survive for long periods in the soil before entering a human or animal host (e.g. *Toxoplasma*). Helminth eggs or larvae (e.g. hookworm) may be found in soil. *Legionella* is often present in potting mix and poses a risk to gardeners through inhalation of the bacteria in dust (see Spotlight box: Legionnaires' disease, page 172). Soil containing bird droppings contaminated with *Chlamydia psittaci* can be the source of psittacosis or bird flu, an atypical pneumonia. Various soil fungi can be the source of hand and nail infections (see Chapter 6).

Water

Water is a reservoir for a number of pathogens that live and multiply best in an aqueous environment. These include protozoa such as *Giardia*, *Cryptosporidia* and *Entamoeba* that are frequently present in natural water environments, especially where sewage pollution occurs. Many different kinds of bacteria can be found in water. *Legionella* is found in natural streams and has been isolated from water in the cooling towers of air-conditioning plants. *Pseudomonas*, which has minimal nutritional requirements, is often present in the water in cooling towers, drinking water coolers and bubblers. Water contaminated with faeces can be the source of a large number of pathogens responsible for infectious diseases, including typhoid, cholera, polio and hepatitis A.

Leptospira may be found in water contaminated with animal urine. In tropical areas, *Burkholderia pseudomallei*, which causes melioidosis, lives in water and enters through skin abrasions (see Chapter 14, page 353). The blood fluke *Schistosoma* has a free-living larval stage in water—the cercariae—and people swimming where these occur are at risk of acquiring the infection. Outbreaks of diarrhoea caused by *Cryptosporidium parvum* have been associated with children's wading pools.

Biofilms

Some bacteria are able to cling to surfaces and form a **biofilm**, so the surface acts as a reservoir or source of infection. Usually these surfaces are immersed in a fluid that supports bacterial growth. This can occur, for example, on the outside of catheters, on endoscopes, on contact lenses and as dental plaque (see Figure 8.2). *Legionella* is able to exist as a biofilm in water tanks. Biofilms are difficult to dislodge by flushing, and contaminated surfaces require special cleaning.

PORTALS OF ENTRY

To be able to cause disease, the infectious agent must first gain entry to the human body. The microorganisms may come from any of the reservoirs described above and can be described as **endogenous** or **exogenous** (see below).

Most pathogens have a preferred **portal of entry**, one that gives ready access to an immediate environment suitable for the establishment of growth, or allows the pathogens to reach their target tissues or organs (see Table 8.2). Sometimes the portal of entry is quite specific for the disease. For example, bacteria such as *Salmonella*, which cause gastrointestinal infections, enter via the mouth. In other cases, pathogenic organisms are able to gain entry to the body via more than one portal. *Mycobacterium tuberculosis* can enter via both the gastrointestinal and respiratory tracts.

Some diseases take different forms depending on the portal of entry. *Yersinia pestis*, the bacterium responsible for plague, produces buboes in the lymph glands (bubonic plague) when it is introduced via the bite of an infected flea. However, it can also be inhaled directly into the lungs, causing pneumonia and giving rise to a much more severe form of the disease (pneumonic plague).

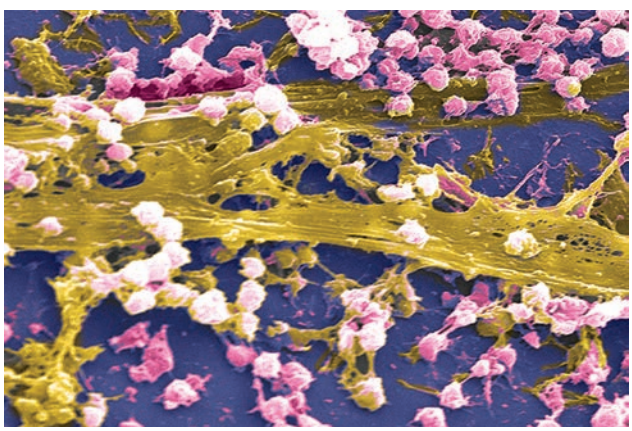


FIGURE 8.2

Staphylococcal biofilm on the outside of a catheter

Bacteria often form biofilms on catheters and other invasive devices, enabling the bacteria to be resistant to the washing action of normal body secretions.

Source: CDC/Rodney M. Donlan, PhD, Janice Carr.

TABLE 8.2 Preferred portal of entry for some common pathogens

PORTAL OF ENTRY	DISEASE	PATHOGEN
Skin		
Infections of skin and mucosal surfaces	Herpes	*Herpes simplex*
	Thrush	*Candida albicans*
	Skin infections	Staphylococci
		Streptococci
		Pseudomonads
Conjunctiva	Trachoma	*Chlamydia trachomatis*
	Gonorrhoea	*Neisseria gonorrhoeae*
Puncture wounds	Tetanus	*Clostridium tetani*
	Gangrene	*Clostridium perfringens*
IV drug users	Hepatitis B	Hepatitis B virus
Needlesticks	Hepatitis C	Hepatitis C virus
	AIDS	Human immunodeficiency virus
Burrowing parasites	Hookworm	Hookworms
	Schistosomiasis	*Schistosoma*
Insect-borne infections	Plague	*Yersinia pestis*
	Malaria	*Plasmodium*
	Arboviral infections	Various viruses (see Table 5.4)
Respiratory tract		
Localised infections of the Respiratory tract and lungs	Diphtheria	*Corynebacterium diphtheriae*
	SARS	SARS-CoV
	Common colds	Various adeno, rhino viruses
	Tuberculosis	*Mycobacterium tuberculosis*
	Pneumonia	Various organisms (see Table 7.3), e.g. *Streptococcus pneumoniae*
	Whooping cough	*Bordetella pertussis*
Systemic infections	Chickenpox	*Varicella zoster* virus
	Measles	Morbillivirus
	German measles	Rubella virus
	Glandular fever	Epstein-Barr virus
	Influenza	Influenza virus
	Meningococcal meningitis	*Neisseria meningitidis*
Gastrointestinal tract		
Gastrointestinal infections	Gastroenteritis	Various organisms, e.g. *Shigella*, *Salmonella*, *Staphylococcus aureus*, *Campylobacter*, *E. coli*, *Cl. perfringens*, enteroviruses
	Cholera	*Vibrio cholerae*
	Hepatitis	Hepatitis A, hepatitis E viruses
Parasitic infections	Tapeworm	*Taenia saginata*
	Hydatids	*Echinococcus granulosis*
	Amoebic dysentery	*Entamoeba histolytica*
	Giardiasis	*Giardia intestinalis*
Systemic infections	Toxoplasmosis	*Toxoplasma gondii*
	Polio	Enterovirus
	Mumps	Paramyxovirus
	Typhoid	*Salmonella typhi*
Urogenital tract		
Sexually transmitted infections (STIs)	Gonorrhoea	*Neisseria gonorrhoeae*
	Syphilis	*Treponema pallidum*
	Trichomoniasis	*Trichomonas vaginalis*
	Chlamydia	*Chlamydia trachomatis*
	Genital warts	Human papillomavirus
	Herpes	*Herpes simplex*
	Thrush	*Candida albicans*
Systemic infections	Hepatitis B and C	Hepatitis B, hepatitis C viruses
	AIDS	Human immunodeficiency virus

Endogenous infections

Endogenous infections are caused by microorganisms already present on the human body, which acts as the reservoir. They include infections such as:

- **cystitis**, an infection of the bladder, usually caused by normal flora from the colon
- **thrush**, an opportunistic infection caused by *Candida albicans*
- recurrent **herpes**, due to the reactivation of a latent infection
- disseminated infections such as those due to *Streptococcus* species, which spread from the throat and set up infections in the ears, lungs or kidneys
- **endocarditis** due to dissemination of mouth bacteria
- some skin and wound infections due to normal skin flora.

Infections acquired by the foetus *in utero* across the placenta are also considered to be endogenous.

Exogenous infections

Other infections are caused by **exogenous** pathogens—that is, microorganisms derived from the external environment.

Skin as a portal of entry

An intact skin provides an important protective barrier for the body, and anything that damages or penetrates this barrier allows the entry of microorganisms. A similar barrier is provided by the mucous membranes lining the walls of the respiratory, gastrointestinal and genitourinary tracts. Infections of the skin are described in Chapter 16. Intact skin is an important barrier to the entry of microorganisms (see Chapter 9). Cracks or splits in the skin, due to swelling, drying or cutaneous mycoses such as tinea, allow other microorganisms to enter and set up infections in the cutaneous or subcutaneous layers (see Case History 7.3: Systemic disease related to tinea, Chapter 7, page 153). The herpes virus also enters through the skin and infects epithelial cells. Bacteria such as *Staphylococcus aureus* and *Streptococcus pyogenes* can infect hair follicles or sebaceous glands, causing pimples and boils. Damage to the skin caused by scratching itchy insect bites or scabies infections allows bacteria to enter, causing infections such as impetigo and sometimes leading to more serious systemic infections.

The conjunctiva of the eye also provides protection against infection, so damage to the conjunctiva by dust or foreign bodies can allow entry of microorganisms. Pathogens such as *Neisseria gonorrhoeae* and *Chlamydia trachomatis* can infect the intact conjunctiva because they have attachment devices to avoid the flushing action of tears. Other important pathogens (e.g. hepatitis B) can gain entry via the conjunctiva as a result of splashes of blood or body fluids into the eyes.

The larvae of helminths such as hookworm enter the body by burrowing through the skin, as do the cercariae of *Schistosoma*. The bite of an insect vector that breaches the skin barrier and carries pathogens directly into the bloodstream is an important method of entry for many infectious agents. Mosquitoes can carry a variety of diseases—malaria, encephalitis, yellow fever, dengue fever and Ross River fever. Fleas carry plague, ticks carry Lyme disease and human body lice may carry typhus.

The use of hypodermic syringes by injecting drug users breaks the skin and provides ready access for microorganisms. Sharing of needles and syringes allows the spread of blood-borne pathogens such as hepatitis B, hepatitis C and HIV, as well as bacterial infections. Tattoos can introduce infections if the equipment has not been properly sterilised (see Case History 7.1: Life-threatening cellulitis after tattooing, Chapter 7, page 143).

Other microorganisms gain entry to the body through puncture wounds in the skin—for example, spores of the agents that cause tetanus and gangrene. They lodge in areas where there has been extensive tissue damage, where they reproduce under the favourable anaerobic conditions that exist in deep or necrotic tissue, causing disease by the production of harmful toxins.

The importance of intact skin is especially apparent in the hospital environment where breaks in the skin due to surgical procedures provide an easy portal of entry, especially if the wound has a drainage device attached postoperatively. Even when correct aseptic techniques are followed, approximately 10 per cent of all surgical wounds become infected while the patient is in hospital.

Invasive procedures such as the insertion of intravenous (IV) cannulas (in particular, central venous lines), and subsequent manipulation and care of these cannulas and catheters by nursing staff, provide further opportunities for microorganisms to enter via breaks in the skin. Central venous lines pose an additional hazard when used for total parenteral nutrition, as the nutritious solutions used are an ideal growth medium for bacteria and fungi.

Burns patients may have large areas of damaged skin and are particularly vulnerable to infections by bacteria, of which *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the most serious.

Respiratory tract as a portal of entry

Infections of the respiratory tract are described in Chapter 17. The mucous membranes of the respiratory tract are a major portal of entry for many pathogens, which may be inhaled as aerosols or spores, or on dust particles. If they are not washed away by the mucous secretions, they adhere to the cells lining the upper respiratory tract and cause infections in the nasopharynx, tonsils, throat and larynx. They may then travel further—to the bronchial tubes, causing bronchitis, or to the lungs, causing pneumonia.

Common bacterial diseases that are acquired via the upper respiratory tract (URT) include whooping cough (*Bordetella pertussis*), diphtheria (*Corynebacterium diphtheriae*), meningitis (*Neisseria meningitidis*), streptococcal infections, tuberculosis (*Mycobacterium tuberculosis*) and Legionnaires' disease (*Legionella pneumophila*). Tuberculosis enters via

the URT and infects the lungs. The viruses of influenza, measles, chickenpox, rubella, mumps and the common cold also enter via the mucous membranes of the URT. From there, some of these organisms spread to other parts of the body and produce systemic symptoms, such as a skin rash or enlarged lymph nodes. Spores of fungi such as *Aspergillus* are able to infect the lungs (see Chapter 6). Certain infections that enter initially via the respiratory tract may be disseminated to other organs of the body, especially in immunocompromised patients.

Pneumonia is an inflammation of the lungs that can be caused by a number of different viruses and bacteria that enter via the respiratory tract and penetrate to the lung tissue (see Table 7.3, Chapter 7, page 146). Among these, SARS (severe acute respiratory syndrome) is the most recent viral infection to be described. It enters via the mucosal surfaces of the URT and causes an atypical pneumonia (see Case History 8.1). Hospitalised patients are particularly susceptible to colonisation by pathogens carried on air currents and aerosols around the hospital. Viral infections such as those caused by the respiratory syncytial virus and influenza virus may be serious and even fatal to infants or elderly patients.

The use of respiratory equipment such as ventilators can create conditions that allow microorganisms to be introduced directly into the respiratory tract. Water from the humidification of the inspired air and the condensation of expired air collects in the ventilator tubing and may support the growth of bacteria, especially Gram-negative rods such as *Pseudomonas aeruginosa*. Tubes should be changed regularly to minimise this risk. Adequate cleaning and disinfection of all respiratory equipment is of the utmost importance.

Another hazard exists in the use of nebulisers for humidification, as the equipment has the potential to spray the patient with contaminated water. Endotracheal intubation for mechanical ventilation bypasses the defence mechanisms normally present in the mouth and throat and allows entry of microorganisms directly to the lungs. The wound associated with the tracheotomy provides another portal of entry for infection as it may become colonised with organisms such as *Staphylococcus aureus* during handling of the tracheal tube. The maintenance of good oral hygiene is important in preventing the spread of bacteria from the mouth to the respiratory tract and lungs.

Gastrointestinal tract as a portal of entry

Infections of the digestive tract are described in Chapter 18.

The ingestion of contaminated food or drink via the gastrointestinal tract (GIT) is a major way for pathogens to enter the body. To gain access by this route, the pathogen must be able to resist the acid conditions in the stomach (e.g. cholera, some toxigenic *E. coli*, and enteroviruses), need only a low infective dose (e.g. *Shigella*), or be ingested in such large numbers that enough organisms survive to penetrate into the intestine (e.g. *Salmonella*). In some cases, the pathogens may be protected by the food in which they are carried. Most of the microorganisms responsible for gastrointestinal infections have specialised structures for attachment to the mucosal cells lining the intestine (see Chapter 10). Some microbes are ingested as eggs (e.g. helminths), cysts (e.g. *Giardia*) or endospores (e.g. *Clostridium perfringens*).

As well as being the entry route for the microorganisms that cause gastroenteritis, such as *Campylobacter*, *Salmonella* and *Shigella*, the GIT also acts as a portal of entry for various bacterial toxins that are produced in food before it is ingested. These include the neurotoxin of botulism and the enterotoxin of *Staphylococcus aureus*. These toxins are resistant to stomach acid and are absorbed in the small intestine, producing symptoms within hours of ingestion.

Other diseases that enter via the GIT, but are disseminated to other parts of the body, include hepatitis A and hepatitis E, which affect the liver, and polio, which is absorbed in the intestine and affects other parts of the body, including nerve cells.

Urogenital tract as a portal of entry

Infections of the urinary and genital systems are discussed in Chapter 21.

The lower section of the urinary tract, the urethra, is frequently contaminated with normal flora, similar to those found in the colon. In females these organisms are likely to ascend the relatively short urethra to the bladder, causing cystitis; if not treated they may reach the kidneys and cause pyelonephritis. A high percentage of all hospital-acquired infections are of the urinary tract and are associated with the use of urinary catheters. The use of indwelling catheters, especially in elderly or incontinent patients, gives bacteria ready access to the bladder, usually on the outside of the catheter where biofilms may form (see Figure 8.2). The infecting organisms are generally derived from the patient's own flora but, in hospitalised patients or patients undergoing long-term antibiotic treatment, there is the likelihood that these infections will be caused by antibiotic-resistant strains of bacteria that have colonised the patient's skin. The

FIGURE 8.3

Cells of *E. coli* contaminating a spinach leaf

Source: Custom Medical Stock Photo/Getty Images Australia Pty Ltd.

CASE HISTORY 8.1

SARS

In early March 2003, newspapers began to carry reports of an outbreak of atypical pneumonia in Hong Kong. The first death was of a doctor from Guangdong province in China who had travelled to Hong Kong in February and stayed in a hotel, before being transferred to hospital. At least 17 other guests and visitors to the hotel became infected, some of whom travelled to Vietnam, Singapore and Toronto, where further transmission occurred, and others to the UK, Germany, the US and Thailand. Hotel guests and their contacts were admitted to a number of hospitals in Hong Kong.

In the first weeks of the outbreak in Hong Kong many of the cases occurred in healthcare workers who cared for the patients. In turn, they spread the infection to their household contacts. An outpatient at one of the Hong Kong hospitals became infected and transmitted the infection to a relative he visited in a block of apartments called Amoy Gardens, in Hong Kong, where 320 residents subsequently became ill. It is thought that the virus was shed in faeces, and that aerosols from contaminated sewage entered the bathrooms of other apartments through open U-traps.

In Toronto it was at first thought that the patients were suffering from pneumonia, so strict infection control precautions were not applied. This resulted in 361 cases and 41 deaths, particularly among healthcare workers and intensive care nurses.

By the middle of March it was apparent that health authorities were dealing with a serious new disease. It was named Severe Acute Respiratory Syndrome (SARS) and the World Health Organization (WHO) declared a global emergency. A team of epidemiologists, infectious disease specialists, public health physicians and microbiologists was assembled to study and contain the disease. Attention was also focused on Guangdong province where, it was revealed, cases of a severe respiratory pneumonia-like disease had been occurring since November 2002.

The speed with which the world community reacted prevented the occurrence of a more serious pandemic. By the middle of April, scientists had isolated the causative organism and identified it as a novel coronavirus—it was named SARS-CoV. (Coronaviruses are a family of enveloped, single-strand RNA viruses that usually cause mild infections such as the common cold in humans.) The viral genome was sequenced and molecular techniques used to identify different strains of the virus and trace its spread.

By the time the epidemic was brought under control in June 2003, there had been over 8000 cases of SARS and 774 deaths worldwide, 21 per cent of cases occurring in health workers. The overall case fatality rate was 9.6 per cent

It is now known that the virus is spread through direct or indirect contact of the mucous membranes of the eyes, nose or mouth with infected respiratory droplets or fomites. Because many of the patients required ventilation, the use of procedures such as suctioning, endotracheal intubation, bronchoscopy and aerosolised medication all contributed to the generation of infected aerosols. Profuse watery diarrhoea is a symptom of the disease and the virus is shed in faeces. Viral shedding is at a maximum on day 10 of the disease and the virus can survive for days in faeces and when dried on surfaces.

The natural reservoir of the virus has been identified as flying foxes (fruit bats) and it is thought to have been transmitted to humans by exposure to infected civet cats; early symptoms of the disease are non-specific, yet the disease is highly infectious. Travel history is very important.

Questions

1. Who was the index case in this epidemic?
2. Why did so many health workers become infected?
3. How has this epidemic changed the infection control protocols that are used in emergency departments?
4. What are the unusual features of this virus that contributed to its spread?

most serious complication of urinary tract infections occurs when damage to the cells lining the mucosa of the urinary tract allows the organisms to gain access to the bloodstream, causing sepsis.

The genital tract is the main portal of entry for a number of infections, usually transmitted by intimate contact during sexual intercourse. Organisms responsible for diseases that enter this way include *Neisseria gonorrhoeae*, *Treponema pallidum* (syphilis), *Chlamydia*, human papillomavirus (genital warts), herpes and *Trichomonas vaginalis*. Some of these microorganisms have special structures such as pili, which enable them to attach to the mucosal cells lining the

genital tract and prevent their being washed away by urine or mucosal secretions (e.g. *Neisseria gonorrhoeae*).

Blood-borne viruses present in semen and vaginal secretions (e.g. hepatitis B and C, and HIV) can enter the bloodstream through breaks in the skin or the mucosal lining of the genital tract, although this is not considered their major portal of entry.

The prevalence of sexual practices other than vaginal intercourse means that sexually transmitted organisms can enter and infect other areas of the body such as the anus or mouth and throat, provided that specific attachment sites for the microorganisms exist in these areas. *Neisseria gonorrhoeae* and the human papilloma virus (HPV) are organisms that can infect any of these sites as well as the genital tract.

Congenital infections

Microorganisms that infect the foetus, *in utero*, gain access by crossing the placenta. The placenta is usually an efficient barrier, allowing nutrients and waste to pass through, while excluding larger, possibly harmful, cells and molecules. However, some microorganisms are able to cross the placental barrier and infect the foetus. This can lead to abortion or various degrees of foetal damage, depending on the type of organism involved and the stage of pregnancy when the infection occurs (see Case History 8.2). Organisms that can cause congenital defects include:

- rubella virus (congenital rubella syndrome)
- *Toxoplasma gondii* (toxoplasmosis)
- cytomegalovirus (congenital CMV)
- *Treponema pallidum* (congenital syphilis)
- *Listeria monocytogenes* (congenital listeriosis).

CASE HISTORY 8.2

Listeria infection

A 29-year-old woman who was 28 weeks pregnant presented to the maternity unit of a large teaching hospital. She was in premature labour and delivered a stillborn baby boy weighing 845 grams. The baby was covered in a rash. Blood cultures on the baby were positive for *Listeria monocytogenes*. The placenta was patchy and discoloured and also tested positive for *Listeria*. The mother recalled having a flu-like illness with a high temperature some days before going into labour.

Questions

1. What is the possible source of *Listeria*?
2. How does this organism affect the foetus?
3. How can pregnant women avoid this infection?
4. What other microorganisms can cause premature delivery?

Hepatitis B and HIV may also infect the foetus, but this usually occurs at the end of pregnancy or during birth and gives rise to a chronic infection in the neonate without congenital defects.

Perinatal infections (infections acquired at birth) are caused by microorganisms present in the mother's birth canal; they infect the baby by entering via any of the portals described above. Most serious for the neonate are herpes infections of the eyes (which can spread to the brain and cause herpes encephalitis), chlamydial and gonorrhoeal infections of the eyes, and pneumonia due to Group B streptococci or *Chlamydia*. Thrush (*Candida albicans*), derived from the mother's genital tract, is a common infection of the mucosal lining of the mouth of the neonate.

PORTALS OF EXIT

In earlier sections of this chapter we described the human body as an important reservoir for infectious microorganisms, and the body's secretions and excretions as a potential source of pathogens.

Microorganisms are continually being shed from the human body during normal activities such as coughing, sneezing, defecating and changing clothing. A healthy person sheds mainly normal flora, which do not generally pose any special risk to other healthy members of the population. When a person is suffering from an infectious disease, the pathogens responsible for that disease are also shed in large numbers through **portals of exit** and may infect other susceptible people. Very often, the pathogen is shed from the same portal by which it entered (see Table 8.3). In the hospital environment, it is important for the nurse to be aware of the way in which pathogens exit the human body in order to ensure the safe and effective disposal of contaminated material and to prevent cross-infection. Health workers also need to be aware of the potential risks to themselves of occupational exposure to pathogenic organisms (see Chapter 13).

Upper respiratory tract: nose and throat

Organisms that infect the nasopharynx, throat and lungs are usually present in large numbers in the watery mucosal secretions and in sputum. They are expelled as aerosols by talking, coughing and sneezing. The fine aerosol droplets allow rapid dispersal of pathogens such as the cold and flu viruses, which do not survive long outside the body. These aerosol droplets are able to infect other people, directly through inhalation, or indirectly by contamination of a fomite. Pathogens, such as those responsible for measles, chickenpox and tuberculosis, are also carried in respiratory secretions for short distances to their next host. Some bacteria, such as *Mycobacterium tuberculosis* (TB), persist in a dried form on dust particles and are carried by air currents for great distances. Viruses such as the mumps virus (which infects the salivary glands), herpes simplex virus, Epstein-Barr virus (glandular fever) and cytomegalovirus (CMV) are secreted and shed in saliva. CMV is also present in urine and other body fluids.

TABLE 8.3 Major diseases transmitted in body substances

SECRETION	DISEASE
■ Sputum, mucosal secretions	Tuberculosis Diphtheria Whooping cough Influenza Measles Rubella SARS
■ Saliva	Mumps Glandular fever Hepatitis B (C?) Cytomegalovirus
■ Semen/vaginal secretions	Sexually transmitted infections Hepatitis B AIDS
■ Urine	Cytomegalovirus Leptospirosis
■ Faeces	Polio Typhoid Cholera Hepatitis A, hepatitis E Gastroenteritis—bacterial and viral
■ Blood	Hepatitis B Hepatitis C AIDS

Gastrointestinal system

A major portal of exit of pathogens from the human body is via the bowel. Some of the pathogens responsible for gastrointestinal infections cause irritation of the lining of the large intestine and interfere with water absorption, giving rise to watery stools or diarrhoea. When this occurs, large numbers of the pathogens are shed in the faeces. Organisms responsible for other diseases such as cholera, typhoid, hepatitis A and polio, as well as protozoal cysts and helminth eggs and larvae, are also excreted in faeces. When these pathogens contaminate the water supply, an epidemic can occur (e.g. cholera outbreaks often occur after natural disasters).

Faeces are a major source of potential pathogens, so nurses need to be aware of the importance of handwashing, the correct method of disposal of faeces, and the correct cleaning and disinfection of contaminated equipment.

In the community, faecal contamination of water is an important public health issue. Pathogens derived from faeces are frequently found in the sea water on Australian beaches and in stormwater run-off. In some countries, untreated sewage is used as a fertiliser for vegetable crops, which creates a serious health risk when unpeeled or unwashed fruit or vegetables are eaten. Faecal contamination of food and water is a major cause of gastrointestinal infections in developing countries.

Skin

The dead outer layers of the skin (dermis) are constantly being shed as skin scales and may carry the bacteria that are resident or transient on the skin. Bacteria and viruses contained in dried particles from crusts or scabs of wounds and exudates from skin lesions, boils and pustular rashes (e.g. chickenpox) may also be shed in this way. In the hospital environment these dried particles and dust containing microorganisms can be disturbed by activities such as bed-making, dry dusting or excessive movement of equipment, and dispersed around the hospital on air currents. Other skin diseases in which microbes are shed into the environment include herpes, warts and syphilis. Some of these organisms survive for a long time in the environment (e.g. chickenpox), while others (e.g. syphilis) are quite fragile and do not survive for long outside the human body.

Urogenital tract

The organisms responsible for most sexually transmitted diseases are present in semen and vaginal secretions and so are discharged from the body in this way. Others such as herpes or warts, which infect the skin, are shed from lesions on the genital organs. Organisms that infect the bladder or kidneys are usually present in urine, but do not pose a significant health risk to others. Cytomegalovirus is a major pathogen shed in urine, but there is no evidence that HIV or hepatitis B or C viruses are present in urine in significant amounts. Urine from animals infected with *Leptospira* contains the bacterium and can be a source of infection when it contaminates water supplies.

Blood

Blood is not usually considered a portal of entry for pathogens unless contaminated blood is given in a transfusion. In Australia, the careful screening of blood donors and blood products has essentially eliminated the risk of transmission of these organisms by blood transfusion. However, blood is a significant portal of exit for several important blood-borne pathogens that are released when bleeding occurs, or when blood is removed from the body for some other purpose.

Viruses such as HIV, hepatitis B and hepatitis C are transmitted in blood, blood-tinged body fluids and blood products. They may be transmitted by the sharing of needles for injecting drug use, or through breaks in the skin or mucous membranes that allow blood containing the virus to enter the body. These pathogens can also be transmitted from an infected patient to another individual (patient or staff) during dental or medical procedures where bleeding occurs, if there is a breakdown in aseptic technique or if contaminated instruments are not adequately cleaned and sterilised.

Another risk for health workers is transmission occurring as a result of needlestick injuries (see Chapter 13).

Some diseases are transmitted in blood by the bite of a bloodsucking insect such as a mosquito. One of the most important of these is *Plasmodium*, the protozoan responsible

for malaria, which is present in the blood of infected humans (see also page 174). In very rare cases, malaria can be transmitted by a blood transfusion. The Ebola virus, responsible for outbreaks of haemorrhagic fever in Uganda, causes massive bleeding from body organs and is shed and transmitted in blood.

TRANSMISSION OF MICROORGANISMS

So far, in this chapter, we have looked at the reservoirs of infection and the ways in which microorganisms enter and exit the human body. For a disease to spread, the causative organism or pathogen has to move or be transmitted from a reservoir or source to a susceptible host. A knowledge and understanding of the possible means of transmission is very important for health professionals, especially when working in a hospital, where there is a huge reservoir of pathogens and a population of susceptible patients. To prevent the spread of infection, it is important to be able to break the **chain of transmission**. This is discussed more fully in Chapter 13 (see Figure 13.14, page 313). In this section we outline the major routes of transmission.

Transmission of microorganisms usually occurs horizontally—that is, from person to person. Organisms can be spread horizontally by **direct contact**, by **indirect contact** with the reservoir or source, by means of a **common vehicle**, or by a **mechanical vector** or **biological vector** (see Figure 8.4). Vertical transmission—that is, from mother to foetus—occurs across the placenta.

Contact transmission

Many pathogens are too fragile to exist for any length of time outside their host. For these organisms to spread, there has to be direct or very close contact between the infected host and a suitable portal of entry in the next host. Organisms may be transmitted from one person to another by actually touching the skin or body secretions of the infected person, or by touching an object that has recently been contaminated with the pathogen.

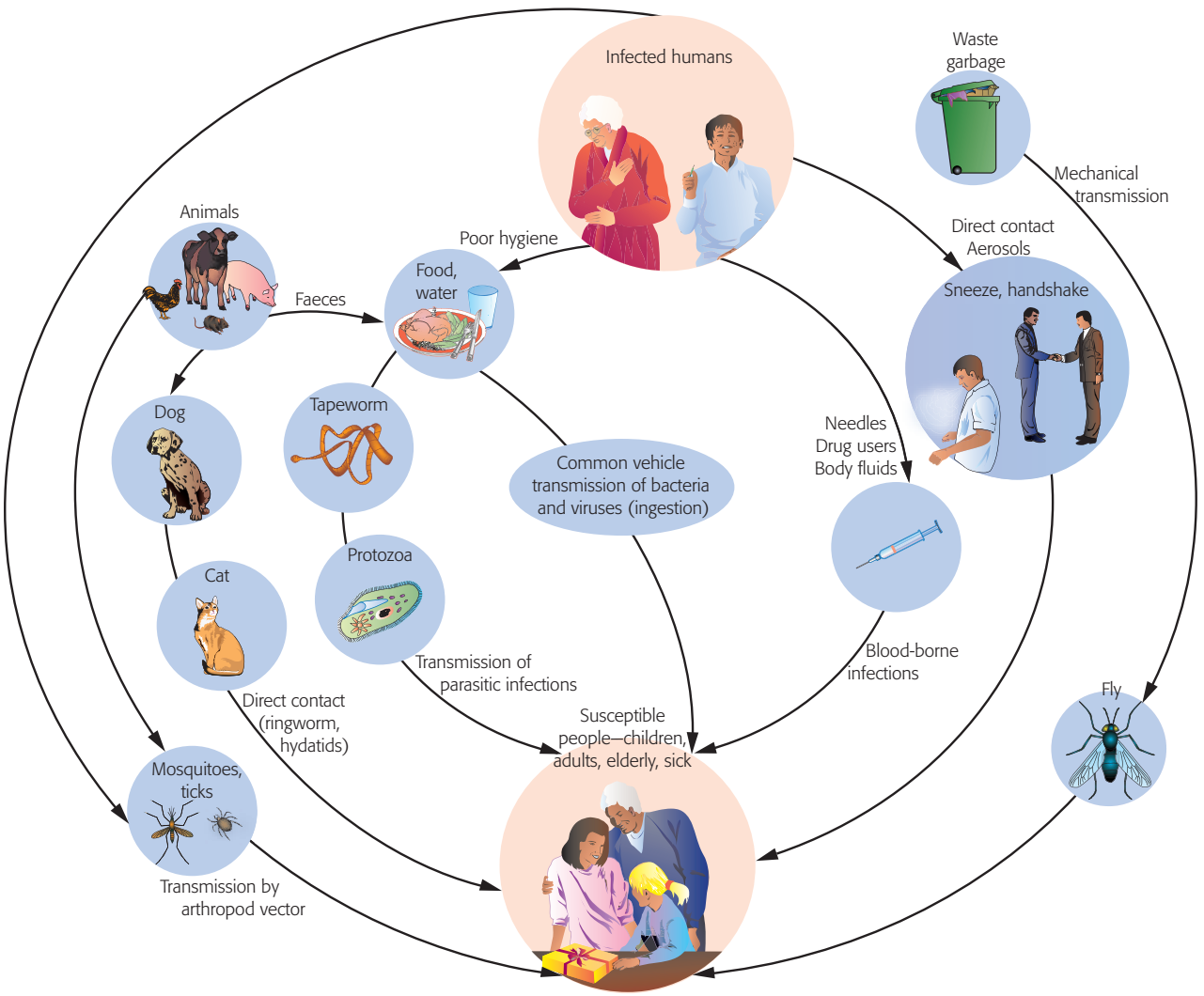


FIGURE 8.4
Methods of transmission

Direct contact

Direct contact refers to close or intimate contact between the infected person and a susceptible individual. Exposure to skin and body secretions, such as occurs during kissing and sexual activity, is a major route of transmission of many infectious diseases. The transmission of pathogens present in the genital area, or in semen or vaginal secretions, can be prevented by adopting 'safe sex' practices, particularly the wearing of a condom. Skin infections such as herpes, cutaneous fungal infections and bacteria from infected wounds can also be transmitted by direct contact.

Infections may also be transmitted from one part of the body to another. For example, if the hands touch the genital region and then the eyes, infections such as gonorrhoea or herpes can be transferred from the genitals to the eyes. Similarly, normal flora from different parts of the body can be transmitted to an open wound. Once the hands are contaminated, organisms can be transmitted to other parts of the body or to another susceptible individual. Health workers should be aware of the need to wash their hands regularly, to wear gloves whenever caring for patients with a communicable infection, and to educate patients about personal hygiene.

Some zoonoses can be transmitted to humans by direct contact with an infected animal. People working in the livestock industry may acquire the zoonoses, Q fever and brucellosis, which can be transmitted by direct contact with cattle as well as by eating or handling contaminated meat. Although not found in Australia, rabies is a serious disease transmitted in the saliva of an infected animal, usually via a bite. Anthrax is transmitted by direct contact with the exudate from the lesions on an infected animal or by contact with or inhalation of anthrax spores. Other zoonoses transmitted by direct contact include tinea (ringworm), which is common in domestic cats and dogs.

A number of viruses (e.g. hantaviruses) that are endemic in the rodent population can find their way from wild rodents to domestic animals and then to humans. They are generally transmitted in saliva and urine by direct contact, or by indirect contact with dried urine or droppings. Infections caused by the 'new' viruses such as Hendra, Menangle, Nipah and SARS-CoV are also transmitted by contact with animals.

Indirect contact

Indirect contact occurs when microorganisms from an infected host or other reservoir are deposited on an inanimate object, or **fomite**, and then transmitted to a susceptible host. This type of transmission can easily occur in the hospital environment unless health personnel take special precautions.

Fomites that can become sources of infection include medical equipment and instruments, eating utensils, bedpans, clothing, bedding, dressings, soap, taps, cupboard handles, refrigerators, sinks, telephones, identity badges, computer keyboards, toilet bowls, keys, money and handkerchiefs. The pathogen leaves the human body by any of the portals described above and is deposited on a fomite. From there it can be spread directly to a new host. The pathogen may also be picked up on the hands of a health worker after handling the contaminated fomite and transferred to a susceptible patient.

Adequate cleansing and disinfecting procedures, careful handwashing and wearing of gloves are the main ways of preventing transmission by this route.

Faecal–oral transmission

Faeces contain a large number of microorganisms. Many pathogens are shed in the faeces and may be transmitted to another person by direct or indirect contact. Usually the pathogen is carried on the hands and may contaminate food which is then ingested. The bacteria and viruses responsible for gastrointestinal infections are easily transmitted person to person unless careful handwashing and good hygiene practices are followed.

Droplet transmission

Droplet transmission is also considered a form of contact transmission. Microorganisms contained in body secretions, usually from the upper respiratory tract, are expelled from the body by coughing, sneezing or talking and may immediately come into contact with a susceptible person. The major pathogens transmitted in this way are the viruses responsible for SARS, colds and influenza, as well as whooping cough, measles and tuberculosis. The organisms are carried in droplets over a distance of less than one metre and inhaled directly into the respiratory tract of the new host. Simple actions such as covering the nose and mouth when coughing or sneezing help to prevent the spread of these organisms.

Common vehicle transmission

Microorganisms can be transmitted over a wide area by means of a vehicle such as air, water or food. When a number of people are infected with the same organism by one of these routes, it is called **common vehicle transmission**.

Airborne transmission

Airborne transmission occurs when disease-causing microorganisms are carried on air currents over distances greater than one metre. The pathogens must be able to survive outside the host and tolerate dry conditions. They include fungal spores, bacterial endospores, and the cysts or eggs of various parasites. Certain bacteria, such as some staphylococci, streptococci and the tubercle bacilli, are resistant to dry conditions and can remain viable (alive) for long periods. The oocysts of *Toxoplasma gondii* can be transmitted by inhalation of the dust from cat faeces in kitty litter.

Pathogens may also be present in fine water aerosol sprays that are too light to settle. The bacterium *Legionella pneumophila*, which is responsible for causing Legionnaires' disease (see Spotlight box), is transmitted in aerosol sprays derived from the exposed reservoirs of water-cooled air-conditioning plants and blown by wind currents over a wide

area before entering a susceptible person via the respiratory tract.

Although pathogens are easily spread by the airborne route, they will cause disease only if they are able to gain access in sufficient numbers to a susceptible host through a suitable portal of entry. Organisms transmitted by the airborne route do not multiply in air, but their spread is facilitated in modern air-conditioned buildings, such as hospitals, which have controlled temperatures and moist, recirculating air.

In the hospital environment there is a large reservoir of disease-causing microorganisms, constantly being shed in the form of aerosols, infected skin scales, and dried bacteria in crusts from infected wounds and herpes lesions. These organisms fall to the floor, some adhering to dust particles or to equipment and clothing. Activities such as cleaning, bed-making or movement of equipment disturb them and they can then be carried on air currents throughout the hospital (see Figure 8.5).

The use of wet mops and dusters when cleaning can reduce the spread of pathogens by this route. Bed linen should be folded carefully and not shaken. The use of masks and special protective clothing helps to prevent airborne transmission in operating theatres, burns wards and intensive care units, where patients are particularly susceptible (see Chapter 13).

Water-borne transmission

Water is a major vehicle for the transmission of microorganisms. Faecal contamination of the water supply used for drinking or bathing is a major source of infectious agents. These include most of the bacterial and viral pathogens responsible for diarrhoeal infections, as well as the causative organisms of hepatitis A, hepatitis E, typhoid, cholera and polio. Protozoa such as *Giardia*, *Cryptosporidia* and *Entamoeba* live and multiply in fresh-water habitats and can be acquired by drinking or swimming in the water. As mentioned above, the bacterium *Legionella pneumophila* multiplies in water reservoirs and is dispersed in water aerosols. Water also acts as a vehicle for transmission of some parasites such as the cercariae of *Schistosoma* (blood flukes). The bacterium *Leptospira* is generally transmitted in water contaminated with animal urine.

A clean, uncontaminated water supply is essential for public health (see Spotlight box: Water quality and public health, Chapter 6, page 126). In developed urban areas, government authorities are responsible for ensuring the quality of the water. However, in remote areas or in developing countries the condition of the water supply is often uncertain. If there is any question about the quality of the water, drinking water should be boiled and people should not swim in areas where pathogens are known to be found.

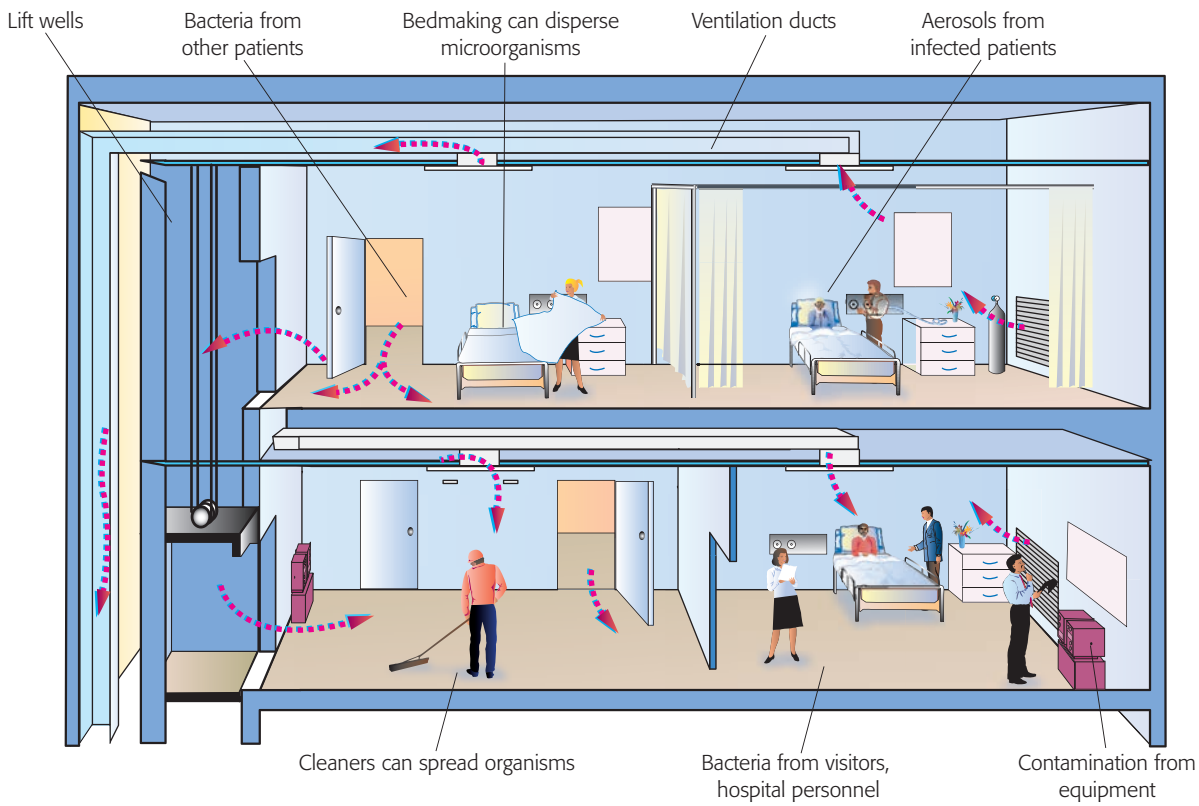


FIGURE 8.5

Air currents in a hospital

Some common modes of transmission of infections in a hospital setting.

SPOTLIGHT ON

Legionnaires' disease: an epidemiological investigation

The discovery of *Legionella pneumophila* as the causative agent of an atypical pneumonia is a classic example of an epidemiological investigation. From time to time there is an outbreak of a disease that has not previously been described. This occurred in 1976 in the United States when 29 people from various parts of the country died from an unusual type of pneumonia. It might have gone unnoticed if it were not for the fact that all the victims were members of the American Legion and had recently attended their annual convention in Philadelphia. When reports of the sudden deaths of a number of their members simultaneously reached Legion headquarters, they decided to investigate the cause.

Epidemiologists from the Centers for Disease Control (CDC) in Atlanta, Georgia, were called in. A total of 182 people reported being ill and 29 died. The common link was that they had all been in or around the hotel where the convention was held. The investigation took on the character of a murder mystery. The hotel, its surroundings, the food that had been served, the water supply, and all the equipment and utensils that had been used were tested for microorganisms, food toxins and poisonous chemicals. There was a Senate inquiry and CDC scientists were accused of a cover-up! Finally, one of the scientists at CDC re-examined some tissue from one of the victims and found an unusual rod-shaped bacterium. DNA testing showed that it was a completely new genus. It was named *Legionella* (after the Legionnaires) *pneumophila* (lung-loving).

Outbreaks in other parts of the country helped to identify the reservoir of the infectious agent and the method of transmission. The bacterium was found growing as a biofilm in the open water tanks that were part of the air-conditioning system on the top of the Philadelphia hotel. Wind blowing across the tanks produced a fine aerosol spray carrying bacteria, which were then blown around the street and into the foyer of the hotel. Anyone who happened to be present when the air currents were circulating in a certain way was at risk of inhaling the bacteria and contracting the infection.

Further investigations revealed that the bacterium occurs throughout the world, living in watery environments. Tests on stored blood and tissue samples of patients who had died from unidentified atypical pneumonia over a number of years revealed the presence of *Legionella.*

Over 40 different species of *Legionella* have now been identified. It is known that the disease has different forms depending on the species. The most severe form of pneumonia is caused by *Legionella pneumophila*, but there is a milder form—known as Pontiac fever—which is characterised by an influenza-like illness.

It has been shown that *Legionella pneumophila* is:

- transmitted by inhalation of the bacteria in fine aerosol sprays
- not transmitted person to person within a household
- sensitive to erythromycin.

One of the largest outbreaks of Legionnaires' disease in Australia occurred in April 2000 and was linked to the Melbourne Aquarium. The number of confirmed cases eventually reached 113, with two elderly patients dying from the disease.

The cases were confirmed by the identification of *Legionella pneumophila* serogroup1 antigen in urine samples from patients and the organism was isolated from cooling towers on the roof of the building. During 2000 there was a record number of 244 notifications in Victoria, with nine deaths.

Public health authorities have also reported that a number of cases of legionellosis occur each year, some of them fatal, after exposure to *Legionella longbeachae*. This particular strain of the bacterium is found in potting mix and soil in Australia and is associated with lower respiratory tract infections. Gardeners are warned to wear a mask and to keep the mix damp to avoid inhaling dust.

Note: Outbreaks of legionellosis occur from time to time in Australia and New Zealand. Usually, there are one or two deaths, mainly in older people who have associated illness or lung impairment. The disease can be successfully treated if diagnosed early. Health authorities require that all water tanks of water-cooled air conditioners be regularly cleaned and chlorinated. Deaths from *Legionella* each year represent only a small percentage of the deaths from pneumonia caused by other organisms.

FIGURE 8.6

Water-cooled air-conditioning plants can be a reservoir for *Legionella*

Food-borne transmission

Food spoilage due to microbial growth is a well-recognised problem in the home as well as in the food industry. In general, people tend not to consume food that is obviously contaminated with microorganisms. We usually discard mouldy bread, rotten fruit and food that smells 'off'. However, food can be contaminated with some microorganisms and still be palatable. The handling, storage and preparation of food provide many opportunities for microorganisms to be deposited in or on the food, and for the organisms then to multiply. In this way, food can serve as an efficient vehicle for the transmission of disease.

Disease-producing microorganisms may be present in food at its source—for example, meat from animals infected with parasites such as tapeworms or *Toxoplasma*. Careful inspection of meat at abattoirs can usually detect helminth infections. Bacteria that cause diseases such as anthrax, brucellosis and listeriosis, as well as the rickettsia, *Coxiella burnetii* (responsible for Q fever), are also sometimes found in or on meat for human consumption. Thorough cooking destroys most pathogens, but the ingestion of raw or inadequately cooked products can cause disease.

Salmonella is commonly found on eggs and raw chicken and can be transmitted to humans in food containing raw eggs (e.g. mayonnaise) or if the chicken is not thoroughly cooked (see Case History 8.3). It can also be transmitted by poor food-handling procedures from raw chicken to other foods such as salads. Health department regulations do not allow the handling of raw meats with the same utensils that are used for processed food that is eaten without further cooking (e.g. sliced meats). Pathogenic, toxin-producing strains of *Escherichia coli*, derived from humans or animals, may be present on raw meat or salad vegetables (see Case history 8.4: Tracing the outbreak of haemolytic uraemic syndrome in Germany, 2011, page 176). Endospores of *Clostridium perfringens* are ubiquitous in the environment and are not destroyed by cooking. They are often the cause of gastroenteritis characterised by stomach cramps due to the toxin produced (see Case History 3.1: Food poisoning due to *Clostridium perfringens*, Chapter 3, page 54).

In South-East Asia, raw fish is often contaminated with the larval stages of parasites such as flukes. Shellfish that are eaten raw (e.g. oysters) can transmit a variety of gastrointestinal infections.

Milk can be a major means of disease transmission. It may be contaminated with pathogenic organisms from the cow, such as *Listeria monocytogenes*, *Mycobacterium* species or *Brucella*. Adequate pasteurisation destroys most pathogens in milk.

The transmission of infectious diseases in contaminated food occurs most frequently when the organisms, mainly bacteria, are introduced into the food by poor hygiene and sanitation, or inadequate handling or storage procedures. The source of the contamination is frequently the food handlers, who may be shedding pathogens from their person or in their faeces. It is therefore essential that people involved in food preparation should wash their hands thoroughly. In institutions such as mental hospitals, daycare centres or nursing homes, outbreaks of food-borne infections such as hepatitis A can occur if staff are not careful about personal hygiene. Once bacteria are present in food, they can multiply and reach the numbers needed for an infective dose, especially if the food is stored at room temperature or left in a warming oven. *Salmonella*, *Campylobacter* and *Shigella* are the most common bacterial causes of gastrointestinal infections.

CASE HISTORY 8.3

Food poisoning at a wedding

A 27-year-old male presented to his doctor with fever, diarrhoea, abdominal cramps and myalgia. He was diagnosed with a *Salmonella typhimurium* phage type 44 infection.

He had attended a wedding two days earlier. Several other people who were also at the wedding had similar symptoms. *Salmonella* is a notifiable disease in Australia, so the Communicable Diseases Control Branch in South Australia was informed and carried out an investigation to determine the source of the outbreak. The case definition included anyone who attended the wedding and subsequently suffered from gastrointestinal illness or who was diagnosed with confirmed *Salmonella* infection. A questionnaire was sent to everyone who had been present, seeking demographic information and details of food and drink consumption. Responses were received from 147 of the 190 people who attended the wedding. Of those, 30 met the case definition. Statistical analysis of the data identified a significant correlation between consumption of garlic aioli or prawns and illness. Food samples of the garlic aioli and eggs from the venue used at the reception were sent for testing. The eggs tested negative, but a sample of the aioli served on the night tested positive for *S. typhimurium* 44.

Source: Adapted from E.J. Denehy et al. 2011, Outbreak of *Salmonella typhimurium* phage type 44 infection among attendees at a wedding reception, April 2009. *Communicable Diseases Intelligence* 35(2): 192–96.

Questions

1. Why is it necessary to have a case definition?
2. What type of food is most likely to be contaminated with *Salmonella*?
3. What component of the aioli would be the most likely source of infection?
4. How can commercial food outlets and caterers reduce the risks of gastroenteritis due to *Salmonella*?

Another common contaminant is *Staphylococcus aureus* (which usually comes from the nose or skin of a carrier). When allowed to multiply at room temperature before the food is eaten, some strains produce an enterotoxin that is heat-stable and resistant to stomach acid. The toxin does not alter the taste of the food but causes rapid, severe gastrointestinal symptoms after ingestion.

Transmission by vectors

Vectors are living agents, usually insects, that are responsible for the transmission of an infectious agent from one host to another. The major vectors are mosquitoes, fleas, ticks, lice, sandflies, and other biting flies and bugs. We can distinguish between mechanical transmission and biological transmission.

Mechanical transmission

Mechanical transmission is the passive transport of microorganisms on the outside of the insect's body from a source of infection to a susceptible host, either directly or indirectly. The source may be rotten food, faeces, or a contaminated wound or dressing; the vector may be a household fly. **Direct mechanical transmission** might involve a fly alighting on the source and then going to a portal of entry such as an open sore, a wound or even eyes. **Indirect mechanical transmission** might involve an insect carrying the pathogen from the source and depositing it on food that is later consumed, or on a dressing or equipment which then comes into contact with a susceptible patient.

Biological transmission

Biological transmission occurs when an insect bites an infected host and ingests some blood or body fluid containing the pathogen. The pathogen then multiplies (or goes through a stage in its life cycle) inside the vector, resulting in an increased number of organisms being present in the vector. When the insect bites the next host, the pathogen may be present in regurgitated blood or in faeces that is deposited at the site of the bite, and is introduced into the body when the bite is scratched. In the case of malaria, the protozoa are present in the saliva of the mosquito and are injected into the bloodstream of the new host when the mosquito feeds.

Insect vectors are the means of transmission for many serious diseases, including malaria, leishmaniasis and sleeping sickness, as well as the arboviral diseases, encephalitis, yellow fever, dengue fever and Ross River fever. Many of these are zoonoses (i.e. they infect animals as well as humans) and can only be transmitted from the animal reservoir to humans by a vector. Mosquitoes are the most important insect vectors. Vector-borne diseases are listed in Table 8.4.

The best method of control of vector-borne diseases is to interrupt the transmission by destroying the vectors or their habitats. Sometimes, the development of an area for farming or settlement interferes with the natural ecosystem and creates areas where the vector can breed, and so the disease may be introduced. Drainage of swamps and emptying of containers of stagnant water help to reduce mosquito breeding grounds. In Australia, the major arboviral diseases are Ross River fever, Murray Valley encephalitis, Barmah Forest fever and dengue fever (see Chapter 5, page 102 and Spotlight box: Arboviruses endemic to Australia, Chapter 14, page 355). Control of the mosquito population and avoidance of mosquito bites are the most effective means of preventing outbreaks of these diseases, for which no vaccines or antibiotics are available. In recent years a number of new or 'emerging' viral infections have been described in other parts of the world. Japanese encephalitis virus and the chikungunya virus are both transmitted by mosquitoes. Although not endemic in Australia there have been some cases imported from Asia and there is the possibility they could become established as the mosquito vector responsible for their spread is present in Australia.

TABLE 8.4 Some diseases transmitted by arthropod vectors

ORGANISM	DISEASE	VECTOR
Bacteria		
Yersinia pestis	*Plague	Flea
Borrelia spp.	Lyme disease	Tick
Rickettsia		
Rickettsia prowazekii	*Typhus	Louse
Rickettsia australis	Queensland tick typhus	Tick
Orientia tsutsugamushi	Scrub typhus	Mite
Protozoa		
Plasmodium spp.	*Malaria	Mosquito
Leishmania donovani	*Leishmaniasis (kala-azar)	Sandfly
Viruses		
Alphavirus	Ross River fever	Mosquito
Alphavirus	Barmah Forest fever	Mosquito
Flavivirus	Murray Valley encephalitis	Mosquito
Flavivirus	Dengue fever	Mosquito
Flavivirus	*Yellow fever	Mosquito

Note: *Not endemic in Australia.

EPIDEMIOLOGY

Epidemiology is the study of the occurrence, spread and control of disease. An epidemiological study can be applied to all kinds of disease, including cancer, heart disease, drug addiction and mental illness, as well as infectious diseases. It involves the systematic collection and processing of information about all aspects of the disease: its **aetiology** (cause), distribution, method of transmission and various other factors that contribute to the spread of the disease or the susceptibility of the population. For infectious diseases, the causative agent is a microorganism.

Infectious diseases have always had a huge impact on the health of the population and they are still the major cause

FIGURE 8.7

Mosquito with blood meal

Source: CDC/Professor Frank Hadley Collins, Director, Center for Global Health and Infectious Diseases, University of Notre Dame.

of morbidity and mortality in developing countries (see Chapter 1). Improvements in public health have come about in the last hundred years due largely to the work of doctors and scientists who investigated the natural history of various diseases, identified causes and developed methods of controlling their spread.

The science of epidemiology is generally regarded as having been founded by John Snow, an English physician who worked in London in the 1850s. He was intrigued by the fact that some of his patients contracted cholera while others did not. Although the causative agent of cholera was not known at that time, he suspected that it was being transmitted in water. His investigations showed that the inhabitants of London who received their water supply from the River Thames *downstream* from London (i.e. after the river had passed through the city and been polluted with all the sewage and wastes from the city) were much more likely to contract cholera than those who received water drawn from the Thames *before* it reached London. He concluded that the agent responsible for cholera was being excreted by people suffering from the disease into the sewage which was dumped in the river, contaminating the water used by other inhabitants.

Snow's work was confirmed by Robert Koch in Germany, who was investigating an outbreak of cholera in Hamburg at about the same time. He noticed that, although there was a high incidence of cholera in Hamburg, the nearby town of Altona, which filtered the dirty river water through sand before use, was essentially free of disease. The work of these two scientists laid the basis for our modern ideas of 'common vehicle transmission' and water purification.

Snow's work illustrates the basic principles of modern epidemiology:

- careful observation
- accurate recording of quantitative data
- thorough analysis of all contributing factors (e.g. environmental exposure and susceptibility)
- development of recommendations for control based on valid data from the study.

Epidemiology refers both to the methods applied to the study of disease and to the knowledge derived from the studies. Epidemiological studies also incorporate data that may have been recorded for purposes other than treatment of the disease. Factors such as age, sex, socioeconomic group, ethnicity and nutritional state all impact on the susceptibility of the patient and the risk of infection occurring (see Chapter 7). They provide the data necessary for informed decision making about the control of disease through public health intervention strategies. Epidemiology includes:

- a description of the natural history of the disease—how the disease occurs and progresses in the human body (signs and symptoms)
- the development of methods of early diagnosis (laboratory tests) so that treatment can begin as soon as possible
- identification of risk factors in order to direct the treatment protocol or nursing management
- development of guidelines to control the incidence and spread of the disease.

It differs from clinical practice in that it focuses on the health of a group or community, rather than an individual.

Epidemiological measurements

In today's society, people share many common sources of food and water and live or work in close proximity to each other. They also travel extensively and are exposed to a wide variety of microorganisms which they may pick up on their bodies and transport from one habitat to another, thus spreading disease. Globalisation of trade has also increased the likelihood of food being transported from one part of the world to another, with the potential to spread disease (see Case History 8.4 and Case History 18.3: Shigellosis, Chapter 18, page 451). When there is an outbreak of disease, or sometimes even an isolated case, the epidemiologist is called in to determine the facts surrounding the incident.

An **outbreak of infection** is defined as the occurrence of a number of cases of the disease in excess of the number expected in a given time or place. During the investigation, the natural history of the disease is determined, the '**case definition**' is established (i.e. a description of the syndrome), questionnaires are distributed to patients to collect information about their lifestyle, risk factors, people they have been in contact with, food they have eaten and places they have visited, in order to pinpoint the source and method of transmission of the disease-causing microorganism. Food samples may be tested and patients' symptoms and clinical

CASE HISTORY 8.4

Tracing the outbreak of haemolytic uraemic syndrome in Germany, 2011

In May 2011 The Robert Koch Institute in Germany reported the occurrence of a cluster of 30 cases of haemolytic uraemic syndrome (HUS) and bloody diarrhoea due to infection with a shiga-like toxin-producing *Escherichia coli* (STEC), also called enterohaemorrhagic *E. coli* (EHEC), and verotoxin-producing *E. coli* (VTEC). This was an abnormally high incidence and sparked concern that there was an epidemic. The outbreak was notified to all Germany's European Union (EU) partners and the WHO. The European Centre for Disease Prevention and Control (ECDC), which coordinates the prevention and control of infectious diseases in the European Union, drawing on experts in public health institutes and laboratories in 30 countries that participate in the network, issued daily updates about the epidemic.

By the end of the first week there were 250 cases. The number of cases of HUS and bloody diarrhoea continued to rise steadily, while public health authorities tried to find the source of the outbreak and the nature of the pathogen. Most of the cases occurred around Hamburg, in northern Germany, or in tourists who had briefly visited the region. The disease was unusual in that it affected mainly adults, rather than children (who are usually very susceptible), and about 75 per cent of cases were in females.

The pathogen was identified as a multi-drug-resistant *E. coli* O104: H4, which was carrying a highly virulent phage-mediated Shiga toxin 2. This was a very rare strain, only eight cases having been reported in Europe previously.

Epidemiological investigations focused on identifying places that patients had visited and the food they had eaten. It quickly became apparent that the source of the disease was fresh salad vegetables, at first mistakenly attributed to cucumbers imported from Spain. Because of widespread media coverage, this had a disastrous effect on Spanish farmers and resulted in political tension between Spain and Germany. This source was soon revised to implicate contaminated bean sprouts, most of which had been supplied to restaurants from an organic sprout farm near Hamburg.

The real breakthrough came when there was a small outbreak of the same *E. coli* strain near Bordeaux, in France, among people who had eaten bean sprouts grown by children in a school. The sprouts had been grown from fenugreek seeds imported from Egypt. There was no link to the German cases. The European Commission withdrew certain seeds imported from Egypt from the market and banned their importation.

By the time the epidemic was over, in July 2011, there had been an estimated 3332 cases of STEC, 818 of HUS and 38 deaths, 95 per cent of which occurred in Germany.

The successful containment of this outbreak illustrates the importance of cooperation among international public health authorities and laboratories. The rapid dissemination of results and technical information on the various alert networks meant that the key data reached scientists and doctors in all areas.

Note: For a review of this outbreak, see the address by the president of the ECDC, Stockholm, September 2011, <http://ecdc.europa.eu/en/aboutus/organisation/Director%20Speeches/201109_MarcSprenger_STEC_ICAAC.pdf>.

Questions

1. Discuss the properties of toxin-producing strains of *E. coli.*
2. Identify the scientific methods that allowed the rapid identification of this disease.
3. What other factors contributed to the containment of the outbreak?
4. Why did the consumption of a small amount of bean sprouts have such serious consequences?
5. What is a possible reason for the fact that a high percentage of the cases occurred in females?

results collated. Statistics may be collected giving the levels of **morbidity** (serious illness) and **mortality** (death) from the disease.

As well as determining the cause and source of an infectious disease, epidemiologists also study the pattern of its occurrence.

The **incidence** of a disease is the number of *new* cases of the disease seen in a specific period of time. Incidence statistics are important in determining whether the number of new cases of the disease is increasing or decreasing; that is, whether the outbreak is being contained.

Attack rate is a measure of the cumulative incidence of a disease among a particular population at risk—for example, in an outbreak of food poisoning, the attack rate is represented by the number of people who become ill out of the number exposed to the pathogen.

Prevalence is the number of people who are infected with the disease at any one time. If a count is made of people suffering from the disease on a particular day, it would include both old and new cases. So, depending on the duration of the disease, a single case may be counted more than once. Thus, if prevalence studies are carried out once a week, but

the disease lasts three weeks, then the same patient will contribute to the prevalence statistics each week (i.e. three times altogether). If only incidence statistics are collected, the patient will only be counted once. Incidence statistics reflect more accurately the occurrence and, therefore, the actual rate of transmission of the disease.

Seroprevalence is the number of people who are carrying antibodies to a particular disease at any time. The presence of antibodies in the blood gives information about a person's immune status or a present or past infection. The person is not necessarily infected or symptomatic. It can also indicate the number of people in a community who are immune to a particular disease (**herd immunity**—see Chapter 14, page 338). In a disease such as AIDS, detection of antibodies to the human immunodeficiency virus (HIV) in the blood gives an indication of the prevalence of the infection in the community. People who are HIV-antibody-positive are infected, even though they may be largely asymptomatic. However, the incidence—that is, the number of new people testing positive—will indicate whether the spread of the disease is being contained.

The use of rates to measure the frequency of disease provides a scientific approach to a systematic study of the distribution of the disease and the determination of causal factors. It can give a measure of the 'burden of disease' in a particular society and its impact on public health. It is important to determine accurately which factors are associated with the occurrence and progression of the disease, so that appropriate control measures can be implemented.

Identification of source and causality

To contain an outbreak of disease, it is essential that the causative agent be identified and its source located so that the chain of transmission can be broken. Sometimes, the source may be a single person who has entered the community and is infected with a particular disease which then spreads. This person is called the **index case**. The exact source can sometimes be determined by identifying the particular strain of the pathogen isolated from the index case and comparing it with the strains causing the disease in later cases.

Modern techniques of DNA analysis have contributed to scientists' ability to distinguish between different bacterial strains and different viral types. In Sydney in 1993, when it was alleged that transmission of the AIDS virus from patient to patient had occurred in a doctor's surgery, the claim was substantiated by viral typing, showing that all four patients had been infected with the same virus type as the original patient (i.e. the index case).

Nucleotide sequence analysis of the different DNA or RNA samples is also used to identify the strain present in a clinical specimen. This technique was particularly useful in the identification of flying foxes as the natural reservoir of the new zoonotic viruses—Hendra virus, Menangle virus, bat lyssavirus and more recently SARS-CoV (see Spotlight box: Viral zoonoses linked to flying foxes, page 160).

Occasionally, the epidemiologist is confronted with a totally new disease, one caused by a previously unknown microorganism. The outbreak of an atypical pneumonia, severe acute respiratory syndrome (SARS), that occurred in March 2003, spread rapidly around the world and involved epidemiologists, infectious disease specialists and health professionals from many countries (see Case History 8.1: SARS, page 166). The causative organism, a new coronavirus, SARS-CoV, was identified within two months and infection control guidelines developed. This rapid result was possible because of international cooperation and the availability of new technologies such as nucleic acid testing. SARS is highly infectious, with most cases occurring in close contacts of the patients (family members and healthcare workers).

In the case of the outbreak of haemolytic uraemic syndrome in Germany in 2011, DNA analysis of the organism allowed it to be distinguished from other toxinogenic *E. coli* strains and identified it as an unusual strain of *E. coli*, carrying a rare virulence factor (see Case History 8.4: Tracing the outbreak of haemolytic uraemic syndrome in Germany, 2011).

Legionnaires' disease, which appeared in the United States in 1976, is another example of an epidemiological investigation that identified a previously unknown organism, *Legionella pneumophila* (see Spotlight box: Legionnaires' disease, page 172). In this case, it was necessary to find the reservoir for the causative agent (the cooling tower for the air conditioner), the mode of transmission (air currents) and the presence of a susceptible host (people around the hotel). Unlike SARS, Legionnaires' disease is not transmitted person to person.

When a number of factors are implicated, a statistical approach to causality is needed. This involves the use of group data, rather than individual data. The causal relationship may be direct or indirect, or there may be multiple factors that are not independent of each other. For example, although it was found that the *cause* of Legionnaires' disease was a new type of bacterium, the *risk* of becoming infected was dependent on the wind currents when the person was in the vicinity of the hotel cooling towers, and the *severity* of the outcome was related to the state of health of the patient.

The ultimate determination of causality is reached through experimentation. The bacterium *Helicobacter pylori* was shown to be implicated as a cause of gastric ulcers when Nobel laureate Barry Marshall purposely swallowed a culture of the bacteria and developed the symptoms of the disease (see Chapter 18, page 458).

Classification of disease

Epidemiologists classify diseases according to their incidence in our society. The incidence can be affected by seasonal variation or climatic changes. A disease is said to be **endemic** in a particular geographical region if a reservoir of the causative organisms is always present in that area and able to give rise to an outbreak of disease at any time. For example, the childhood diseases of measles, mumps and chickenpox are endemic in most parts of the world, but may have seasonal variation. Most outbreaks of chickenpox occur in the spring. Rotavirus, which causes severe diarrhoea

in infants, is usually seen in winter. Outbreaks of endemic diseases may be **sporadic**; that is, they may occur as unrelated isolated cases, anywhere in the country.

Epidemics occur when there is a sudden rapid rise in the incidence of a disease in a particular locality. This can be due to a number of factors. For example, an epidemic may occur when a new organism or strain of an organism is introduced into a population that is not immune, such as occurs with the regular outbreaks of influenza each winter. Travellers may carry organisms to areas where they were not previously found. The early settlers in Australia brought with them European diseases, such as smallpox and measles, which killed many of the Australian Aborigines who had not previously been exposed to them.

Between 1992 and 1994 there were several serious outbreaks of measles in Australia that were classed as epidemics. The reasons for this are discussed more fully in Chapter 14 and relate to the lack of vaccination coverage in the population, as well as a lack of previous exposure to the virus. (For another example of the importance of herd immunity, see Spotlight box: Eradication of polio, Chapter 14, page 339.) Endemic diseases can become epidemics given the right conditions—for example, lack of immunity in a large proportion of the population of the community. Sometimes, epidemics are due to the emergence of a particularly virulent strain of the pathogen, such as occurs in some influenza epidemics.

A **pandemic** is a series of epidemics that occur when the disease spreads worldwide. Examples include the devastating influenza epidemic of 1918, which killed 21 million people worldwide, the AIDS epidemic, which has spread from Africa throughout the rest of the world, the 2004 SARS epidemic and the 2009 swine flu pandemic.

Surveillance

Ongoing **surveillance**—that is, the collection and analysis of data on all aspects of the occurrence and spread of a disease—is an important aspect of epidemiology that is necessary for effective control of disease. Included in surveillance data are the laboratory results detailing the isolation and identification of the causative organisms, data on antimicrobial drug susceptibility, morbidity and mortality statistics, results of field investigations, and reports of adverse reactions to immunisation or exposure to chemical or biological hazards. Epidemiologists are also concerned with all the factors that determine a person's susceptibility to a particular disease. They collect information about age, sex, lifestyle, occupation and history of immunisation, together with data about exposure to common vehicles (food, water) or sources of the infectious agent. All of these data can be analysed and used to predict or prevent future outbreaks of the disease.

Public health authorities are responsible for collecting and recording data on outbreaks of infectious diseases. In Australia a number of diseases are classified as *notifiable*. When a patient is diagnosed with a notifiable disease, the doctor or hospital involved is obliged to inform the appropriate health authority. In this way, it is possible to keep statistics on the incidence of infectious diseases, which assists health authorities to formulate policies and guidelines to control their spread.

Surveillance can be used to predict an outbreak of disease. Public health authorities in Australia use sentinel chicken flocks to monitor the appearance of antibodies to arboviruses such as Ross River virus and Murray Valley encephalitis virus which, together with observations of mosquito populations, can give warning of potential outbreaks. This is discussed further in Chapter 14.

Epidemiology is the scientific discipline underlying much infection control work and is the science on which public health guidelines are based. Infectious diseases are one type of disease where intervention strategies of proven efficacy are available to treat and prevent the spread of those diseases. Among these strategies are immunisation programs, antibiotic therapy and prophylaxis, infection control programs in hospitals, and public health programs for infection control. The surveillance data from the community at large are used to develop health department policies and provide guidelines to prevent or contain the spread of infectious diseases.

Levels of prevention

Three levels of prevention are generally recognised:

- **Primary prevention** involves the maintenance of good health by good nutrition, the elimination of infection risks by good hygiene, and the provision of protection by vaccination.
- **Secondary prevention** refers to strategies such as the development of tests for early detection of infection, and screening programs.
- **Tertiary prevention** involves actions to prevent further complications of the disease or deterioration in health, such as secondary infections or transmission in healthcare facilities. This is especially relevant to good nursing care.

EVIDENCE-BASED PRACTICE

This brief overview of epidemiology is included to provide the reader with some understanding of an important discipline that has applications in many areas of health. A knowledge of the principles of epidemiological investigation is of use in the critical evaluation of research papers and in the development of research projects to assist clinical practice.

There is an increasing trend towards '**evidence-based practice**' in all health-related areas. In other words, it is widely accepted that the development of new procedures or changes in protocols should be based on valid research that has evaluated the improvement to be gained in terms of clinical outcome, patient well-being, cost benefit and community health. Such research should embrace the principles of epidemiology and quantitative assessment of outcome. Anecdotal or qualitative description based on individual cases is not sufficient grounds for change in practice.

Frequently, a study is inconclusive because the data collection is flawed or incomplete. An understanding of epidemiology and the use of quantitative methods will allow valid conclusions to be reached.

Retrospective studies can identify the source of an outbreak and the practices that contributed to transmission. **Prospective studies** are used to evaluate the effect of a change in practice on infection rates.

Experienced clinicians often recognise deviations from normal occurrences—for example, an outbreak of infection or an increase in wound infection rates. A surveillance project, with data collected and analysed over a defined time period, allows them to determine whether it is a real or perceived variation, to investigate the cause and to implement appropriate change.

Reliable data are required to evaluate the effectiveness of an intervention program and provide impetus for change. Surveillance studies can be divided into 'processes' (e.g. compliance with guidelines) and 'outcomes' (e.g. adverse or positive health events).

Quality surveillance is dependent on a number of factors. These include:

- accuracy—a clear definition of the event to be measured
- the use of trained personnel to collect and manage data
- the use of specific, unambiguous and practical definitions for data collection so the results can be validated
- the determination of appropriate risk factors and confounding events.

When evaluating research findings, it is important to be able to detect bias in a study. Bias may occur:

- in the selection of the sample group to be monitored, or
- by not correctly identifying all confounders (i.e. factors that influence the outcome, such as underlying illnesses), or
- by selective collection of data.

Determining risk factors requires prior knowledge of *all* the determinants of a particular disease. The move towards evidence-based practice in healthcare relies on epidemiological studies that involve good surveillance and quantitative data collection and analysis, to ensure that optimum care is given to patients. Good surveillance is therefore an essential part of clinical research.

SUMMARY

COMMUNICABLE AND NON-COMMUNICABLE DISEASES

- Communicable diseases are easily spread from one host to another.
- Non-communicable diseases are not usually spread during the normal course of activities. They may be opportunistic, require a vector or be caused by a toxin.

RESERVOIRS OF INFECTION

- For a disease to persist in a community, there has to be a permanent reservoir of the infectious agent. It may be human, animal or non-living.
- The source of an infection is the individual or object from which the infection is acquired.
- Human reservoirs include patients who have an acute, latent or chronic infectious disease.
- Normal flora of the body also act as a reservoir of infection.
- Diseases that occur in both animals and humans are called zoonoses.
- Transmission of the infectious agent from animal to human may be by direct contact with fur, feathers or body secretions, by the ingestion of cysts, or via the bite of an insect vector.
- Soil and water are major reservoirs of infectious agents.

PORTALS OF ENTRY

- Endogenous infections are caused by flora that are already present on the human body.
- Exogenous pathogens are microorganisms derived from a source outside the human body; they enter via a characteristic portal of entry.
- Many pathogens have a preferred portal of entry. It may be the skin, respiratory tract, GI tract or genitourinary system.
- Intact skin is a major barrier to the entry of pathogenic microorganisms.
- Puncture wounds, injuries, insect bites and the use of hypodermic syringes break the skin and facilitate the entry of microorganisms.
- Hospital-acquired infections may occur in surgical patients or patients subjected to procedures such as the use of central lines, IV lines and catheters.
- Microorganisms that are inhaled into the respiratory tract gain entry by adhering to the mucosal cells.
- The use of respiratory equipment creates conditions where microorganisms can be introduced directly into the respiratory tract.
- A major portal of entry for pathogens is the ingestion of contaminated food or drink.
- Urinary tract infections are very common in catheterised patients in hospital.
- The genital tract is the portal of entry of sexually transmitted diseases.
- Some microorganisms cross the placenta and infect the foetus *in utero*.
- The neonate is susceptible to infections acquired during passage through the birth canal.

PORTALS OF EXIT

- Microorganisms are continuously shed from the body.

- Health workers need to be aware of the ways in which pathogens are shed.
- Organisms such as viruses are expelled as fine aerosols by coughing and sneezing.
- Faeces contain normal flora as well as pathogens that may contaminate food and water.
- Microorganisms are shed on dead skin scales, as particles from dried crusts on wounds, and from skin lesions.
- Organisms responsible for sexually transmitted diseases are present in semen and vaginal secretions.
- Urine contains bacteria from urinary tract infections, as well as pathogens such as cytomegalovirus and *Leptospira*.
- Blood-borne pathogens such as HIV, and hepatitis B and C, are present in blood and body fluids.

TRANSMISSION OF MICROORGANISMS

- Transmission of microorganisms occurs horizontally from person to person or vertically from mother to baby.
- Transmission may be by direct or indirect contact, via a common vehicle such as air, water or food, or by insect vectors.

EPIDEMIOLOGY

- Epidemiology is the study of the occurrence, spread and control of disease.
- There is an outbreak of infection when the number of cases that occur is in excess of that expected in a given time or place.
- The index case is the person who is the primary source of the infection.
- The incidence of a disease is the number of new cases of the disease seen in a specific period of time.
- Prevalence is the number of people infected at any one time.
- Seroprevalence is the number of people carrying antibodies to a particular disease at any time.
- A disease is endemic if there is a constant reservoir of causative organisms in the area.
- Epidemics occur when there is a sudden rapid rise in the incidence of disease in a particular locality.
- A pandemic is a worldwide epidemic.
- Surveillance data are used to develop public health guidelines and infection control policies.
- Three levels of prevention of infection are generally recognised.

EVIDENCE-BASED PRACTICE

- Evidence-based practice derived from valid research is of increasing importance for the development of new procedures or practice.

STUDY QUESTIONS

1. What is meant by a 'reservoir' of infection?
2. Give examples of some of the major reservoirs of infection for human pathogens.
3. What is the difference between the source of an infection and the reservoir of infection?
4. Give some examples of fomites found in the hospital environment.
5. What is a zoonosis? Give examples.
6. Describe three diseases caused by members of the genus *Clostridium*.
7. What are the major non-living reservoirs for microorganisms?
8. What is the difference between endogenous and exogenous infections?
9. What type of organisms cause 'cutaneous infection'? Give two examples.
10. Which diseases flourish in deep puncture wounds?
11. Give an example of an iatrogenic infection.
12. Describe how microorganisms gain entry to the body via the respiratory tract.
13. List four organisms that can cause pneumonia.
14. What are the risks to patients associated with the use of mechanical ventilation?
15. What is cystitis?
16. What are the major organisms that can cross the placenta?
17. How are pathogens shed from the human body?
18. Which diseases are likely to be transmitted in blood or blood products?
19. What is meant by 'vertical transmission'?
20. What is the difference between direct contact and indirect contact?
21. What is meant by 'common vehicle transmission'?
22. How can the spread of airborne microorganisms be controlled in the hospital?
23. Why is it important to use different utensils for handling raw and cooked food?
24. Why should milk be pasteurised?
25. Which insects can act as vectors for infectious disease?
26. What is the best method of controlling vector-borne diseases such as malaria?
27. What is the difference between the rates of morbidity and mortality?
28. What is meant by 'the index case'?
29. What is meant by 'seroprevalence'?
30. What is the difference between an epidemic and a pandemic?

TEST YOUR UNDERSTANDING

1. What are the risks of transmission of an infectious disease from a person who is a carrier?
2. Under what circumstances can the normal flora of the body cause an infection?
3. Why do microorganisms have a preferred 'portal of entry'?
4. Why is an intact skin so important?
5. What are the characteristics of microorganisms that are able to cause gastrointestinal infections?
6. What risks are associated with the use of urinary catheters?
7. What is the difference between incidence and prevalence statistics?
8. What is the value of carrying out surveillance programs?

FURTHER READING

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CHAPTER
9

The body's defence systems

CHAPTER FOCUS

- What are the body's three lines of defence against infection?
- What are the innate defences of the body?
- How do the skin and mucous membranes protect the body against infection?
- What cellular components of the innate immune system protect the body against foreign particles and microorganisms?
- What is inflammation and what is its role in defence?
- What are the characteristics of the acquired immune system?
- What cells and tissues comprise the acquired immune system?
- What is humoral immunity and what are the functions of antibodies?
- What is cell-mediated immunity?
- What are the principles of immunisation?
- What disorders are associated with the failure or improper function of the immune system?

INTRODUCTION

During normal, everyday activities, humans are exposed to a multitude of microorganisms in the air they breathe, in foods and fluids they consume, and on objects with which they have physical contact. Whether or not a person becomes infected by any of these microorganisms depends on the balance between the attributes of the microbes that enable them to cause disease and the ability of the body to resist them. In this chapter, we examine the ways by which the human body protects itself from infection. In Chapter 10 we look at some of the mechanisms that microorganisms possess that enable them to overcome or evade the body's defences and cause disease. The ability of the body to prevent infection occurring is called **resistance**, while a lack of resistance, or vulnerability to an infection, is called **susceptibility**.

OVERVIEW OF BODY DEFENCES

Given the large number of microorganisms that we are continually exposed to, it is remarkable that we are not constantly infected. In addition, we are continually exposed to other potentially harmful substances in our environment, such as pollutants, chemicals, pollens and other types of air-borne particles. We are protected from these threats by a highly complex, multilayered system of defences. The body needs a battery of different types of defences because it is exposed to such a wide range of microbes and foreign substances that possess different characteristics and mechanisms for causing disease. The body's resistance to infection depends on three major groups of defences (see Figure 9.1), which may be categorised under two terms:

1. **Innate immunity** refers to the body's first two lines of defence, which offer general protection against all potentially harmful agents, including pathogens (infectious agents) and foreign substances. They are called innate defences because they are inborn, or natural, defences. These defences do not discriminate one agent from another, and act in a similar way each time the same agent enters (or attempts to enter) the body.
2. **Acquired immunity** refers to the body's third line of defence. These defences help to eliminate those microbes that succeed in circumventing the innate defences, and also protect the host against future attack by the same organism. They are called acquired defences because they develop (or are acquired) as a result of contact with the microbe or foreign substance. These defences rely on the detection of the structural and chemical features of microbes or substances that mark them as distinct from the body's own cells.

Although the innate and acquired defences are usually considered separately, it is important to recognise that they overlap and interact substantially with each other to protect the body (see Table 9.1). Some innate components are critical regulators in the production of acquired responses, and many of the acquired defence mechanisms direct or enhance the

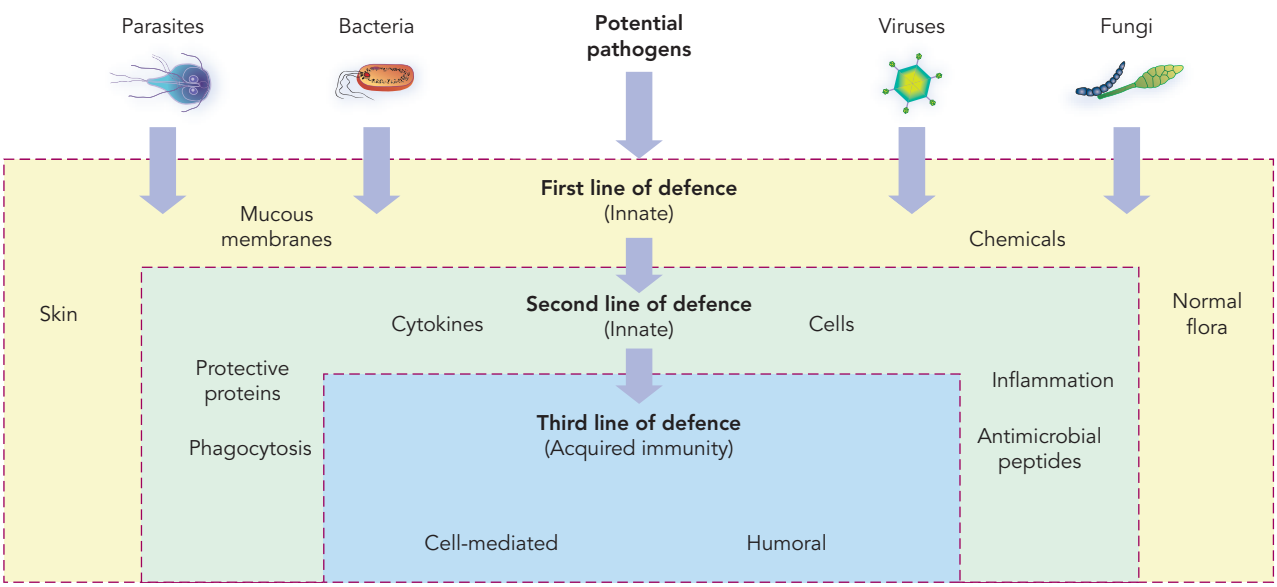


FIGURE 9.1

The body's three lines of defence against infection

TABLE 9.1 **The involvement of defence mechanisms in innate and acquired immunity**

	INNATE IMMUNITY	ACQUIRED IMMUNITY
Skin	✓	
Mucous membranes	✓	
Neutrophils	✓	
Acute inflammatory response	✓	
Normal microbial flora	✓	
Dendritic cells	✓	✓
Macrophages	✓	✓
Natural killer cells	✓	✓
Complement	✓	✓
Antimicrobial peptides	✓	✓
Cytokines	✓	✓
T lymphocytes		✓
B lymphocytes		✓
Antibodies		✓

action of innate defences, especially the body's phagocytic cells. The acquired defences, and some of the innate defences, comprise the body's **immune system**, the protective system that eliminates foreign microorganisms and substances and provides the body with long-term immunity to them.

INNATE IMMUNITY

The three major functions of innate immunity (also called **non-specific immunity** or **natural immunity**) are to:

- prevent the entry of pathogens and other foreign materials into the tissues of the body
- destroy microbes and foreign materials quickly if they do manage to enter the tissues
- assist in the activation of the acquired immune system if the innate defences are breached.

The defences of innate immunity may be divided into:

1. *The first line of defence:* the skin and mucous membrane barriers (and their secretions) and the body's normal microbial flora. These defences are always present, unless damaged in some way (e.g. a skin wound).
2. *The second line of defence:* cellular defences, inflammation, antimicrobial proteins, antimicrobial peptides and cytokines. These defences have the important characteristic of speed—that is, they are activated within minutes of a microbe entering the tissues.

Skin and mucous membrane barriers

The body's first line of defence against invasion by microorganisms is the skin and the mucous membranes. These are very resilient physical barriers which protect against the entry of potentially pathogenic microorganisms and other foreign particles. The skin comprises two distinct layers, the epidermis and the dermis (see Figure 9.2). The epidermis is the outer layer and consists of multiple layers of tightly packed epithelial cells. The outermost of these cells contain the protein **keratin**. Keratin is not affected by weak acids and bases and is resistant to bacterial enzymes and toxins. As long as the epidermis is unbroken, this tough layer represents a formidable barrier to most microorganisms. However, if the epidermis is damaged (e.g. in wounds) the skin and underlying tissues become much more susceptible to infection.

In the dermis there are sebaceous glands that secrete sebum, an oily substance containing fatty acids that lowers the pH of the skin and inhibits the growth of some bacteria and fungi. There are also sweat glands, and the high salt concentration in sweat inhibits many microorganisms.

Intact mucous membranes provide physical barriers within the body. Mucosal membranes line all the body cavities that are open to the environment—that is, the digestive, respiratory, urinary and reproductive tracts, and the inner surface of the eyelids and outer surface of the eye. Mucous membranes comprise two distinct structures: a thin layer of tightly packed cells (sometimes only one cell thick) called the *epithelium*, and an underlying layer of tissue that provides support and nutrients for the epithelium. Although these surfaces suffer considerable wear and tear, the damaged cells are rapidly replaced and the integrity of the membrane is quickly restored. A film of sticky mucous secretion traps foreign particles and microorganisms that might enter the body via these routes. The mucus helps to prevent microbes from attaching to the epithelial surface and entering the tissues. Inside the nose there is a meshwork of tiny hairs that, together with mucus, traps inhaled particles. There are mechanisms (described below) which then move the mucus and entrapped particles out of the body.

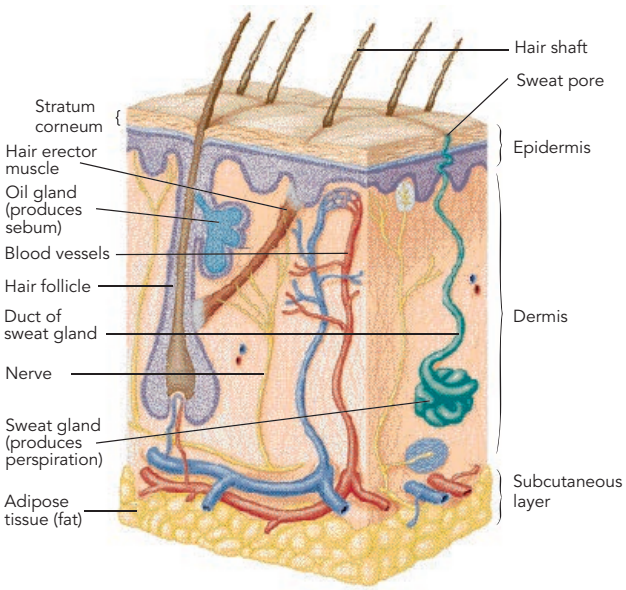


FIGURE 9.2

A section through skin

The top layers of the cells contain the tough protein called keratin.

The mucosal membranes are the sites of secretion or formation of a large variety of substances that contribute to defence. For example:

- **Lysozyme**. This enzyme breaks down the cell wall of many bacteria. It is found in saliva, sweat, tears, and nasal and vaginal secretions.
- **Acids**. The hydrochloric acid secreted in the stomach (pH 2) kills most microorganisms, and is a highly effective protection against the enormous numbers of microbes that enter the body in food and beverages. The pH of the vagina in adult females is acidic (pH 4), which inhibits the growth of some harmful microbes.
- **Digestive enzymes and bile salts**. These substances, which are secreted into the gastrointestinal tract for digestion, also kill microorganisms and inactivate viruses.
- **Lactoferrin**. This substance inhibits the growth of some microorganisms by binding free iron, a necessary growth factor for them. It also impairs the motility and ability to form biofilms of some bacteria. It is found in a number of secretions, including saliva, milk and seminal fluid.
- **Spermine**. This is an antibacterial substance found in semen.

The movement of fluids over mucosal surfaces is a highly effective mechanism of the upper respiratory tract that eliminates many potential pathogens from the body. A vital non-specific defence mechanism is the ciliated epithelium (see Figure 9.3). The cilia move mucus and anything trapped in it upwards towards the throat, either to be expelled or swallowed, thus preventing it from entering the lower respiratory passages. This **mucociliary escalator** is enhanced by two other forms of movement: coughing and sneezing.

FIGURE 9.3

Cilia of the respiratory tract

The cilia of the upper respiratory tract sweep mucus and any foreign material caught in it upwards away from the lungs.

Source: Eye of Science/Science Photo Library.

The movement of fluids over mucosal surfaces can also have a flushing effect, helping to eliminate foreign particles (including microbes) from the body. Thus, tears wash particles from the eyes, and the flow of urine in the urinary tract flushes the urethra. In fact, normal urine flow is one of the most important defences in the urinary tract, and people with urinary obstruction often suffer urinary tract infection. Other forms of movement, such as peristalsis in the digestive tract and defaecation, also help to remove microbes from the body.

The skin and mucous membranes are highly effective barriers. However, sometimes microorganisms are able to break through these barriers, either because their integrity is disrupted by cuts, wounds or abrasions, or because the particular microbes possess special properties that enable them to bypass or penetrate these barriers. The thin epithelium of the mucous membranes is not as effective a barrier as the multiple layers of dead cells in skin, so microbes more often invade through mucous membranes than through skin. When microorganisms breach these barriers they are able to gain entry to the tissues. Fortunately, other defence mechanisms then come into play.

Normal microbial flora

The normal microbial flora of the human body may also be considered as part of the body's first line of defence because of the considerable protection they provide. The organisms that comprise the normal flora are described in detail in Chapter 8. These organisms suppress the growth of potentially pathogenic bacteria and fungi by:

- competing for essential nutrients
- changing environmental conditions to be unfavourable to other microorganisms, but favourable to themselves (e.g. low pH created by lactobacilli in the vagina)
- secreting toxic substances (e.g. colicins of *Escherichia coli*, which inhibit other microbes)
- forming bacterial layers over tissue surfaces (e.g. in the intestine).

The importance of the normal flora in preventing infection is clearly demonstrated when broad spectrum antibiotics are administered to a patient. Such drugs can reduce or change the normal flora and thus allow infection to occur. Thrush, caused by the yeast *Candida albicans*, is an infection of the oral cavity, or gastrointestinal or genital tracts, and often follows broad spectrum antibiotic therapy.

Non-specific cellular defences

When microorganisms or foreign substances penetrate the skin or mucous membranes and enter the tissues, they will encounter a number of second-line defences. Major components of this second line are defensive cells found throughout the body's fluids and tissues. The most important of these are the phagocytes and the natural killer cells. The vital role played by phagocytes is reflected in the serious infections suffered by people with a deficiency in these cells.

Phagocytes

Phagocytes are body cells that actively ingest and digest foreign particles. They also break down dead tissue cells and remove cellular debris from the tissues. The major types of phagocytes in the body are the macrophages, dendritic cells and the neutrophils, although other white cells in blood, such as eosinophils, also have some phagocytic activity.

Macrophages are large phagocytic cells derived from blood monocytes. Some travel around the body looking for foreign material and are called wandering macrophages. Other macrophages stay in specific tissues and organs of the body, and some of these are given different names—for example, Kupffer cells in the liver, microglia in nerve tissue and histiocytes in connective tissue (see Table 9.2). At sites of infection, extra blood monocytes can migrate from the circulation into the tissue where they mature into macrophages.

A distinct group of cells, called **dendritic cells**, also has a significant phagocytic function. They have numerous finger-like projections of their cytoplasm (see Figure 9.4) that help them to trap foreign particles. Dendritic cells are derived from the bone marrow and reside in skin (where they are called **Langerhans cells**), mucous membranes and lymphoid tissues. Dendritic cells and macrophages play a critical role in presenting antigen to T lymphocytes and hence in the initiation of specific immune responses. They are discussed in more detail later in this chapter (see 'Acquired immune system', page 196).

Neutrophils, the most abundant type of white blood cell, are highly phagocytic and actively motile cells. Neutrophils are also referred to as **polymorphonuclear leukocytes** (**PMNs**), **polymorphs** or **pus cells**. Neutrophils phagocytose foreign material in the bloodstream, but also migrate from the bloodstream into tissues in the early stages of infection and inflammation.

The process of phagocytosis involves four major phases (see Figure 9.5):

1. chemotaxis
2. attachment
3. ingestion
4. killing/destruction.

TABLE 9.2 The monocyte/macrophage system of the human body

ANATOMICAL SITE	CELLS
Alveoli	Macrophages
Spleen	Macrophages
Blood	Monocytes
Lymph nodes	Macrophages
Brain	Microglia
Liver	Kupffer cells
Connective tissue	Histiocytes
Bones	Osteoclasts

Phagocytes are attracted to sites of damaged tissue or microbial invasion by chemicals released at the site. This attraction is called **chemotaxis**. A variety of chemical substances act as attractants, including substances released from damaged tissues and some components of microorganisms. These chemical attractants cause the phagocytes to move

FIGURE 9.4

A scanning electron micrograph of a dendritic cell

Source: David Scharf/Science Photo Library.

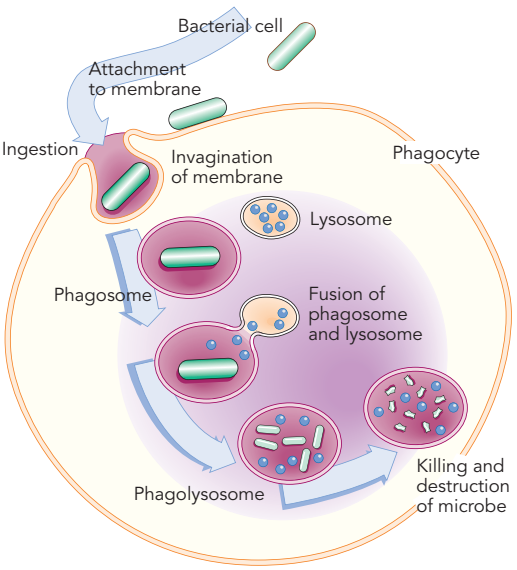


FIGURE 9.5

The mechanisms of phagocytosis

Phagocytosis of a bacterial cell, showing the major steps of attachment, ingestion and killing/destruction of the microbe.

along an increasing concentration gradient of the attractant towards its source—the site of infection or tissue damage.

Phagocytes detect and attach to an invading microorganism via non-specific receptor sites on the surface of the phagocyte. Phagocytes have a limited number of such receptors, called **pattern recognition receptors**, including an important group referred to as **toll-like receptors** (**TLRs**). TLRs are found particularly on macrophages and dendritic cells, but also on other cell types including neutrophils, eosinophils and epithelial cells. TLRs and other similar receptors recognise **pathogen associated molecular patterns** (**PAMPs**), which are types of molecules found on microorganisms but not on host cells. PAMPs include bacterial lipopolysaccharide, peptidoglycan, flagellin and DNA, as well as viral envelope proteins and nucleic acids. The innate immune system relies on a limited repertoire of receptors to detect invading microbes, but is effective because it targets chemical components that are common to many microbes.

Recently, other cell receptors of the innate system have been discovered that enable the cells to sense tissue damage. Thus, the innate immune system can detect both infectious threats as well as the results of infection—that is, stress signals from damaged or dying body cells. These receptors detect substances, such as endogenous alarmins, that have been referred to as **danger associated molecular patterns** (**DAMPs**). These are molecules that are released when cell lysis or tissue damage occurs.

Phagocytes ingest microorganisms and foreign material by a process called **endocytosis**. The cytoplasm invaginates at the point where the foreign particle is attached and then closes around it. Having been taken up, the foreign material is contained in a membrane-bound vacuole called a **phagosome** within the cytoplasm of the phagocyte.

Destruction of the microorganism or foreign material is brought about by fusion of the phagosome with lysosomes, to form a **phagolysosome**. Inside the phagolysosome, the foreign material is exposed to a variety of digestive enzymes (e.g. proteases, lipases) which can break it down. Microbes are also exposed to a wide range of toxic oxygen substances (or free radicals), acids and defensins (small antimicrobial peptides). Some microbes, such as the bacteria that cause tuberculosis and Legionnaires' disease—which appear to be resistant to intracellular digestion—are thought to prevent the fusion of lysosomes with the phagosome. Other organisms, like the *Rickettsiae*, appear to avoid destruction by escaping from the phagosome before it merges with the lysosome.

The importance of phagocytosis as a defence mechanism is clearly reflected by the number of microorganisms that have developed means of escaping the process. This is discussed more fully in Chapter 10.

Natural killer cells

Natural killer (NK) cells are a unique group of cells that are capable of destroying tumour cells and virus-infected cells. They are part of a distinct group of lymphoid cells called *large granular lymphocytes*. NK cells have a complex system of receptors that allows them to recognise changes on the surface of abnormal cells. The name 'natural killer' reflects the fact that they do not recognise specific cells or foreign substances, but are able to attack a variety of such targets. NK cells bring about killing of the target cell by the insertion of protein molecules, called **perforins**, into the cell membrane, creating a pore through which toxic substances can be injected into the cell.

NK cells can be stimulated by factors (called cytokines—see page 195) released in specific immunity, and they then show greater killing activity (see 'Cell-mediated (cellular) immunity', page 209). They also release cytokines (e.g. interferon-γ) which activate macrophages. NK cells also have receptors for antibodies (see 'Humoral immunity', page 202) and so can also kill antibody-coated target cells. Although classified as innate immune cells, there is increasing evidence that NK cells have some attributes similar to lymphocytes of the acquired immune system. For example, NK cells have been shown to exhibit some antigen specificity, to undergo clonal expansion following antigen stimulation, and to generate memory cells (see later section on the acquired immune system).

Eosinophils and mast cells may also be considered cellular components of the innate immune system. The activities of these cells are described later in this chapter.

Inflammation

The **acute inflammatory response** is a second line of defence that is the body's response to tissue injury. It occurs in response to any injury, including physical damage (e.g. cuts and abrasions), burns, radiation (e.g. sunburn) and chemical injury (e.g. acids), as well as to infection by microorganisms. Although the physiologic events are similar each time, the inflammatory response produced is somewhat tailored to the cause and extent of the injury. Inflammatory conditions are named by adding the suffix *-itis* to the affected organ or tissue (e.g. conjunctivitis for inflammation of the conjunctiva of the eye, appendicitis for inflammation of the appendix).

The major function of inflammation is to clear the injured site of cellular debris and any foreign material or microbes (if present), thereby preparing the area for repair processes. If the invading microbes are not able to be destroyed, inflammation may act to confine the microbes inside a wall of fibrin, preventing their spread to adjacent areas. When effective, the inflammatory response eliminates any foreign material, removes injured tissue components and enables tissue repair processes to occur.

The four main signs of acute inflammation are:

- redness
- heat
- swelling
- pain.

The major events of the inflammatory process are described below and summarised in Figure 9.6.

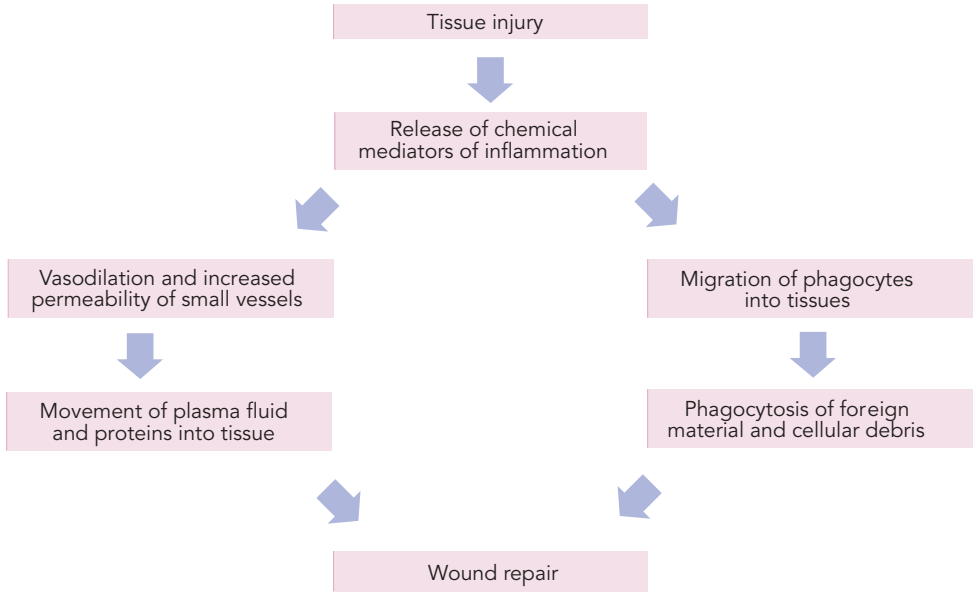


FIGURE 9.6

A summary of the events in acute inflammation

Chemical mediators of inflammation

Once an injury has occurred, several events take place, leading to the activation or release of a variety of chemicals. These chemicals are responsible for the physiological events that occur in inflammation and are called the chemical mediators of inflammation. Derived from a variety of sources, the most important are histamine, kinins, prostaglandins, leukotrienes, tumour necrosis factor and complement. The sources and activities of inflammatory mediators are summarised in Figure 9.7.

The different chemical mediators of inflammation come from a variety of sources. **Histamine** is present in many different body cell types, especially in mast cells in connective tissue, and in basophils and platelets in the bloodstream. It is released when these cells are injured or stimulated, and it has a potent action on small blood vessels (arterioles and capillaries) causing them to dilate (see Figure 9.8) and increase their permeability to plasma fluid and proteins. **Antihistamines** are used to alleviate the redness and swelling prominent in such clinical conditions as hay fever and hives.

Kinins (e.g. bradykinin) are small peptides present in blood and other body fluids in an inactive form (kallikrein). The kinin system is activated by tissue injury. Kinins cause vasodilation and increased vascular permeability as well as inducing pain.

Prostaglandins and **leukotrienes** are vasoactive mediators synthesised from a long-chain fatty acid called *arachidonic acid*, which is released from mast cell membranes. They cause dilation of post-capillary venules, increased vascular permeability and neutrophil chemotaxis. Prostaglandins also induce pain. Several of the leukotrienes cause sustained constriction of bronchioles, as occurs in asthma.

The importance of prostaglandins and leukotrienes as inflammatory agents is reflected in the action of some commonly used anti-inflammatory drugs. Aspirin, indomethacin and certain other non-steroidal anti-inflammatory agents act by blocking the synthesis of prostaglandins, as shown in Figure 9.9. Their analgesic (pain-reducing) effect is also due to inhibition of prostaglandin synthesis. Steroidal anti-inflammatory drugs are very potent partly because they block both prostaglandin and leukotriene synthesis.

Macrophages involved in an inflammatory process also secrete cytokines, such as IL-1 and tumour necrosis factor a (see page 195), that contribute to the progress of the inflammation. Tumour necrosis factor a (TNF-a) is particularly potent because it not only induces inflammation, but also induces inflammatory cells to secrete more TNF-a, thus amplifying the inflammatory response. Continuous and excessive production of this cytokine can result in disorders related to prolonged inflammation, such as rheumatoid arthritis.

Vasodilation and increased vascular permeability

Many of the inflammatory mediators cause dilation of the arterioles, directly or indirectly, in the area of damage. This causes more blood to flow into the area, accounting for the redness and warmth of inflamed tissue.

Some mediators increase the permeability of local capillaries and post-capillary venules by causing retraction of the endothelial cells of these vessels. As a result, plasma fluid and proteins are able to move from the bloodstream into the tissue spaces. This fluid is called an **exudate**. An accumulation of exudate in tissue causes **oedema**, or swelling. The pain that is usually associated with inflammation is due to compression of nerve endings by this swelling, as well as to the direct effects of some inflammatory mediators on pain

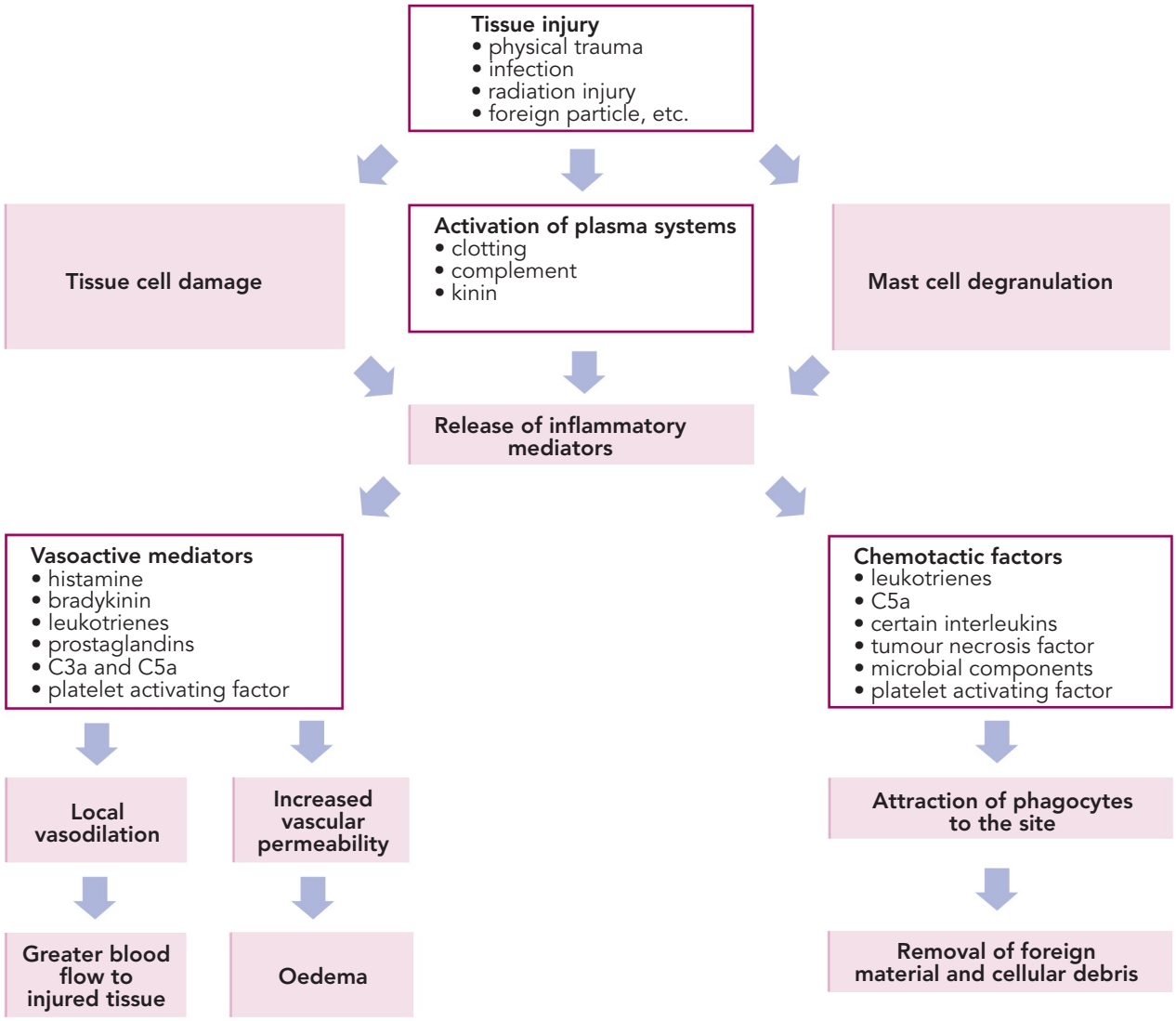


FIGURE 9.7

The sources and activities of the chemical mediators of acute inflammation

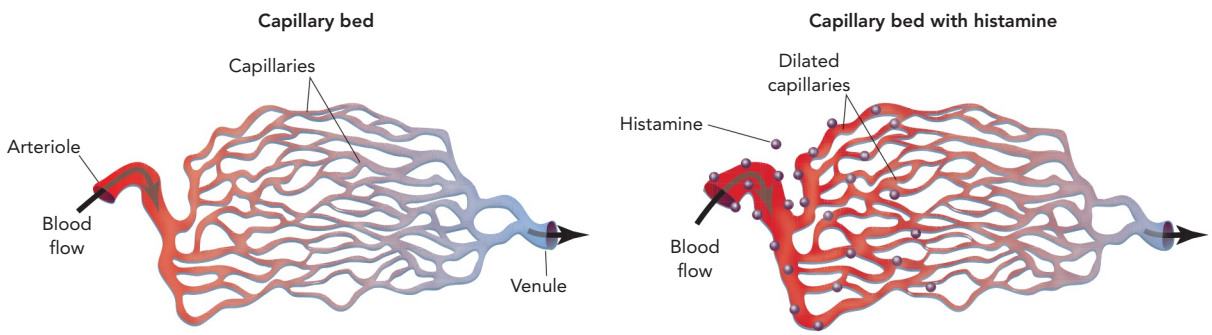


FIGURE 9.8

The dilating effect of histamine on arterioles and capillaries

receptors. Depending on the site of injury, swelling and pain may cause temporary loss of function of a body part (e.g. in a joint), and this is sometimes regarded as the fifth sign of acute inflammation.

The purpose of local vasodilation and increased vascular permeability is to provide the injured site with a variety of blood-borne substances that assist in tissue repair. The plasma fluid that enters injured tissue contains oxygen and

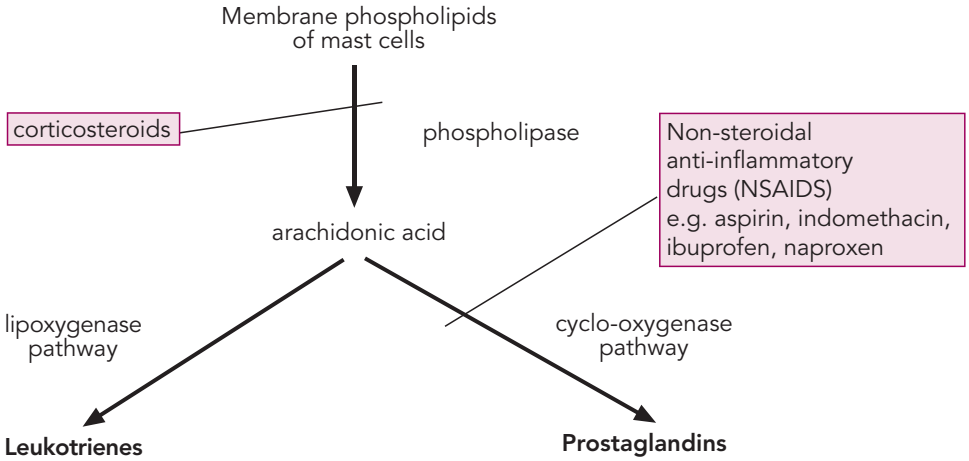


FIGURE 9.9

The synthesis of prostaglandins and leukotrienes and the actions of some anti-inflammatory drugs

nutrients necessary for the repair process, as well as important proteins like antibodies, complement and clotting proteins. In some infections, the clotting proteins form a fibrin barrier around the site to isolate the injured area and prevent the spread of bacteria or other harmful agents into surrounding tissues. As described later in this chapter, the antibodies and complement in plasma fluid are substances that help to eliminate foreign organisms.

Although inflammation is an important defence mechanism, there are situations where it can actually harm the body. For example, the swelling and pain associated with a disorder such as rheumatoid arthritis cause severe discomfort because an excessive degree of inflammation occurs. The role of inflammation in the production of disease is discussed later in this chapter.

Phagocyte migration

During inflammation there is an influx of phagocytic cells into the injured tissue. First, the neutrophils arrive and, later, the macrophages. Activated mediators from plasma and injured cells stimulate the release of extra neutrophils from the bone marrow into the blood and then their attraction to the site of injury. In severe infections, the number of neutrophils in the blood may increase four or five times within several hours. A significant increase in the number of neutrophils in blood is called **neutrophilia**, which is a characteristic sign of severe bacterial infection.

Some of the inflammatory mediators, such as complement factors, prostaglandins and kinins, act as chemotactic agents for phagocytes, attracting them to the site of the injury. During inflammation, the blood flow in the region of the injury slows and neutrophils begin to stick to the inner walls of the capillaries—known as **margination** (see Figure 9.10). Neutrophils do this because the endothelial cells lining the blood vessels exhibit specific adhesion molecules in response to some inflammatory mediators. The neutrophils then squeeze between the endothelial cells of the capillary walls (a process called **diapedesis**) and move into the tissue

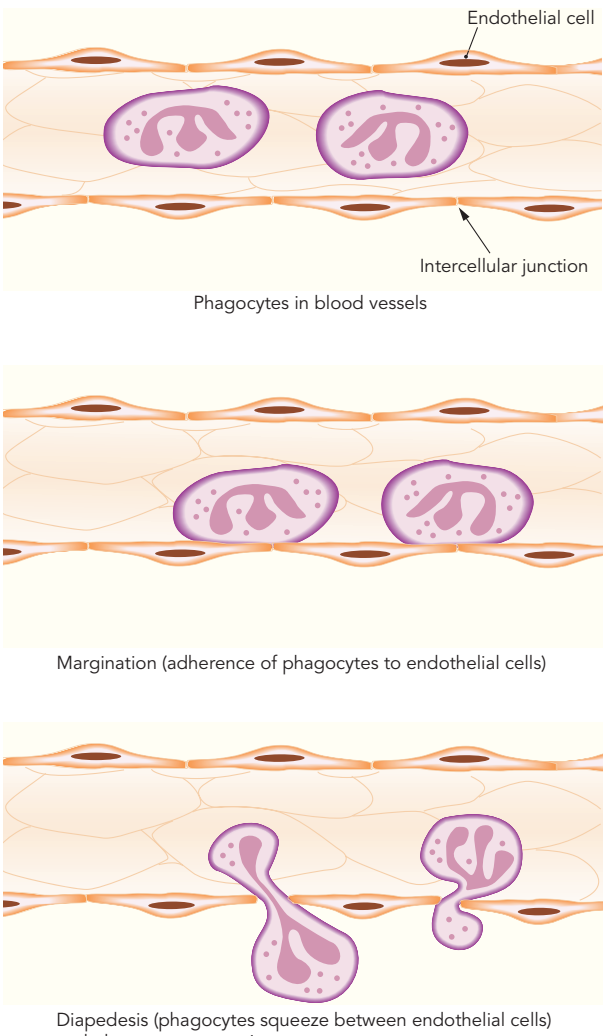


FIGURE 9.10

Margination and diapedesis of phagocytes

During inflammation, phagocytes stick to the insides of capillaries (margination) at the site of injury and then enter the tissue spaces by passing between the endothelial cells (diapedesis).

spaces, where they can devour dead or damaged tissue cells and any foreign particles or microbes that are present.

Late in the inflammatory response, monocytes from the bloodstream enter the area, replacing the neutrophils, which leave or die *in situ*. After entering the tissues the monocytes swell and develop large numbers of lysosomes to become mature, highly active macrophages. They are responsible for the final clearing of cell debris and foreign matter from the injured area.

In sites of bacterial infection, pus may accumulate in the wound. **Pus** is a thick, creamy-coloured fluid comprising a mixture of dead and dying neutrophils, tissue debris and remaining pathogens. Neutrophils are the major component of pus because many die *in situ*, having expended themselves in voracious phagocytic activity. Pus formation is typically induced by bacteria, but not by viruses. This can sometimes be helpful in distinguishing between bacterial and viral infections.

Certain bacteria (e.g. staphylococci, gonococci) are particularly strong attracters of neutrophils and are termed **pyogenic**, or pus-forming, bacteria. When the sac of pus is walled off by a layer of fibrin, an **abscess** is formed (see Figure 9.11). This represents the body's attempt to localise the infection and the potentially damaging neutrophil enzymes and other toxic substances that are released from these cells as they die. The pus remains locked up in the abscess. Thus, physical drainage of abscesses is often necessary before healing can occur.

Wound repair

Wound repair is a complex process, involving a variety of cells, cytokines and repair substances. Classically, wound repair is divided into three phases—inflammation, proliferation and remodelling—but the processes within each of these phases vary according to the nature of the wound and the amount of damage that has been caused. The most favourable outcome is a complete removal of microorganisms and tissue debris and a return of the tissue to normal structure and function.

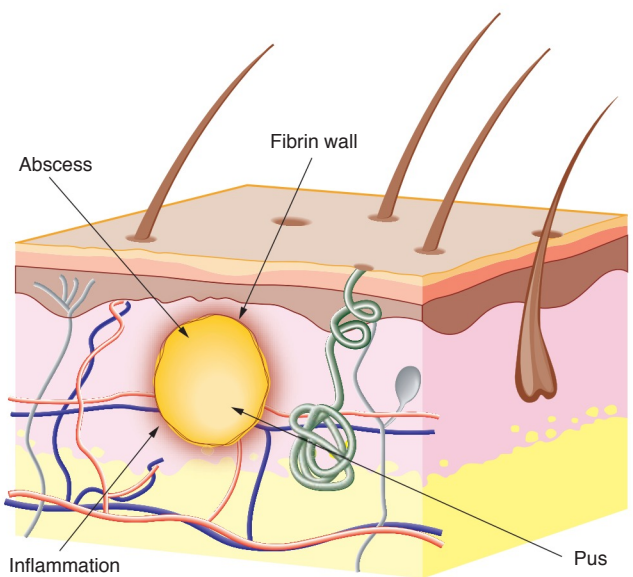


FIGURE 9.11

An abscess is a sac of pus contained within a wall of fibrin

As stated earlier, in the latter stages of the acute inflammatory response monocytes migrate into the wound and transform into macrophages. The macrophages have a phagocytic role, and also secrete a wide array of factors involved in tissue clearance and repair. While inflammation is critical for wound repair, prolonged or intense inflammation can cause further injury to tissues. Failure of an inflamed wound to progress to the proliferation phase results in a chronic wound (see below).

The proliferative phase begins towards the end of the inflammatory phase when various substances are produced within the wound space to form a temporary wound matrix. Fibroblasts migrate into the wound from the wound margins and begin secreting collagen into the wound matrix. Granulation tissue (comprising fibroblasts, collagen, new capillaries and ground substance) grows into the wound from surrounding healthy tissue.

In surface wounds, a process of re-epithelialisation occurs soon after injury. Re-epithelialisation relies on the migration of epithelial cells into the wound. Epithelial migration and proliferation continue until the wound is covered and an intact epithelial barrier is re-established. Some fibroblasts are stimulated to transform into myofibroblasts, which are involved in wound contraction.

Fibroblasts continue producing new collagen, while tissue proteases digest it. However, there is a net increase in collagen over time. This formation and breakdown of collagen is a remodelling process, during which the collagen is progressively reformed into a more organised structure with increased strength.

The most favourable outcome is the restoration of the tissue's original structure and physiologic function by **regeneration**, the proliferation of remaining cells by mitosis. This occurs if the destroyed tissue is capable of regeneration, if the damage is minor and if there are no other complications. If the tissue is incapable of regeneration (e.g. nerve tissue) or is extensively damaged, or if the clean-up is incomplete (e.g. in abscess formation), the tissue is repaired by **scar tissue replacement**. Scar tissue is comprised mainly of collagen. It fills in the lesion but it does not have the physiological functions of the destroyed tissue.

Chronic inflammation

Chronic inflammation occurs if the acute inflammatory response is unsuccessful in eliminating the foreign material from the tissues. For example, if particles (e.g. glass, dirt) and/or bacteria persist in a wound, the inflammatory response may continue beyond two weeks. Pus may be formed continuously, sometimes for months or even years. Large numbers of lymphocytes and macrophages are involved in chronic inflammation, in contrast to acute responses in which neutrophils predominate. Effective wound repair can also be compromised by many other

factors, such as poor blood supply, diabetes, malnutrition, or advanced age, which may result in the development of a chronic wound. Chronic wounds generally occur in skin and are commonly called skin ulcers. Pressure sores are a common type of skin ulcer.

Chronic inflammation can also occur as a distinct process from the outset, in the absence of acute inflammation. Certain microbes, such as the bacteria that cause tuberculosis and leprosy, induce chronic inflammation because of their resistance to intracellular digestion by phagocytes. The persistence of these organisms continues to stimulate an inflammatory response. Other agents that typically evoke chronic inflammation are low-grade, persistent irritants such as talc, silica and asbestos. These substances persist in tissues but do not penetrate or spread.

A **granuloma** is a special kind of chronic inflammation produced in response to certain microorganisms, such as the bacteria that cause leprosy, tuberculosis and syphilis. Granulomas are formed in these infections because the macrophages are unable to eliminate the organisms. Granulomas typically consist of a mass of different types of cells arranged in fairly discrete layers, completely walled off (encapsulated) by fibrous deposits of collagen. At the centre of the granuloma are macrophages or 'epithelioid cells' (modified macrophages) clumped in a mass, or coalesced together to form multi-nucleated cells. Around these giant cells is a layer of epithelioid cells and then a layer of lymphocytes. This mass of cells represents the body's attempt to surround and isolate the foreign agent. Granulomas are sometimes given special names, such as 'gummas' in syphilis and 'tubercles' in tuberculosis.

In the past, surgical gloves were dusted with talc powder so that they would slip on easily, but it was found that particles of talc often entered the surgical field and caused granulomas to develop there after surgery. Gloves are now dusted with an absorbent starch to avoid this problem.

Fever

Fever, a higher-than-normal body temperature, is a systemic response to infection and often accompanies severe or generalised inflammation. It is also considered to be a component of the second line of defence of the innate immune system. Body temperature is regulated by a cluster of neurons in the hypothalamus, considered to be the body's thermostat. Normally, the thermostat is set at approximately 37°C. Fever occurs when the set-point of the thermostat is raised to a higher temperature.

Pyrogens are chemical substances that produce fever. They are referred to as **exogenous pyrogens** if they come from outside the body, and **endogenous pyrogens** if they are formed inside the body. Exogenous pyrogens are, in the main, components of microorganisms, the best characterised being the lipopolysaccharide (endotoxin—see Chapter 10) of the cell wall of Gram-negative bacteria. Endogenous pyrogens are released from macrophages and neutrophils during inflammation or in response to the presence of exogenous pyrogens. The best-known endogenous pyrogens are interleukin-1 (IL-1) and tumour necrosis factor (TNF). These are the substances that actually alter the set-point in the hypothalamus, whereas exogenous pyrogens act indirectly by causing their release. This process is summarised in Figure 9.12.

A person experiencing a fever due to infection may experience cyclical periods of feverishness (feeling hot) and chills (feeling cold). This occurs because the resetting of the body temperature set-point by pyrogens can be unstable, with the set-point fluctuating over time. Thus, if a person has a body temperature of 39°C, but their set-point is suddenly reset to 40°C, they will actually shiver and feel cold—that is, experience chills—even though they have a higher-than-normal body temperature. The body's temperature-sensing mechanisms have detected a body temperature that is lower than the set-point. In contrast, if the set-point is suddenly dropped, the person will feel hot, or feverish.

The clinical approach to the management of fever has changed in recent years. In the past, **antipyretic** (fever-reducing) drugs and physical measures (e.g. tepid baths) were routinely used to reduce fever. Now, it is common practice to allow a slight to moderate fever to run its course. Although high fevers (above 40°C) are dangerous because of the possibility of damage to nerve cells and convulsions, mild or moderate fever may have some benefit for the body. The rate of cell division of bacteria is slowed at temperatures above 37°C. Also, bacteria require iron and zinc for growth, but during fever the liver and spleen sequester these nutrients, making them less available. A small increase in body temperature may also increase the metabolic rate and activity of defence cells, such as T lymphocytes and phagocytes, and is also thought to speed up repair processes.

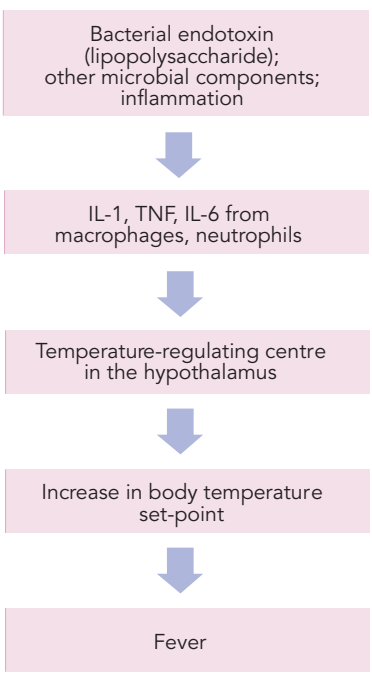


FIGURE 9.12

The production of fever

It is accepted, however, that high fever, or fever in patients with cardiovascular disease, seizures or respiratory disorders, should be treated immediately. Antipyretic drugs such as aspirin appear to reduce fever by restoring the temperature set-point in the hypothalamus to normal.

Antimicrobial proteins

In addition to the various protective chemicals mentioned earlier in this chapter, the body has certain antimicrobial proteins that act non-specifically. The most important of these are the complement proteins and acute phase proteins.

Complement

The complement system, or simply **complement**, comprises a complex system of plasma proteins that are present in the blood in an inactive state. The major components are designated as factors C1 to C9 and factors B, D and properdin. It is an enzymatic cascade system, similar to the blood coagulation system. When it is activated, the complement system enhances the inflammatory response and is largely responsible for the destruction of foreign cells in the body. Its major functions are to:

- enhance phagocytosis
- induce inflammation
- directly lyse (break down) foreign cells.

Complement enhances the action of many non-specific and specific defences, and is in turn enhanced by some specific defences. Hence, it is a major interface between the non-specific and specific defence systems.

Complement can be activated by one of three pathways (outlined in Figure 9.13). The *classical pathway* is initiated by the binding of antibodies to antigen. Complement proteins are activated and bound to a receptor on antibody molecules in a step called **complement fixation**. The *alternate (properdin) pathway* is initiated by an interaction between properdin and factors B and D, with the polysaccharides that are present in the cell walls of many microorganisms. The third pathway is the *lectin pathway*. Lectins are carbohydrate-binding proteins found in serum that are able to bind to a wide range of microorganisms. This complex causes activation of the complement system as shown in Figure 9.13. Together, these three pathways enable the complement system to clear or destroy a wide range of microorganisms and foreign molecules.

In all three pathways the complement proteins are activated in a particular sequence, with each protein activating the next one in the sequence. All pathways lead to the cleavage of C3 into two fragments (C3a and C3b). These fragments are responsible for the induction of three different processes that result in the destruction of foreign cells and substances. They are cytolysis (cell breakage), opsonisation (enhancement of phagocytosis) and inflammation.

The complement proteins are able to destroy foreign cells by damaging their cell membranes. This **cytolysis** is initiated by the C3b fragment and other components which activate

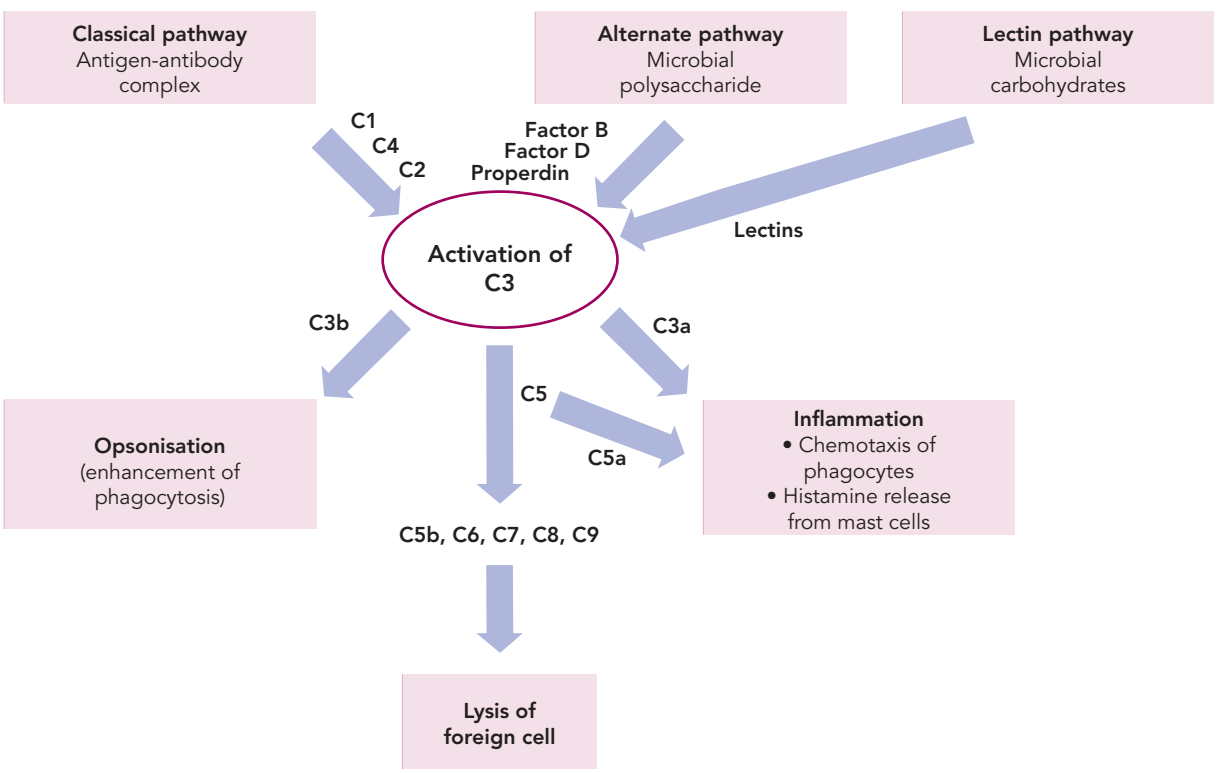


FIGURE 9.13

The pathways of complement activation and its biological activities

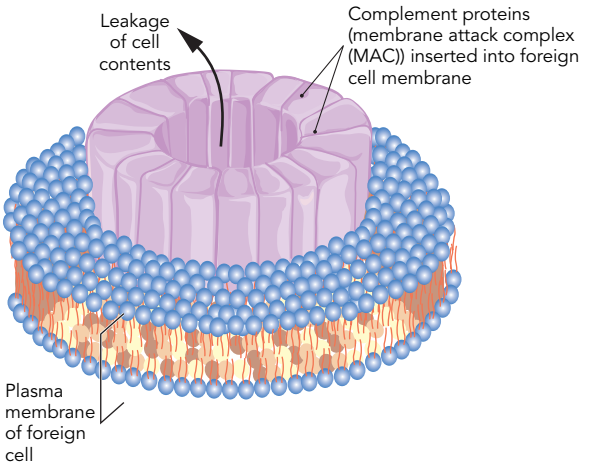


FIGURE 9.14

The membrane attack complex (MAC) of complement

Complement proteins are inserted into the membrane of the foreign cell, forming a pore through which the cell contents can leak out.

the C5 to C9 sequence of reactions. The activated proteins produced from these reactions form a complex which is inserted into the foreign cell membrane (see Figure 9.14). This **membrane attack complex** acts as a trans-membrane channel which allows the leakage of fluid and electrolytes out of the cell and, ultimately, its destruction. Complement-mediated cytolysis is important in protection against microorganisms, and is also the basis for other reactions, such as transfusion reaction following transfusion of incompatible blood, transplant rejection and some autoimmune diseases.

The C3b fragment can also bind to the surface of microorganisms and then bind with specific C3b receptors on phagocytes. By binding to both the microbe and the phagocyte, the C3b effectively holds the microorganism against the surface of the phagocyte (see Figure 9.15), thereby promoting the phagocyte's ability to ingest the organism. This enhancement of phagocyte activity is termed **opsonisation** and C3b is called an **opsonin**.

C3a (the other cleavage product of C3) and C5a (the cleavage product of C5) are **anaphylatoxins**; that is, they bind to mast cells, basophils and platelets, triggering the release of histamine and other substances (see Figure 9.13).

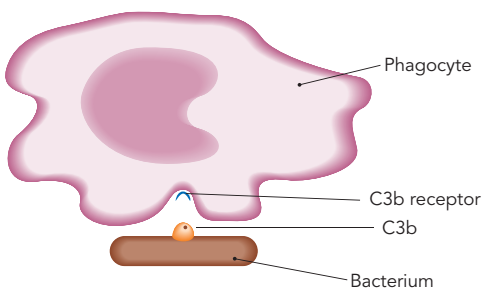


FIGURE 9.15

Opsonisation of a bacterial cell by complement

As mentioned earlier in this chapter, histamine is one of the major inducers of inflammation. C5a also contributes to inflammation by acting as a potent chemotactic factor, attracting phagocytes to the site of injury.

Acute phase proteins

Acute phase proteins are a group of plasma proteins that enhance resistance to infection, limit tissue damage due to infection, trauma, malignancy and so on, and promote tissue repair. Plasma levels of these proteins increase in response to infection, inflammation or tissue injury. One of the proteins, C-reactive protein, is measured in serum to indicate current disease activity in people with certain inflammatory conditions (e.g. rheumatoid arthritis).

Antimicrobial peptides

Antimicrobial peptides (**AMPs**) are small peptides containing fewer than 100 amino acid residues, which protect against a broad range of microorganisms. These substances are produced not only in humans, but also in other organisms including insects, plants and even bacteria. In humans, AMPs are found on the external barriers (skin and mucous membranes), where they act like endogenous antibiotics, by direct killing of microorganisms. Some AMPs have broad activity, being able to attack multiple types of microorganisms, including Gram-positive and Gram-negative bacteria, fungi, parasites and viruses. They are also stored within granules in phagocytes, where they assist in the killing of engulfed microorganisms.

Several AMPs have been identified in humans, the best-known families of substances being the cathelicidins, defensins and histatins. However, there are other important AMPs that are not included within these families, such as granulysin, lactoferrin and hepcidin. The mechanisms by which AMPs exert their antimicrobial effects are not fully understood, but some are believed to kill bacteria by pore formation in bacterial membranes, by binding to cell wall structures, or by inhibiting intracellular processes of the microorganisms.

In addition to their direct antimicrobial actions, AMPs appear to have a range of other biologic effects which contribute to the control of infectious and inflammatory diseases. Other activities that have recently been attributed to these multifunctional molecules include neutralisation of endotoxins, chemoattraction of immune cells, including monocytes, neutrophils and T lymphocytes, and induction of angiogenesis and wound repair. And there is increasing evidence that AMPs play an important role in activating the acquired immune response, and take part in the interconnections between the innate and acquired immune systems.

The increasing incidence of antibiotic resistant bacteria is a major challenge of modern medicine (see Chapter 12). New agents and new therapeutic approaches are clearly needed to combat this resistance problem. It is considered that AMPs could become useful alternatives to antibiotics because of their broad antimicrobial activity. Also, because they operate by diverse mechanisms, the likelihood of

microorganisms becoming resistant to them is considered low, since naturally occurring resistance to them is rare. Furthermore, their immunomodulatory functions and low toxicity adds to their attractiveness as potential therapeutic agents for other conditions, including cancer.

Cytokines

Cytokines are a group of chemical substances that act as messengers within the immune system and between the immune system and other systems of the body. As a group, cytokines are involved in many facets of non-specific immunity, especially in inflammation and phagocyte chemotaxis and activation. As you will see, they also play a critical role in the development of specific immune responses by inducing cell activation and proliferation. Overall, these substances serve many functions, with critical roles in inflammation, haematopoiesis, cell proliferation and differentiation, and the mediation and regulation of the immune response. Cytokines are secreted by a variety of cells, including white blood cells, fibroblasts, endothelial cells and epithelial cells. However, T helper cells and macrophages are the major producers. A single cytokine can have multiple sources, and can act on many different cell types and have different effects on them. The major groups of cytokines are interleukins, interferons, colony stimulating factors, tumour necrosis factors and chemokines (see Table 9.3 for examples).

Interleukins are released by leukocytes and some other cell types. There have been almost 30 different interleukins identified, referred to as interleukin-1 (IL-1), interleukin-2 (IL-2), and so on. Interleukins have numerous actions including cell activation, cell proliferation, cell differentiation and antiviral activity.

Interferons were first identified in the 1950s as antiviral proteins produced by virus-infected cells to help other cells resist infection. One of the important features of interferons is that they are *not virus-specific*; that is, interferon produced against a particular virus protects against a variety of other viruses. Three major types of interferon, produced by different cell types, have been identified. Virus-infected leukocytes produce **alpha interferon** (**IFN-α**), whereas fibroblasts, epithelial cells, macrophages and other body cells secrete **beta interferon** (**IFN-β**) when infected by viruses. IFN-α and IFN-β do not protect already infected cells but, when secreted from them, protect neighbouring cells from infection. They do this by binding to interferon receptors on neighbouring cells, causing them to produce intracellular antiviral proteins which interfere with viral replication in those cells (see Figure 9.16).

The third type, **gamma interferon** (**IFN-γ**), is not produced by virus-infected cells, but is secreted by activated lymphocytes and NK cells. As you will see later in this chapter, IFN-γ has an important role in the development of acquired immune responses by enhancing the actions of macrophages, neutrophils, natural killer cells and lymphocytes.

TABLE 9.3 **Examples of cytokines with their sources and functions**

CYTOKINE	SOURCE(S)	MAJOR FUNCTION(S)
Interleukin 1 (IL-1)	Macrophages, dendritic cells, endothelial cells	Induction of fever; T cell activation; macrophage activation; inflammation
Interleukin 2 (IL-2)	T cells	Proliferation of T cells; activation and proliferation of NK cells
Interleukin 4 (IL-4)	T cells, mast cells	B cell activation; differentiation of Th cells into Th2
Interleukin 5 (IL-5)	Th2 cells	Activation of eosinophils
Interleukin 6 (IL-6)	T cells, macrophages	Differentiation of lymphoblasts into plasma cells
Interleukin 9 (IL-9)	T cells	Stimulates Th2 cells; enhances mast cell activity
Interleukin 10 (IL-10)	T cells, macrophages	Inhibits macrophage functions
Interleukin 12 (IL-12)	Macrophages, dendritic cells	Activates NK cells
Interleukin 18 (IL-18)	Macrophages	IFN-γ production by NK cells and T cells
Granulocyte colony stimulating factor (G-CSF)	Fibroblasts, endothelium	Granulocyte production
Granulocyte-macrophage colony stimulating factor (GM-CSF)	T cells, macrophages	Increases production of granulocytes, macrophages and dendritic cells
Tumour necrosis factor-α (TNF-α)	Macrophages, dendritic cells, NK cells, T cells	Kills tumour cells; induces inflammation and fever; endothelial cell activation
Tumour necrosis factor-β (TNF-β)	T cells, B cells	Kills tumour cells; endothelial cell activation
Interferon-α (IFN-α)	Leucocytes, dendritic cells	Induction of antiviral state in cells; NK cell activation
Interferon-β (IFN-β)	Fibroblasts	Induction of antiviral state in cells; NK cell activation
Interferon-γ (IFN-γ)	T cells, NK cells	Macrophage activation; NK cell activation; promotes antigen presentation

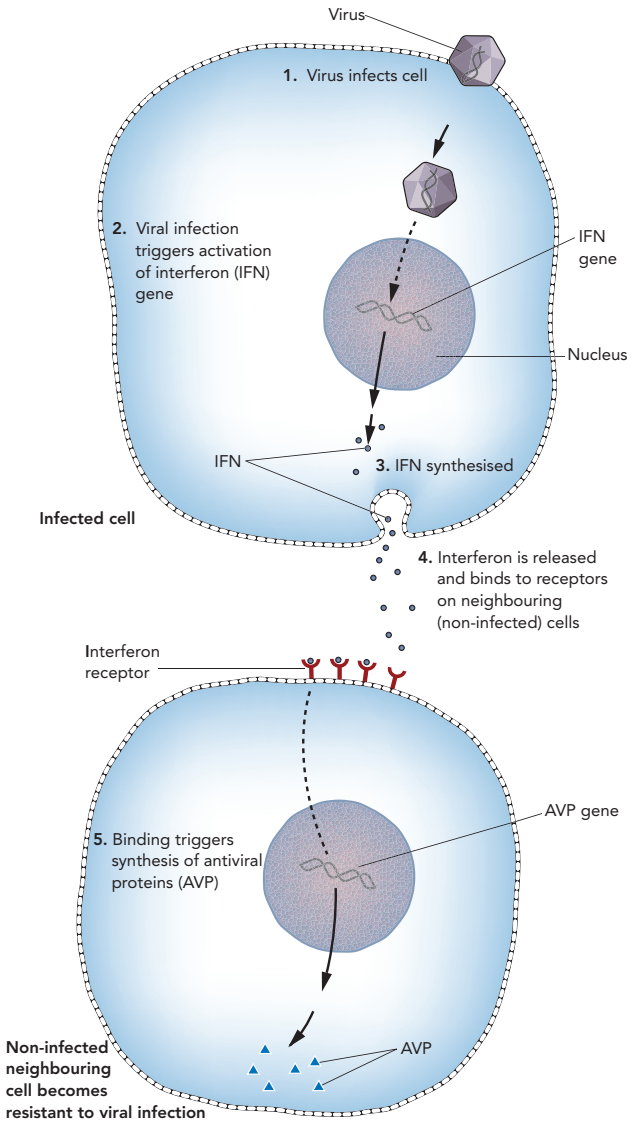


FIGURE 9.16

The antiviral action of interferon

Interferons can be produced in large quantities using recombinant DNA technology, and have been used with some success in the treatment of certain viral infections, such as hepatitis B, hepatitis C and genital herpes, and against some tumours, such as malignant melanoma and Kaposi's sarcoma. They have also been effective to a degree in slowing the progression of autoimmune diseases, such as rheumatoid arthritis and multiple sclerosis (see later in this chapter). However, the clinical use of interferons is not without problems, including unpleasant side effects, toxicity in high concentrations, and a short half-life in the body.

Colony stimulating factors (or growth factors) are cytokines that stimulate certain cells to divide and differentiate. Examples are GM-CSF (granulocyte-monocyte colony stimulating factor), which stimulates monocytes, dendritic cells and granulocytes, and G-CSF (granulocyte colony stimulating factor) which stimulates neutrophils.

Tumour necrosis factors (TNF) are cytokines that kill tumour cells, but they also have other functions such as regulation of immune responses, endothelial cell activation and induction of inflammation. The two main types are TNF-α and TNF-β.

Chemokines are cytokines that are responsible for the attraction of phagocytes to a site of injury—that is, they are chemotactic factors. Chemokines also activate certain leukocytes and control the migration and homing of lymphocytes in the body. More than 60 different chemokines have been identified.

Some cytokines that enhance immune responses are now used in the treatment of certain disease conditions. For example, IL-2 and IFN-γ have been used in the treatment of certain tumours; G-CSF has been used to increase the neutrophil numbers in some people (e.g. those undergoing chemotherapy) who are deficient in these cells; and IFN-α is now part of the standard treatment for chronic hepatitis B and C.

ACQUIRED IMMUNE SYSTEM

The specific body defences, or **acquired immune system** (also called the **adaptive immune system** or **specific immune system**), is a functional system consisting of a variety of cells, especially lymphocytes and macrophages, and various organs such as the thymus gland and lymph nodes. It is the body's third line of defence and protects it from infection and from damage by foreign cells and substances that enter the body; it is also active against tumour cells. If it fails or is disabled, serious diseases such as cancer or life-threatening infections may result. **Immunity** is the capacity of the immune system (the acquired immune system plus some components of the non-specific immune system) to successfully defend the body against a potentially infectious agent. **Immunology** is the study of the immune system and immunity.

Characteristics of the acquired immune system

The innate immune system is highly effective in quickly removing many microorganisms from the body, but some microbes find ways around these defences. For instance, microbes that have a high mutation rate (e.g. the human immunodeficiency virus, HIV) or that replicate intracellularly (e.g. *Mycobacterium tuberculosis*) can often escape the innate defences. When a microbe is able to evade these defences (i.e. the first and second lines of defence) and persist in the tissues, the body reacts by producing a response specifically directed against the invading organism. This is the **immune response**—the third line of defence.

A major principle of immunity is that, once a person has had an infectious disease, they are unlikely to contract the same disease again. The basis of this immunity was revealed in 1890, when Emil von Behring and Shibasaburo Kitasato demonstrated that animals that survive an infection have protective factors (now called *antibodies*) in their blood that protect them against future attacks by the same

pathogen. It was further shown that serum from surviving animals, when injected into other animals, protected the recipients from the same infection. These and subsequent studies revealed some important characteristics of the acquired immune system:

- It is able to distinguish between host substances (self) and foreign substances (non-self)—that is, it *recognises* foreign substances.
- It does not normally respond to self substances—that is, it exhibits *tolerance* to self.
- It is directed only against the particular pathogen or foreign substance that stimulated it—that is, it is *specific*.
- It recognises and mounts a more rapid and stronger response to a previously encountered pathogen—that is, it has *memory*.

The immune response also amplifies the inflammatory response and provides a mechanism for the activation of the complement system.

The two arms of the acquired immune system

In the 1960s it was found that, for some diseases, injection of antibody-containing serum did not protect recipients from the disease the donor had survived, but that injection of the donor's lymphocytes did provide immunity. Jacques Miller in London, and Noel Warner and Aleksander Szenberg (of the Walter and Eliza Hall Institute in Melbourne) determined that there are two different populations of lymphocytes, called **B lymphocytes** (or **B cells**) and **T lymphocytes** (or **T cells**). Each type is responsible for a different form of immunity.

Humoral immunity is immunity provided by **antibodies**, which are present in the body's 'humors' (Latin for 'fluids'). The antibodies are produced and secreted by plasma cells (derived from B lymphocytes) and they then circulate in the blood and other body fluids. Antibodies are most effective against bacteria and their toxins, and against viruses *before* they enter host cells. They act by binding to the invading microbes or their products, leading to their inactivation, destruction or elimination by various means.

Because antibodies are unable to enter cells, another type of defence is needed to deal with intracellular pathogens. This is called **cell-mediated immunity**, or **cellular immunity**, because of the direct involvement of cells in the immune processes. In cell-mediated immunity, T lymphocytes act against target cells by directly killing the cells, or by releasing chemicals that enhance the inflammatory response and/or activate other defence cells to bring about destruction of the target cells. Cell-mediated immunity provides the major form of defence against viruses and bacteria that have invaded host cells, and also against fungi, other eucaryotic parasites and cancer cells.

Before describing the humoral and cell-mediated responses separately, we consider the cells involved in these two components of the immune response and the foreign substances that trigger their activity.

Cells and tissues of the acquired immune system

Lymphocytes

The cells that are largely responsible for a specific immune response are the lymphocytes and macrophages. As stated above, the *B lymphocytes* are mainly responsible for humoral immunity and the *T lymphocytes* are the primary cells of cell-mediated immunity. These two types of lymphocytes interact with other defence cells to produce a specific immune response. A third type of lymphocyte, the *NK cell*, is described earlier in this chapter in relation to its role in innate immunity. NK cells also have a prominent role in acquired immunity, since they are activated by cytokines produced in a specific immune response.

Lymphocytes are the second most numerous type of white cell in blood. Like all blood cells, lymphocytes originate in the bone marrow. Immature lymphocytes are released from the marrow and mature into B cells or T cells depending on the site in the body where they undergo final maturation and become immunocompetent.

T cells are lymphocytes that migrate from the bone marrow to the thymus, and they undergo a maturation process brought about by hormones within the thymus (hence the term T cell). After puberty the thymus begins to regress in size and is progressively replaced by fatty tissue, but it continues to be responsible for T cell maturation until late in life. B cells are believed to mature in the bone marrow itself (or in the liver in the foetus). B cells are so called because they were first identified in the 'bursa of Fabricius', a pouch of lymphatic tissue associated with the digestive tract in birds. Since the thymus and the bone marrow are the sites of lymphocyte maturation (see Figure 9.17) they are referred to as the **primary lymphoid organs**. The collection of lymphoid organs and tissues (described in the next section) where immune reactions occur are referred to as the **secondary lymphoid organs**.

On the surface of each mature lymphocyte there are numerous receptors of a single type that enable the cells to recognise and bind to a single, specific foreign substance.

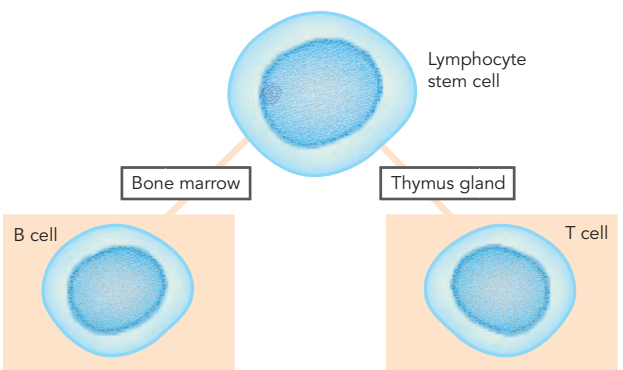


FIGURE 9.17

The maturation of lymphocytes

The maturation of immature lymphocytes occurs in the thymus and bone marrow, where they become antigen-specific T cells and B cells, respectively.

A foreign substance to which lymphocytes react is called an **antigen**. (Antigens are discussed in detail later in this chapter.) Each lymphocyte reacts with one particular antigen, and no others. That is, each lymphocyte is mono-specific. For example, receptors of one lymphocyte can recognise only the toxin of the tetanus-causing bacterium, whereas those of another lymphocyte might bind only to the cold virus. Generally, there are populations of both T lymphocytes and B lymphocytes that recognise each type of antigen.

The exact processes involved in lymphocyte processing and maturation are not entirely clear, but it is known that lymphocytes become immunocompetent and specific for a particular antigen before actually coming into contact with that antigen. That is, the specific foreign substances that the immune system is able to recognise and respond to are predetermined. Importantly, they are predetermined by genes, not by the antigens that are contacted. This means that the immune system consists of pre-committed lymphocytes for all the possible antigens that a person is likely to contact over the whole of their lifetime. Obviously, only some of the possible antigens our lymphocytes are programmed to recognise will ever be encountered and, therefore, only a proportion of the immunocompetent cells present in the body will ever be utilised. The obvious benefit of this diversity of lymphocytes is that together they can recognise virtually any antigen. The downsides of this are the relatively large amount of time required to generate an acquired response and the risk of autoimmunity. Both of these issues are discussed later in this chapter.

Mature lymphocytes that have not yet encountered their specific antigen are referred to as **naive lymphocytes**. Some naive lymphocytes circulate around the body, giving them maximum opportunity to contact their specific antigen. These lymphocytes move about freely between lymphoid organs and tissues, transported in the bloodstream and lymphatic system. Other naive lymphocytes remain in secondary lymphoid organs, such as lymph nodes and the spleen, where they lie in wait for their specific antigen to be brought to them by antigen-presenting cells. When a naive lymphocyte contacts and responds to its specific antigen, it becomes an activated lymphocyte.

Lymphocyte subsets and their functions

Several different functional types of T cells are involved in the process of acquired immunity. T cells possess a number of different glycoproteins on their surface called CD, for clusters of differentiation. Two of these glycoproteins that distinguish between the major functional groups of T cells are the CD4 and CD8 markers. T cells that possess the CD4 marker, called **CD4+ cells** (or **T4 cells**), are primarily T helper cells; whereas T cells with the CD8 marker, the **CD8+ cells** (or **T8 cells**), are cytotoxic T cells.

T-helper cells (**Th cells**) play a central role in the immune response. Once activated by antigen, they release cytokines to help B cells, T cells and other cells to produce an immune response (Figure 9.18). Three major types of Th cells can be distinguished according to the types of cytokines they produce. The **type 1 T helper cells** (**Th1**) mainly secrete the cytokine IFN-γ that helps to activate other T cells and phagocytes in cell-mediated immunity. IFN-γ also has potent antiviral effects. **Type 2 T helper cells** (**Th2**) release cytokines (IL-4, IL-5 and IL-13) to activate eosinophils and mast cells in defence against large extracellular parasites such as helminths. Th2 cells are also involved in the production of IgE antibodies in allergic reactions. **Follicular T helper cells** (**fTh**) are found closely associated with B lymphocytes in follicles in lymphoid organs and promote antibody secretion from B cells. The fTh cells produce IL-21, a cytokine that

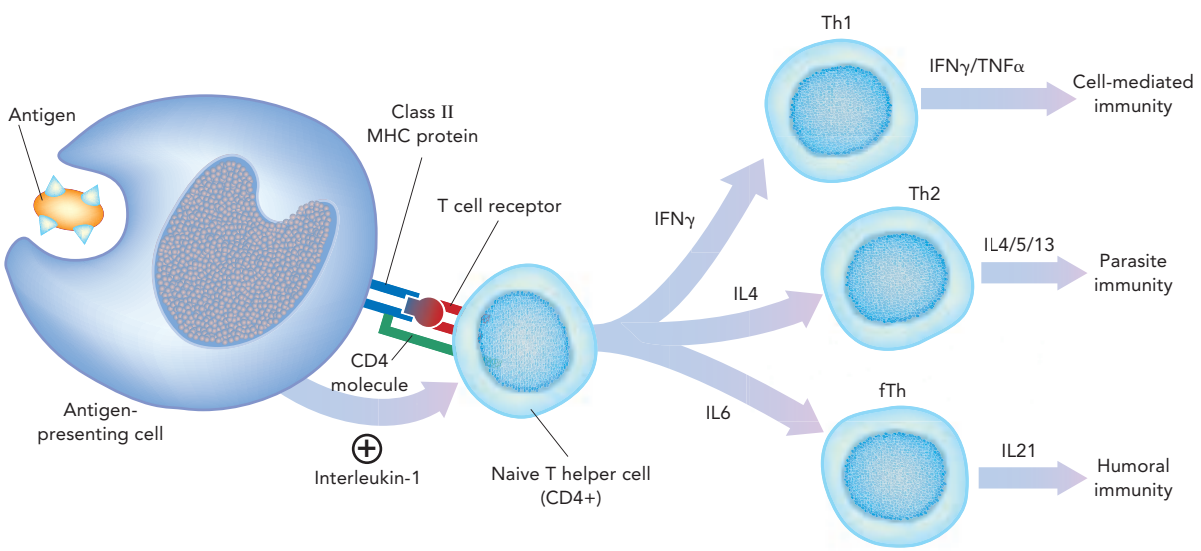


FIGURE 9.18

The central role of T helper cells in humoral and cell-mediated immunity

T helper cells are activated by antigen presented by an antigen-presenting cell; the activated T helper cells then release various cytokines which help to activate other T helper cells, cytotoxic T cells and B cells.

promotes humoral responses. Other subsets of T helper cells (e.g. Th17, Th21) with effector functions against specific pathogens are also known to exist. The differentiation of naive CD4+ T cells into the above T helper types is controlled by cytokines during antigen stimulation of the CD4+ cells. Most antigens require the involvement of Th cells for the activation of B cells and other T cells to occur. These are called **T dependent antigens**.

CD4+ T cells are so important in the activation of other lymphocytes that, without them, the immune response to most antigens would be severely diminished. This is starkly demonstrated in infections caused by the human immunodeficiency virus (HIV). The HIV attacks the CD4+ T cell population, rendering the infected person incapable of mounting immune responses to many organisms, and thus highly susceptible to infection and cancer.

Although the existence of cells that suppress immune responses has been postulated for many years, these cells have only recently been identified and characterised. **T regulatory cells** (**Treg**) are a subset of CD4+ T cells that have a key function in down-regulating (inhibiting) some immune responses. Thus, they have an important role in the prevention of autoimmunity and allergy, by suppressing the immune reactions that are responsible for these conditions. Their importance is illustrated in the severe autoimmune syndrome suffered by individuals with a genetic deficiency in Treg cells.

Cytotoxic T cells (**Tc cells**) bind directly to and kill target cells. Activated cytotoxic T cells circulate in the bloodstream and lymphatic system in search of cells displaying the antigens they specifically recognise. They attack mainly virus-infected cells, but they are also involved in defence against intracellular bacteria and tumour cells.

The role of B cells in the immune system has historically been considered to be restricted to the production of antibodies. However, the existence of B cell subsets that are not involved in humoral immune responses has recently been proposed. While still somewhat controversial, there is evidence to suggest that some B cells have a regulatory function in cell-mediated immune responses. These regulatory B cells appear to have a role in the development and control of certain T cell types, including CD4+ T cells and memory T cells. That is, they actively participate in the quality and magnitude of T cell responses.

The lymphoid system

The lymphoid system comprises a network of vessels, cells and specialised organs. Lymph is the fluid that is formed in tissues from blood components that enter the extracellular spaces. The lymph is drained from the tissues and carried in lymph capillaries that converge into larger vessels, ultimately returning the lymph to the bloodstream. On its way, the lymph is filtered through numerous lymph nodes. The **lymph nodes** are small, bean-shaped organs that tend to occur in clusters along lymphatic channels. Because lymph capillaries carry substances and pathogens absorbed from nearly all body tissues, immune cells in lymph nodes are in a strategic position to encounter antigens that have entered tissues.

The *secondary lymphoid organs* comprise the lymph nodes as well as the spleen, tonsils and clusters of lymphoid cells in the gastrointestinal tract, respiratory tract and other mucosal surfaces. The clusters of lymphoid cells lining the mucosal surfaces are called gut-associated lymphoid tissue (GALT), bronchi-associated lymphoid tissue (BALT) and so on, or are collectively referred to as the mucosa-associated lymphoid tissue (MALT).

The secondary lymphoid organs have a common structure that is a loose framework housing numerous lymphocytes and macrophages. They are strategically placed throughout the body to provide defence where it is likely to be needed (see Figure 9.19). For example, lymphocytes and macrophages in the tonsils act primarily against microorganisms that invade the oral and nasal cavities, whereas the spleen acts as a filter to trap blood-borne antigens. Collections of lymphocytes underlying mucous membranes provide local defence at those sites.

The **thymus** is found in the upper thoracic region beneath the sternum (see Figure 9.20). It continues to grow

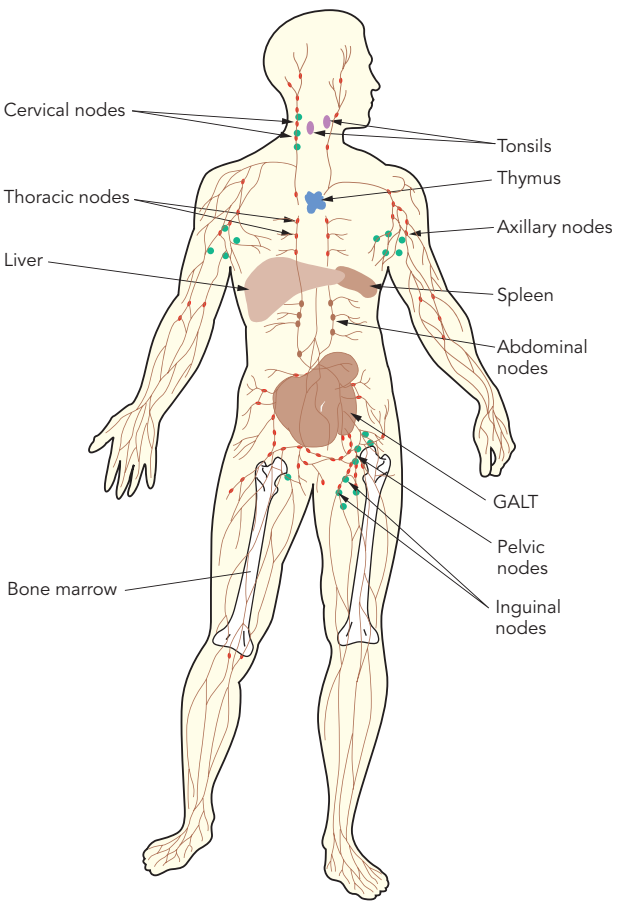


FIGURE 9.19

The components of the lymphoid system

The bone marrow, thymus, lymph vessels, lymph nodes, spleen, tonsils and aggregates of lymphocytes lining the small intestine (gut-associated lymphoid tissue, GALT), bronchi-associated lymphoid tissue (BALT) and other mucosal surfaces.

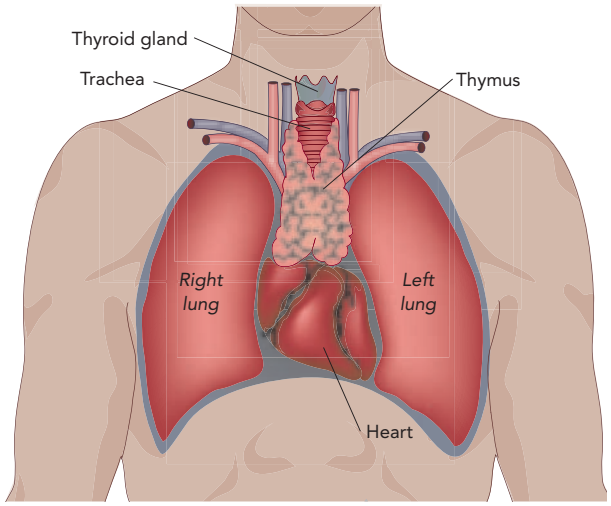


FIGURE 9.20

The thymus gland

until puberty, after which it progressively shrinks to become largely replaced by fatty tissue in later adulthood. As mentioned earlier, immature lymphocytes develop into mature T cells under the influence of thymic hormones. The importance of the thymus gland is clearly demonstrated in babies born without a functional thymus (DiGeorge syndrome). These children are highly susceptible to infection—even common childhood infections such as chickenpox can be very serious, and sometimes fatal.

Some lymphocytes circulate continuously between the bloodstream and lymphoid organs. This enables them to come into contact with antigens present in different parts of the body, as well as with other lymphocytes and antigen-presenting cells.

To summarise: the acquired immune system's ability to identify and destroy foreign substances is based on the ability of its cells, the lymphocytes, to recognise and respond to foreign substances (antigens) in the body. Different types of lymphocytes interact with one another and with other cells to mount a concerted response specific to each antigen.

Antigen-presenting cells

While B and T lymphocytes are considered to be primarily responsible for immune responses, they cannot do this alone. They actually require the help of other cell types, particularly a group known collectively as **antigen-presenting cells**. These cells play a critical role in the activation of lymphocytes. They do this by ingesting foreign antigen, processing the antigen by partially degrading it, and then presenting the antigen to lymphocytes in a form that the lymphocytes can respond to. The major antigen-presenting cells are the dendritic cells and activated macrophages. We have already discussed, in the section on phagocytosis earlier in this chapter, the capacity of these cell types for ingestion of foreign material, including microorganisms.

Dendritic cells, which are found in blood and tissues such as skin, are recognised as having a key role in the activation of naive T helper cells. The dendritic cells appear to perform this function in three distinct steps. First, they phagocytose and process the foreign antigen in the peripheral tissues where they normally reside. Once they have captured antigen, they then migrate to lymphoid organs where naive lymphocytes are located. Once in the lymphoid organ they can present antigen and a necessary co-stimulatory signal to naive T lymphocytes that are specific for that antigen. These lymphocytes are then transformed into activated T helper cells.

Dendritic cells are considered components of the innate immune system, but they clearly form a critical link between the innate and acquired immune systems. Via their array of receptors, including TLRs, dendritic cells are able to aid in the initiation of specific immune responses to a wide range of microorganisms. They are therefore considered to be necessary elements in the initiation of an immune response.

Macrophages are bone-marrow-derived cells that reside throughout the body. They can also have an antigen-presenting function, similar to dendritic cells, once they have become activated by ingestion of foreign material.

Therefore, dendritic cells and macrophages form a sentinel network that is continually on the lookout for foreign substances in the body, ingesting them, processing them and transporting them to lymphoid tissues for interaction with lymphocytes.

The mechanisms by which antigen-presenting cells process and present antigens to lymphocytes are described on pages 202–3.

Antigens

The acquired immune system does not direct its activity against whole microorganisms, but instead focuses against parts of them. The parts of microorganisms that are foreign (i.e. different from substances in the body) and that activate the acquired immune system are called *antigens*. The word 'antigen' is in fact a contraction of the term '*anti*body *gen*erating'. Most antigens are large, complex molecules (macromolecules) of the following types: proteins (and polypeptides), nucleic acids, some lipids and polysaccharides. Antigens are present on the surfaces of viruses, bacteria and other microorganisms, as well as on human, animal and plant cells.

Complete antigens and haptens

Complete antigens are foreign substances that stimulate specific T and B lymphocytes, inducing them to produce an immune response. Complete antigens are thus said to be **immunogenic** (or **antigenic**)—that is, capable of inducing an immune response. Proteins and macromolecules containing proteins tend to be the strongest antigens. Microorganisms are strongly immunogenic because they possess many different antigens, many of which are proteins exposed on the cell surface.

In contrast, smaller molecules, such as peptides, nucleotides and saccharides, are generally not immunogenic, even if they are foreign. However, some of them become immunogenic if they become attached to the body's own proteins and the immune system recognises the combination as foreign. A small foreign molecule such as this is called a **hapten**.

Although haptens cannot initiate an immune response on their own, they can react with antibodies or activated T cells produced against them. A good example of a hapten is penicillin, which can bind with a serum protein in some people and induce an immune response. In this case the immune response is manifested as an allergic reaction (see page 217).

Epitopes

Although antigens are foreign molecules of a certain minimum size, only a small part of the whole antigen molecule is immunogenic. This important part of the molecule is called the **epitope** (or **antigenic determinant**) and is the part to which free antibodies or activated B or T lymphocytes can bind.

Most naturally occurring antigens are very complex molecules and consist of a number of different epitopes (see Figure 9.21). Different epitopes are recognised by different lymphocytes; thus, a single, complex antigen may activate many different lymphocytes, stimulating the formation of many different kinds of antibodies. Large proteins often comprise hundreds of different epitopes, which is why these substances are so strongly immunogenic. In contrast, large molecules formed from many identical repeating units, such as plastics, are often non-immunogenic. Such substances are ideal for making artificial body implants (e.g. bone replacements) because they are not recognised as foreign by the immune system and therefore are not rejected.

The major histocompatibility complex

The **major histocompatibility complex** (**MHC**) is so named because of its importance in determining the compatibility of tissues when attempting to transplant an organ or tissue from one person to another. The MHC is a set of genes on chromosome 6 that code for a large number of proteins with a variety of functions in the body. Two groups of these proteins have a critical role in the immune system, and are referred to as class I and class II MHC proteins. As you will see in the following sections, these proteins are vital for certain cell-to-cell interactions that must occur for an immune response to be produced. Furthermore, MHC proteins are self-recognition molecules that help to prevent the immune system from mounting a response against self-antigens. MHC proteins are particularly abundant on the surface of leucocytes, and hence are also referred to as **human leucocyte antigen** (**HLA**) proteins. Class I MHC proteins are found in the membranes of all nucleated cells, whereas class II MHC proteins are found only on B lymphocytes and antigen-presenting cells (see Figure 9.22).

Different individuals have different HLA proteins (unless they are genetically identical, as in identical twins), although the more closely two people are related the more likely they will share some common HLA proteins. A difference in HLA proteins between donor and recipient is the basis for rejection of organ transplants. Thus, HLA proteins are our personal antigens, a mixture of those inherited from each of our parents.

Antigen processing by antigen-presenting cells involves the intracellular degradation of foreign proteins into small peptides and the combining of these peptides with MHC proteins. Presentation involves the transport of this peptide-MHC protein complex to the surface of the antigen-presenting cell for display to lymphocytes (see Figure 9.23).

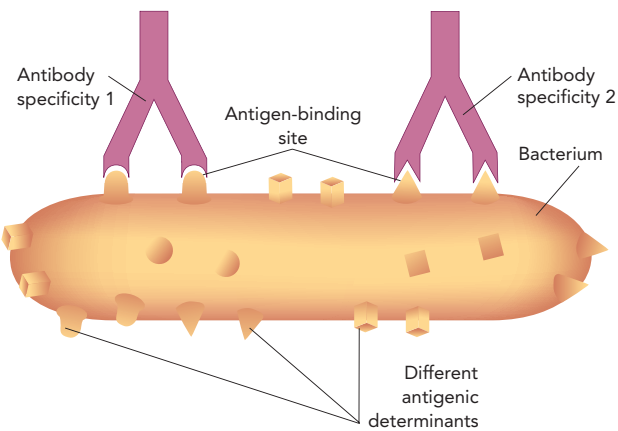


FIGURE 9.21

Different epitopes (antigenic determinants) on a bacterial cell

Bacteria generally have multiple epitopes on their surface, each one capable of stimulating a different antibody response.

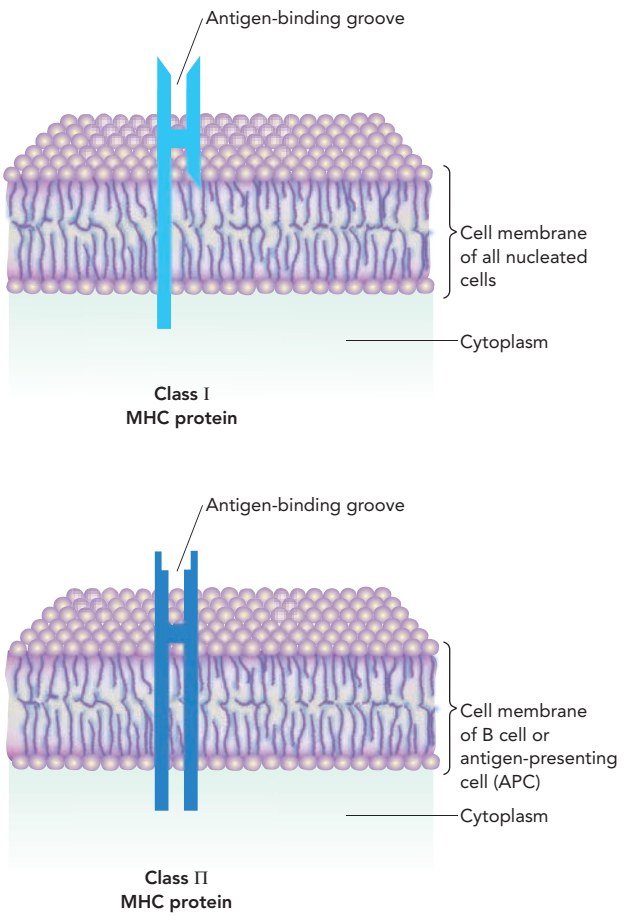


FIGURE 9.22

The two classes of MHC proteins involved in cell-to-cell interactions

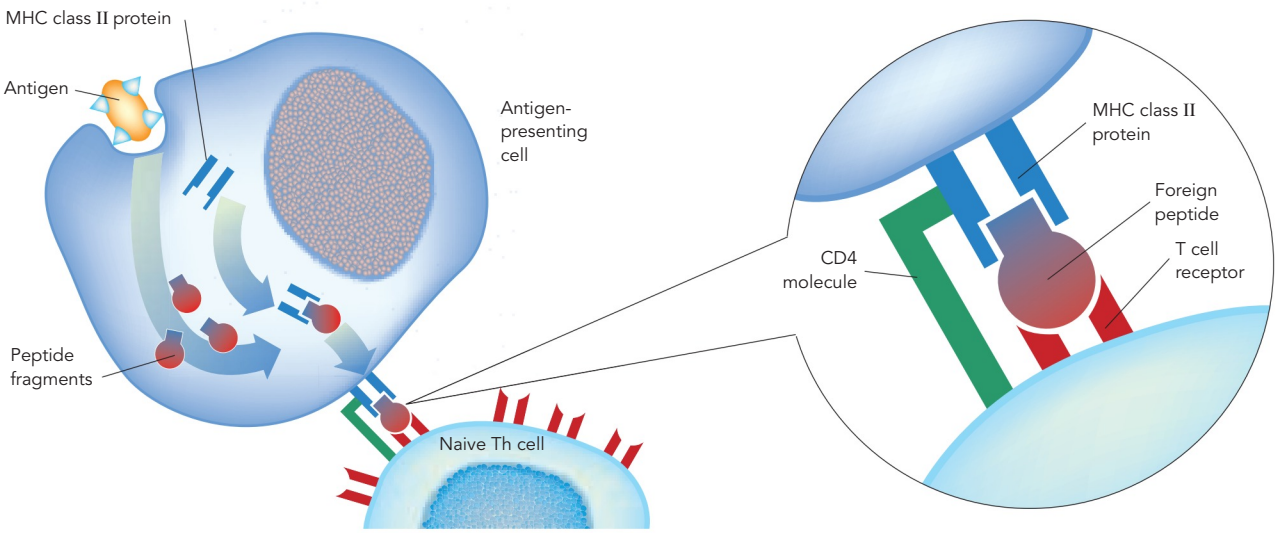
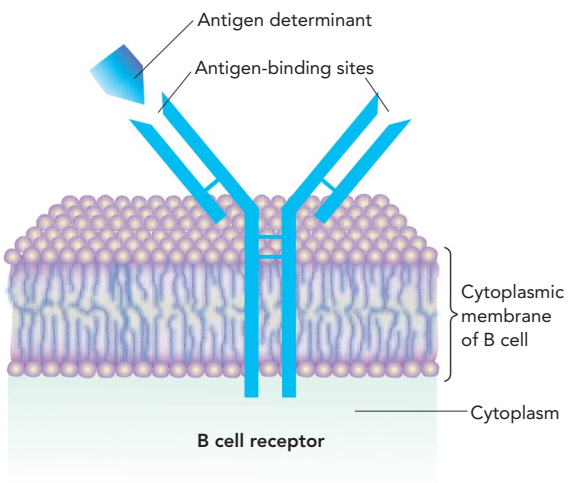


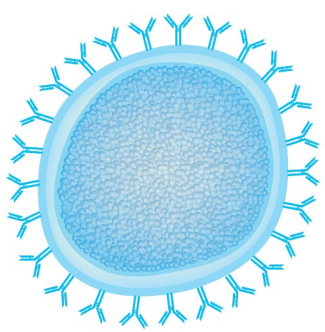
FIGURE 9.23

Antigen processing

Specialised antigen-presenting cells, mainly dendritic cells and macrophages, ingest antigens, process them and join them to a recognition molecule (called an MHC protein) so that lymphocytes can respond to the antigen.



(a) The B cell receptor (an antigen receptor)



(b) The B cell has hundreds of thousands of antigen receptors on its surface

FIGURE 9.24

The antigen receptor of B cells is an antibody molecule

Humoral immunity

If foreign cells or antigens enter the body and evade the innate defences, they then encounter the cells and tissues of the lymphoid system.

Activation of B cells and formation of antibodies

Recall that each lymphocyte is mono-specific. So, each naive B lymphocyte carries thousands of molecules of a specific antigen receptor (actually an antibody molecule) on its membrane. This is known as the **B cell receptor** (see Figure 9.24). Activation of a B lymphocyte is a multistage process involving antigen-presenting cells, T cells, B cells and cytokines (see Figure 9.25):

1. The antigen is phagocytosed by an antigen-presenting cell (APC) and this APC then presents the antigen fragment together with an MHC II molecule to a naive CD4+ T helper lymphocyte., which is then activated.
2. The activated T cell differentiates into T helper cell following its binding to the presented antigen and MHC II molecule and due to secretion of specific cytokines by the antigen-presenting cell. Two types of T helper cells involved in antibody production are thought to be generated following naive T cell activation: follicular T helper cells and another T helper subset that has not yet been fully characterised, but was formerly thought to be Th2.
3. Antigen is also bound to a specific B cell via its antibody receptors. This antigen is internalised and processed and the antigen fragment is then presented on the surface of the B cell together with an MHC II molecule.
4. The B cell presents the antigen fragment and MHC II molecule to T helper cells which then secrete cytokines, resulting in the activation of the B cell. Thus the T helper cells promote specific B cell activation and high levels of antibody secretion.

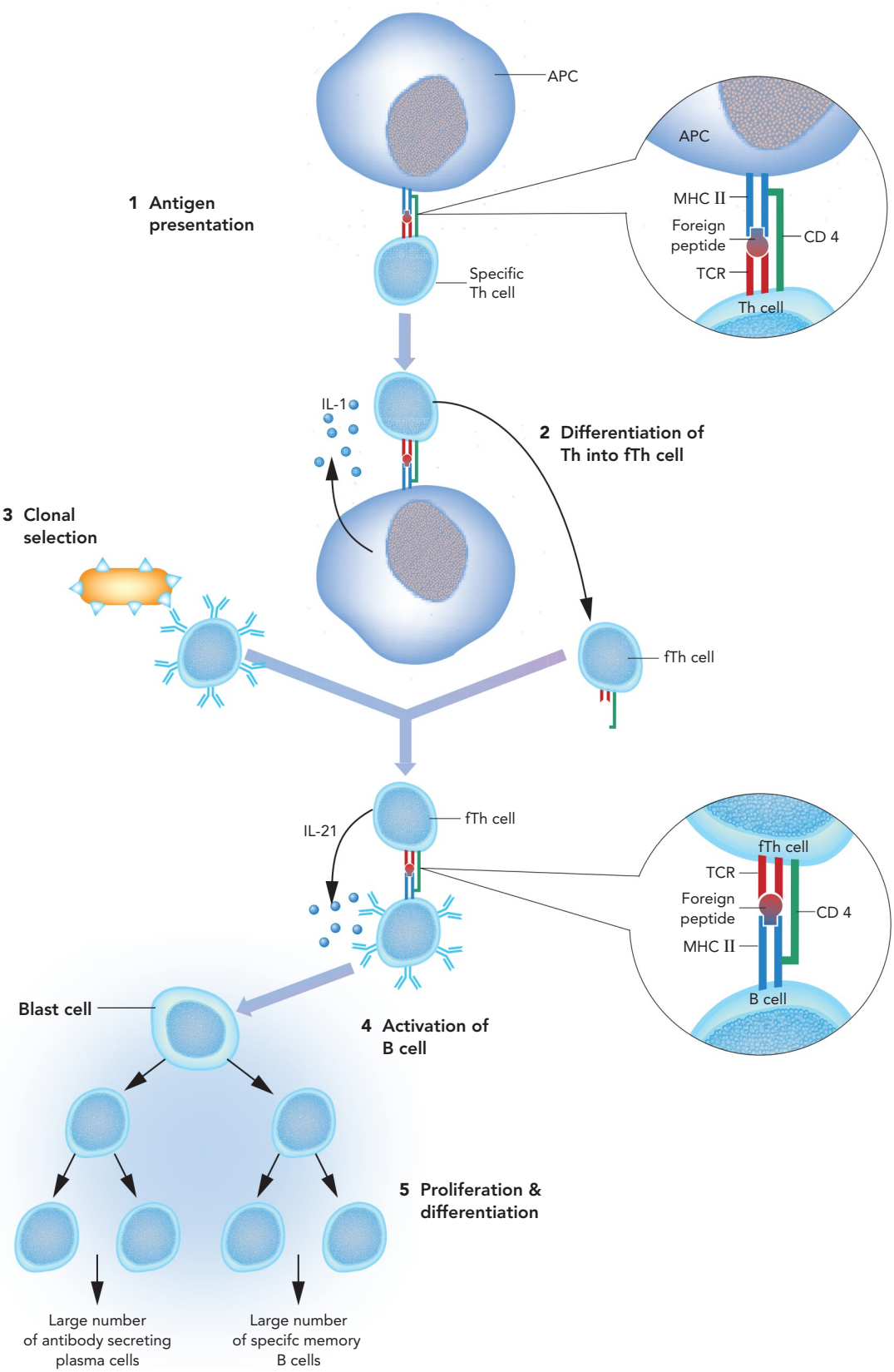


FIGURE 9.25

The humoral immune response

Activation of a specific T cell and a specific B cell by antigen leads to a large number of antibody-secreting cells and memory cells.

The above describes the activation of B cells specific for protein antigens. These are referred to as T-dependent antigens, because of the need for T helper cell involvement. The vast majority of antigens are proteins, and hence T-dependent antigens. A different B cell activation process occurs, without the involvement of a T cell, for polysaccharides, lipids and other non-protein antigens. These are referred to as T-independent antigens.

Following the activation of B cells, there are two important processes:

- Proliferation of the B cells—to expand the number of cells capable of reacting to the particular antigen.
- Differentiation of the cells in the expanded population—to obtain large numbers of clone cells (see below).

The activating event first stimulates the B cell to transform into a *blast cell*, which is an enlarged, highly active cell prepared for mitotic division. The blast cell multiplies through successive mitotic divisions to produce a large population of cells all bearing the same antigen receptors (Figure 9.26). This population of cells, all with the same specificity, is called a **clone**. Thus, it is the foreign antigen that does the selecting by 'choosing' a lymphocyte with complementary receptors. This is the basis of the **clonal selection** theory, first proposed by the Australian immunologist Sir Frank Macfarlane Burnet in the 1950s. There are thought to be approximately 10^{12} different antibody receptors—that is, 10^{12} different B cell clones.

After multiplication, the clone cells differentiate into functional cells. Some of them become **plasma cells**, which are responsible for synthesis and secretion of antibodies. Plasma cells are able to synthesise enormous amounts of antibody (as many as 10 million antibody molecules per hour!) for several days and then they die. Each antibody has the same antigen specificity as the receptor molecules on the surface of the parent B cell. The antibodies are secreted by the plasma cells into the blood and other body fluids, where they can bind to specific antigens and thus prepare them for destruction by other specific or non-specific mechanisms.

The clone cells that do not become plasma cells become long-lived **memory cells**, which persist in the body for months to years, and can react with the same antigen if it enters the body again at a later time. Memory cells respond to antigenic stimulation in the same way that the original parent B cell responded to antigen, again increasing the clone size, producing even more cells with the same specificity. The larger population of memory cells is more easily activated and therefore is able to respond more rapidly and more strongly compared to the group of cells in the original B cell population.

The events described so far in this section constitute the **primary immune response**; that is, the response of the acquired immune system the first time it is exposed to a particular antigen. Immediately after antigen challenge in

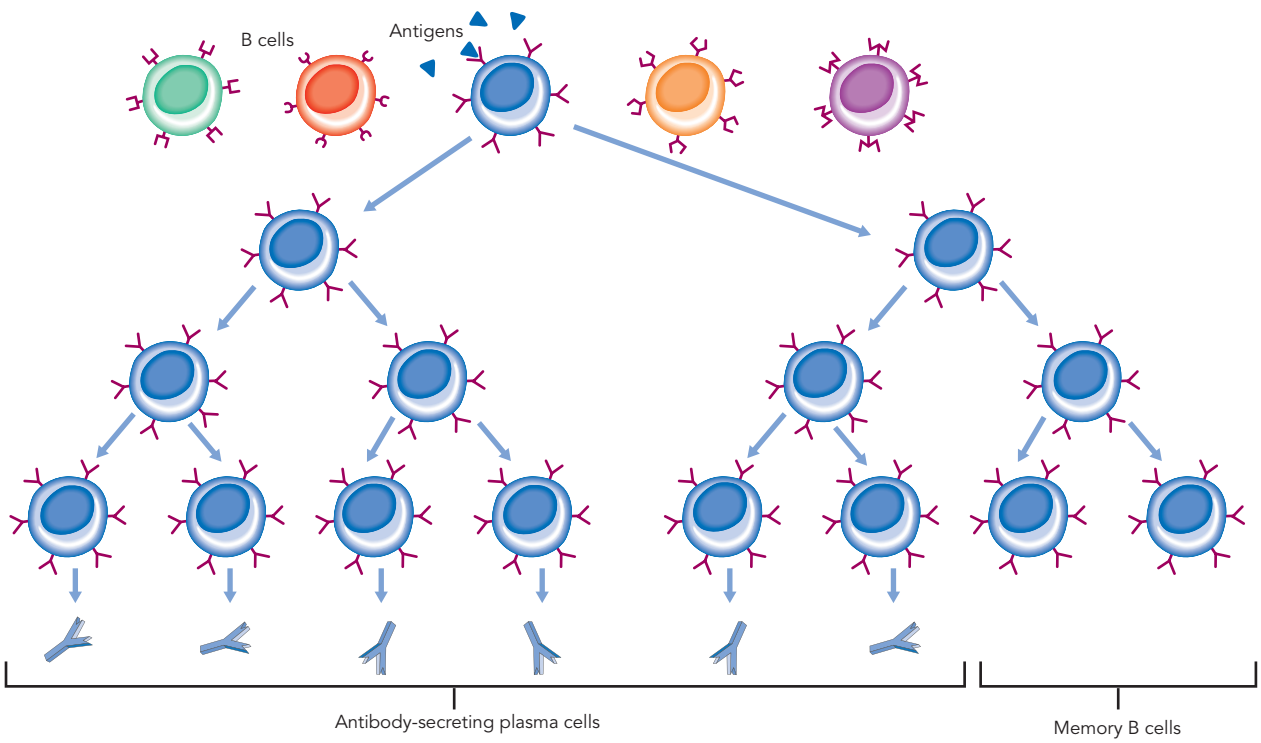


FIGURE 9.26

Clonal selection

There is a multitude of different lymphocyte populations in the body, each population being able to recognise a single antigen. When an antigen enters the body it virtually selects the clone that is specific to it. When activated by antigen, that small clone proliferates and then differentiates to produce a much larger clone, comprising antibody-secreting plasma cells and long-lived memory cells. All cells in this large clone are specific for the same antigen.

a primary immune response there is characteristically a lag period of 5–10 days (sometimes longer) in which there is no observable increase in the specific antibody levels in blood (or serum). However, during this time there is much activity; the lag period is the time required for processing of antigen, for the small number of B cells specific for the antigen to be activated and proliferate, and for the newly formed cells to differentiate into plasma cells. Once antibodies start to be produced in large amounts, the specific serum antibody level begins to rise, peaking within several weeks; it then declines over the next few weeks to months (Figure 9.27).

If a person is exposed to the same antigen a second time, a **secondary immune response** occurs that is:

- faster
- stronger
- longer-lasting (Figure 9.27).

The secondary response is very different because the immune system has been primed to the antigen in the primary response. The increased numbers of specific memory lymphocytes and their easier triggering are responsible for this powerful secondary response. These memory cells provide what is commonly called **immunological memory**. This memory effect is the basis for giving boosters when using some vaccines. When a booster is given, even more memory cells are produced and the antibody levels in the blood can remain high for many years, due to continuous production of antibodies by long-lived plasma cells. A secondary antibody response provides not only greater amounts of antibody, but also more effective antibodies. This improvement is due to a switch in antibody class and an increase in affinity (strength of binding) of the antibodies to their antigen.

It should be stressed that the primary antibody response described above relates to the injection of non-living (and therefore non-replicating) antigen. In the case of infection or vaccination with a living organism, the antibody response is more like that of a combination of primary and secondary responses. The reason for this is that living organisms stimulate the immune system repeatedly as they replicate in the body. Effectively, it is the equivalent of having multiple boosters. Vaccines containing attenuated organisms (see later in this chapter), which are alive and replicate but do not cause disease, stimulate the immune system in this way. Thus, infection or a single vaccination of attenuated organisms can provide long-term and sometimes lifelong immunity.

Basic antibody structure

Antibodies, also called **immunoglobulins**, are proteins found in blood and other body fluids, including lymph, urine, CSF and mucosal secretions. They constitute the **gammaglobulin** fraction of blood proteins. All antibodies in humans can be grouped into one of five immunoglobulin types (called isotypes, or classes), based on differences in their structure and function. We describe how these immunoglobulin classes differ in Table 9.4.

All antibody molecules have a basic structure consisting of four polypeptide chains linked together by disulphide bonds (Figure 9.28). The two longer chains are identical, and are called **heavy (H) chains**. The other two chains, the **light (L) chains**, are also identical but much shorter. The molecule has a Y-shaped structure, which can change to a T-shape because of a flexible hinge region. This is the basic, single antibody molecule or *monomer*.

As shown in Figure 9.28, the two 'arms' of the antibody molecule are called **antigen-binding fragments** (**Fab**), and at the ends of the arms are grooves called the **antigen-binding sites**, which is where the molecule binds to its antigen. The remainder, or tail part, of the molecule is called the **crystallisable fragment** (**Fc**). There are two antigen-binding sites per monomer. The high specificity of the antigen-binding sites for their antigens is similar to that of the active site of enzymes for their substrates.

The binding sites are complementary to the antigen. These binding sites have to be different for different antigens, and are part of what is called the *variable (V) region* of the molecule. The specific sequence of the amino acids in the variable region

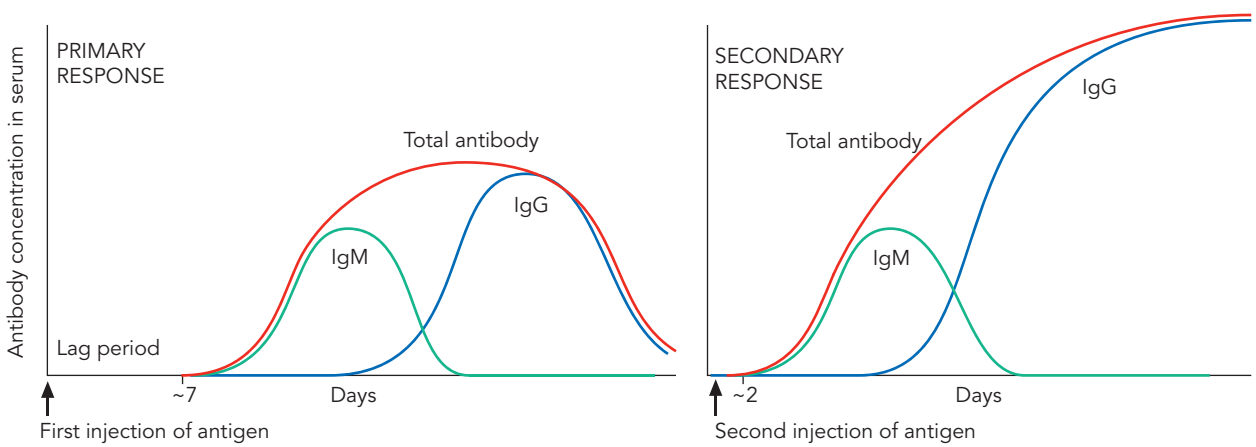


FIGURE 9.27

The primary and secondary antibody responses to injection of a non-replicating antigen

The secondary response is faster, larger and longer-lasting.

TABLE 9.4 The biological properties of human immunoglobulins

ANTIBODY CLASS	STRUCTURE	PROPERTIES/FUNCTIONS
IgG *Subclasses: IgG_1, IgG_2, IgG_3, IgG_4*	monomer	Major type of circulating antibody Crosses placenta (mother to foetus) Activates complement system Major type produced in secondary response
IgM	pentamer or monomer	First type to be produced in primary response Monomer is B cell surface receptor Activates complement system
IgA *Subclasses: IgA_1, IgA_2,*	monomer or dimer	Monomer found in blood Dimer is major type in secretions (saliva, mucus, etc.)
IgE	monomer	Triggers allergic reactions Involved in immunity to parasites (e.g. helminths)
IgD	monomer	B cell surface receptor

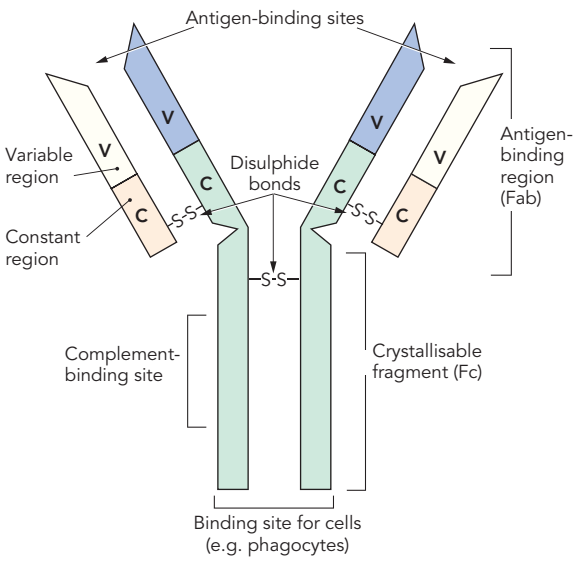


FIGURE 9.28

The basic structure of an antibody molecule

The four polypeptide chains (two heavy and two light) are joined together by disulphide bonds. In each chain there is a variable region (V) and a constant region (C). Antigen binding occurs in the variable region of the antigen-binding fragment (Fab). The molecule binds the complement proteins and binds to a phagocyte via parts of the Fc region.

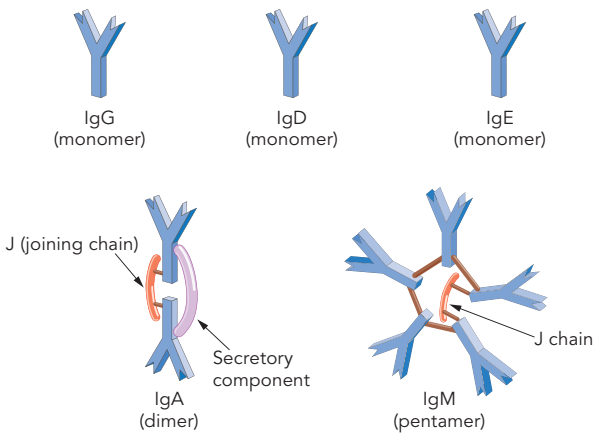


FIGURE 9.29

The structures of the five classes of human immunoglobulins

determines the antigen specificity of the antibody. The other end of the arm of the molecule is the *constant (C) region*, where the amino acid sequence is the same (or very similar) in all antibodies of the same class. The Fc fragment is involved in binding to other molecules, such as complement, and cells of the immune system.

Antibody classes

Five major immunoglobulin classes have been identified in humans and are designated IgD, IgM, IgG, IgA and IgE, based on five different types of C-region. 'Ig' stands for immunoglobulin, followed by a letter designating the different types of heavy chains. As illustrated in Figure 9.29, IgG, IgD and IgE have the single, basic Y-shaped structure and therefore exist as monomers. IgA exists as either a monomer or dimer (two linked monomers). IgM is made from five linked monomers—hence called a pentamer.

Each class of antibody has a different biological function. For example, IgM is the first type of antibody that is produced after primary antigenic stimulation, and lasts for only a short time (weeks to months) in body fluids. IgG is the most abundant antibody in blood and is the type that persists for months to years after antigenic stimulation (see Figure 9.27), thus providing long-term protection. IgG antibody can cross blood vessel walls and enter tissue fluids in areas of inflammation, and is the only antibody class that is able to cross the placental barrier from mother to foetus.

IgA is found primarily in the mucus that covers the body's mucosal surfaces. In these secretions it exists as a dimer and has an extra component, called a secretory piece, which makes it resistant to proteolytic digestion by enzymes in these fluids. IgA plays a major role in preventing pathogens from gaining entry into the body by stopping them from crossing the mucosal epithelium. IgA aggregates antigens

and keeps them in the secretions; when the secretions are expelled so, too, are the antigens.

The monomeric forms of IgM and IgD are membrane proteins present on the surface of B lymphocytes where they act as antigen receptors. IgE antibodies are involved in immunity to worm infections of the intestinal tract, and also in allergic reactions. These and other characteristics unique to each of the immunoglobulin classes are summarised in Table 9.4.

The basis of antibody diversity

It is thought that the acquired immune system of a person can make antibodies to millions of different antigens. Given the number of possible antigens in the world that the body might contact, this would seem to be necessary if the immune system is to protect us against them. Since antibodies, like all proteins, are coded for by genes, it might be expected that an individual will have millions of genes just for antibodies. However, this cannot be the case, since humans are thought to have only about 100 000 genes for all the proteins that the body's cells must make. There are believed to be only about 1000 genes for antibodies.

A question that vexed immunologists for many years was how so few genes could produce so many different antibody proteins (around 10^{12}). The answer began to unfold in the 1970s when it was shown that, instead of containing a complete set of antibody genes, cells contain sets of mini-genes that act as 'building blocks' for antibody genes. Antibody diversity comes from the shuffling and combining of these gene segments in different ways by each B cell as it becomes immunocompetent. The products of these assembled genes are then expressed as the surface receptors of B cells, and in the antibodies later released by their clone members.

This process has been likened to using a child's building block set—the set contains a small number of different blocks, but they can be used to build numerous different objects. It is estimated that the diversity in the light and heavy chains allows for almost two million different antibody genes.

However, the random mixing of gene segments to make antibody genes, plus the diversity that results from different combinations of H- and L-chains, account for only part of the huge number of antibody specificities. The joining process adds even more diversity. The enzymes that combine the gene segments add random DNA bases to the segments as they are being joined.

As a result of gene shuffling and mutation on a large scale, the lymphoid system is thought to be capable of producing millions of different clones of lymphocytes. And according to the clonal selection theory:

- each mature lymphocyte is preprogrammed
- each mature lymphocyte can respond to one antigen only
- each clone of specific lymphocytes is selected and expanded only when the appropriate antigen enters the body.

Because the total lymphocyte population must express such a large number of different specificities, it follows that there can only be a relatively small number of lymphocytes (perhaps a few hundred) that have the same specificity before first contact with the antigen. A considerable part of the lag period in the antibody response is spent increasing this small number of cells to a larger population, capable of producing large amounts of antibody.

Activities and functions of antibodies

The basic function of antibody molecules is to bind specifically with their antigens and form **antigen-antibody** (or **immune**) **complexes**. Once they have bound to antigen, antibodies help to bring about removal of the antigen from the body in a number of different ways:

- opsonisation
- complement activation
- neutralisation
- agglutination or precipitation.

Different actions of antibodies are necessary for different types of antigens. These functions are summarised in Figure 9.30.

Antibodies can **opsonise** antigens, as shown in Figure 9.31. Microorganisms or other particles, when coated with opsonising antibody, become more readily recognised and ingested by phagocytes. Opsonising antibodies are particularly important in defence against microbes that have capsules or other structures that enable them to avoid being ingested by phagocytes (see Chapter 10).

CASE HISTORY 9.1

Glandular fever diagnosis

Stephanie, a 20-year-old apprentice chef, attends the local medical clinic because she has been feeling unwell for the last week. Her symptoms of headache, fever, fatigue and a sore throat have been gradually worsening. On examination, her doctor notices inflammation of the pharynx and tonsils and swollen axillary lymph nodes, and suspects that she has glandular fever (infectious mononucleosis). The doctor takes a sample of blood and sends it to the pathology laboratory, requesting an IgM antibody test for Epstein-Barr virus.

Questions

1. Why is an antibody test necessary for the diagnosis of Stephanie's condition?
2. What is the reason for a specific IgM antibody test to be performed?
3. Is there any urgency for the laboratory results to be obtained?
4. If Stephanie is found to have antibodies to the virus, does that mean she is immune to it?

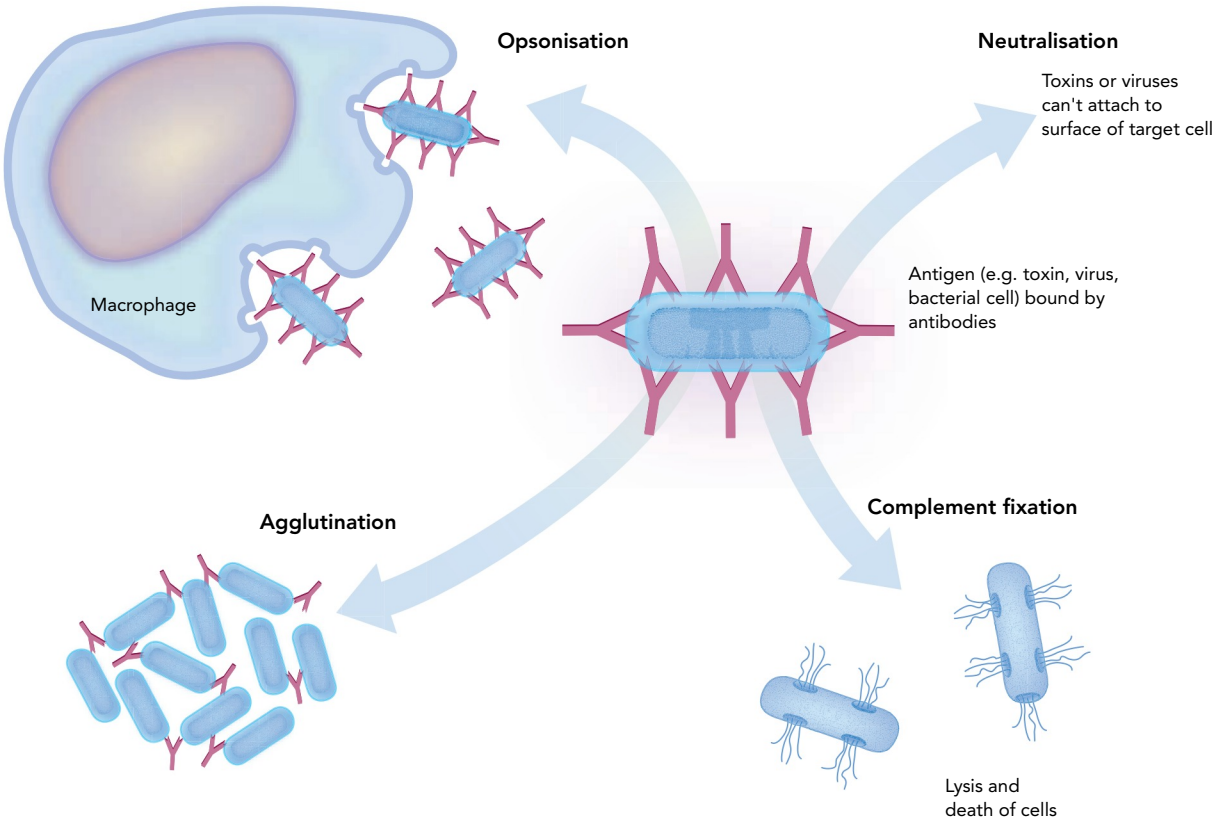


FIGURE 9.30

The functions of antibodies

Antibodies have different functions depending on the class of the antibody and the nature of the antigen. Once bound to antigen, antibodies can enhance the phagocytosis of particulate antigens (called opsonisation), aggregate particulate antigens (called agglutination), coat toxins and viruses (called neutralisation) or, in conjunction with the complement system, cause lysis and death of foreign cells.

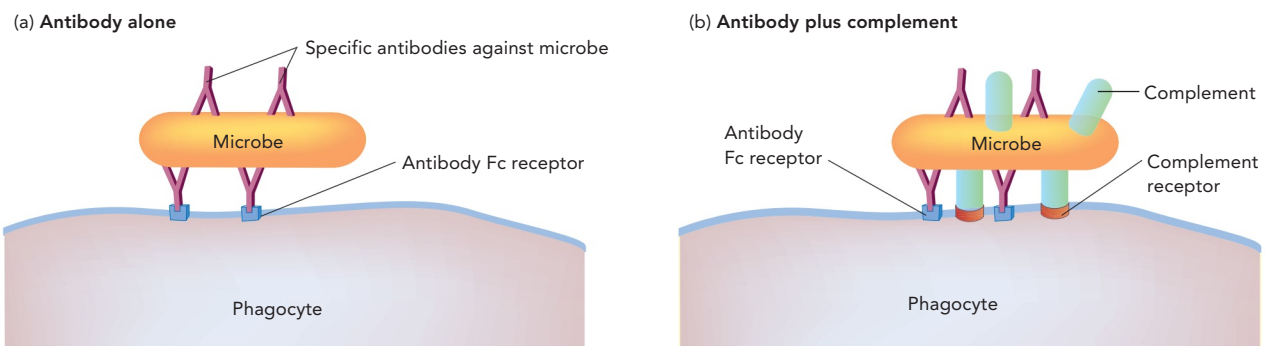


FIGURE 9.31

Opsonisation of microorganisms

Adherence of microbes to phagocytes can be facilitated by (a) antibodies, or (b) antibodies together with complement. The mechanisms are similar to opsonisation with complement alone (illustrated in Figure 9.15).

Antibody attachment followed by **complement activation** (or fixation) is an important defence against foreign cells, such as bacteria and cancer cells. When antibodies bind to cellular targets, complement-binding sites are exposed on the Fc fragments of the antibody molecules. This triggers complement activation, which leads to the insertion of complement proteins (the membrane attack complex) into the plasma membrane of the foreign cell, creating holes in the membrane and resulting in cell lysis.

Neutralisation is a process in which antibodies bind to and block specific attachment sites on viruses or bacterial exotoxins (see Chapter 10). As a result of being coated by antibody, the virus or toxin is prevented from binding to receptor sites on tissue cells, thus preventing invasion

or injury. The antigen-antibody complexes are eventually destroyed by phagocytes. **Antitoxin** is the name given to antibodies that neutralise bacterial toxins.

Because an antibody molecule has two or more antigen-binding sites, it can bind to different antigen molecules. In this way, antibodies can cross-link antigens to form large lattices (see Figure 9.30). When cell-bound antigens are cross-linked, clumping of the foreign cells occurs, and this is called **agglutination**. IgM, with (theoretically) ten antigen-binding sites (see Figure 9.29), is a very potent agglutinating antibody. In **precipitation**, soluble molecules (instead of cells) are cross-linked into large complexes which then precipitate out of solution. The protective benefits of these reactions are that agglutinated bacteria and precipitated foreign molecules are much more easily identified and captured by phagocytes.

The Fc part of the antibody molecule is also functional. Once antigen is bound, areas of the Fc fragment become activated and can bind to receptors on some host defence cells, including macrophages, neutrophils, mast cells and NK cells. As discussed earlier in this section, the Fc region is involved in the opsonisation of antigen (see Figure 9.31). Also, IgE antibodies bind specifically to mast cells and basophils via the Fc region. When antigen subsequently enters the body and is bound to the IgE molecules, release of allergic mediators such as histamine from the cells occurs (see later section on immediate hypersensitivity). NK cells also have receptors for the Fc part of antibodies, so foreign cells coated with antibodies are readily recognised and destroyed by the NK cells.

Cell-mediated (cellular) immunity

Usually, at the same time that B cells are responding to an antigen, a clone of specific T cells is similarly activated. The cell-mediated immune response involves the activation, differentiation and actions of T cells, and the actions of chemical mediators produced by them. This arm of the acquired immune system is designed to recognise body cells that have viruses or bacteria within them—that is, it is particularly important for microbes that antibodies cannot access. Because of their size, antibodies are not able to cross the plasma membrane and enter infected cells. Cellular immunity also provides defence against tumour cells, fungi and parasites (helminths and protozoa).

As for humoral immunity, the stimulus for clonal selection and lymphocyte activation, proliferation and differentiation in cell-mediated immunity is the binding of foreign antigen. The T cell receptor (Figure 9.32) is not an antibody, but is similar, consisting of two trans-membrane polypeptide chains (α and β). T cells are unable to recognise free antigens, but work carried out by Australian Nobel laureate Peter Doherty showed that they do recognise and respond to processed fragments of foreign antigens displayed with MHC proteins, on surfaces of the body's own cells. T cell activation therefore requires a simultaneous recognition of non-self (the antigen) and self (an MHC protein).

A cell that is infected by a virus or intracellular bacterium usually displays microbial antigen fragments in conjunction

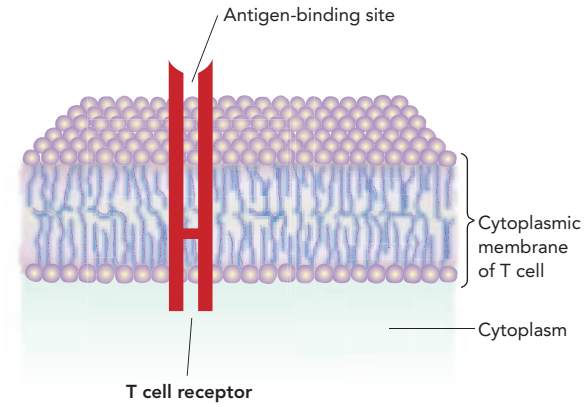


FIGURE 9.32

The T cell receptor

A surface molecule composed of two polypeptide chains (α and β) that form the antigen-binding site.

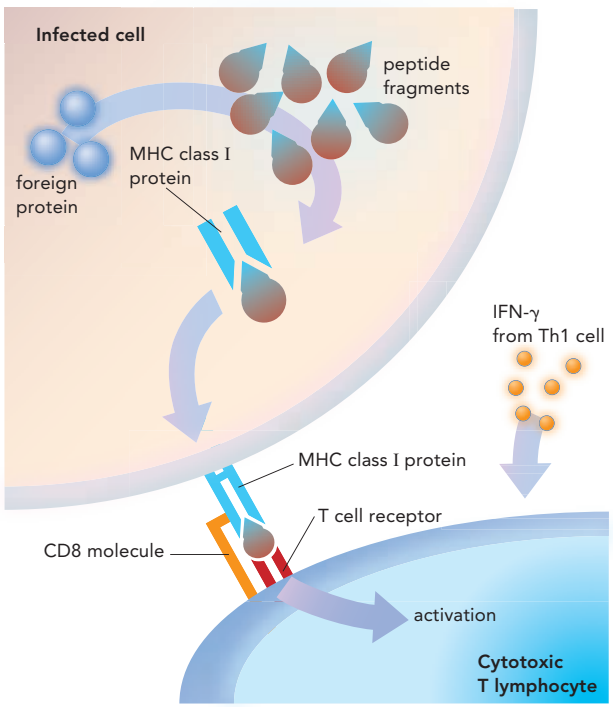


FIGURE 9.33

Activation of cytotoxic T cells

Cytotoxic T cells are activated by antigen in combination with a class I MHC protein on an infected cell.

with class I MHC proteins on its surface. If a CD4+ T lymphocyte with receptors for that antigen contacts the infected cell, the antigen-class I MHC protein combination causes the T cell to differentiate into a Th1 cell, which then secretes IFN-γ. This cytokine enables cytotoxic T cells to be activated (see Figure 9.33) by the antigen on the infected cell. The CD8+ molecule on cytotoxic T cells in fact binds to part of the class I MHC protein.

Once the cytotoxic T cell (Tc or CD8+ cell) is activated, it enlarges and proliferates to form more Tc cells and

memory T cells of the same antigen specificity. The new Tc cell population targets and kills infected cells. The Tc cell binds tightly to the target cell and then releases molecules of the protein perforin, which are inserted into the plasma membrane of the target cell (see Figure 9.34). The proteins form a pore in the cell membrane through which enzymes, called *granzymes*, can be injected into the target cell. These granzymes cause the target cell to commence a process of programmed cell death, called **apoptosis**, in which the cell begins to kill itself from within. Additionally, the perforin pore disrupts the integrity of the cell membrane, which also leads to cell death. By killing the infected cells, virus (or bacterial) replication is disrupted and the microbe can then be exposed to extracellular defences. However, the infected host cell is also destroyed. Recently, another type of CD8+ cell has been identified that can inhibit viral replication within a cell without damage to the cell. By injecting granzymes into neurons infected with herpes virus, specific CD8+ cells are able to interfere with the replicative cycle of the virus.

Tc cells also secrete potent cytokines (see page 195), including gamma interferon (IFN-γ), which limits viral replication inside an infected cell and also attracts phagocytes to the cell to destroy it.

The memory T cells persist for a long time, thereby providing a reservoir of cells that can mediate secondary cell-mediated responses to the same antigen if reinfection by the same microbe occurs in the future.

In addition to the cytokines that Th1 cells secrete to activate Tc cells, they also secrete a number of other cytokines to enhance the production and activity of other cell types involved in cell-mediated immunity (see Figure 9.35). These include TNF-β, which activates neutrophils; GM-CSF, which activates macrophages and increases production of macrophages and dendritic cells; and IL-3, which increases production of macrophages and granulocytes. IL-2 and IFN-γ activate not only Tc cells, but also NK cells.

The natural killer T cell (NKT cell) is a unique type of T lymphocyte that appears to be involved in cell-mediated immunity to tumours and infectious microorganisms. NKT cells also appear to suppress some cell-mediated immune reactions, thereby preventing some autoimmune diseases. Overall, little is known about the functions of these cells, except that they appear essential for certain immune processes. The fact that they have such contrasting actions suggests that there are likely to be a number of distinct NKT cell subsets.

The functions of the major cells involved in cell-mediated immunity are outlined in Table 9.5.

Active versus passive immunity

Specific immunity can be acquired either actively or passively (see Figure 9.36). **Active immunity** is the immunity developed when the acquired immune system responds to microorganisms or other foreign substances that enter or are introduced into the body. Active immunity is *naturally acquired* during microbial infections, including sub-clinical or inapparent infections, and *artificially acquired* as a result of vaccination (see next section). Generally, active immunity is long-lasting, often for a lifetime, especially when naturally acquired.

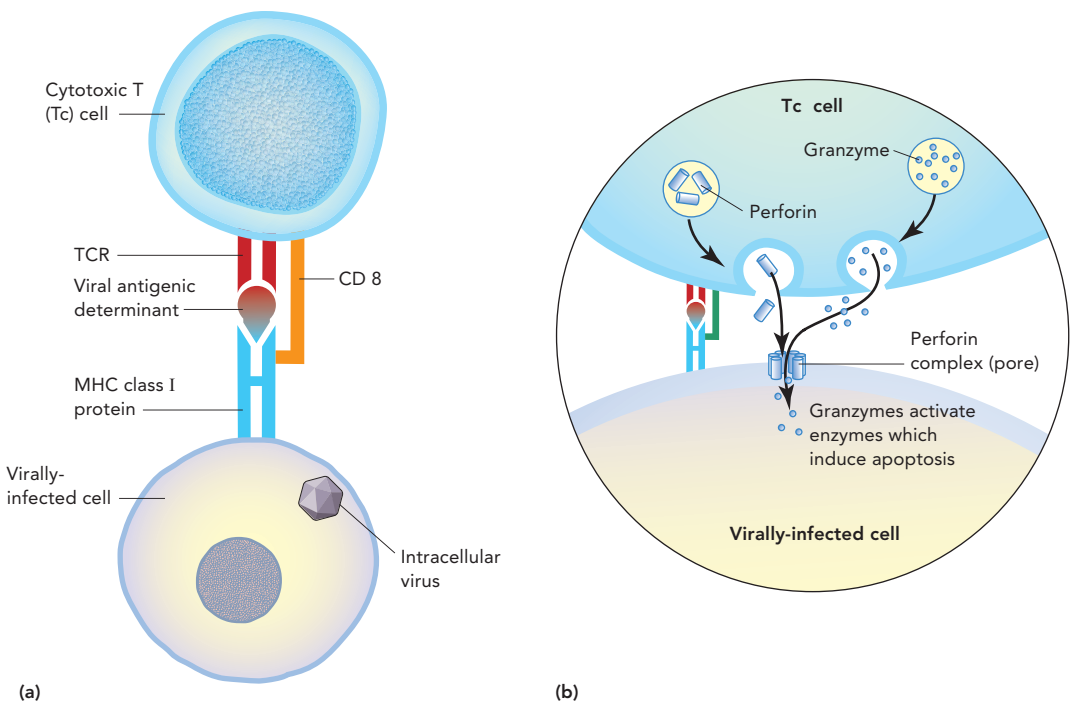


FIGURE 9.34

Killing of a target cell by a cytotoxic T cell (Tc)

(a) Cytotoxic T cell is activated by antigen in combination with a class I MHC protein on an infected cell; (b) cytotoxic T cell releases perforin and granzymes to kill infected cell.

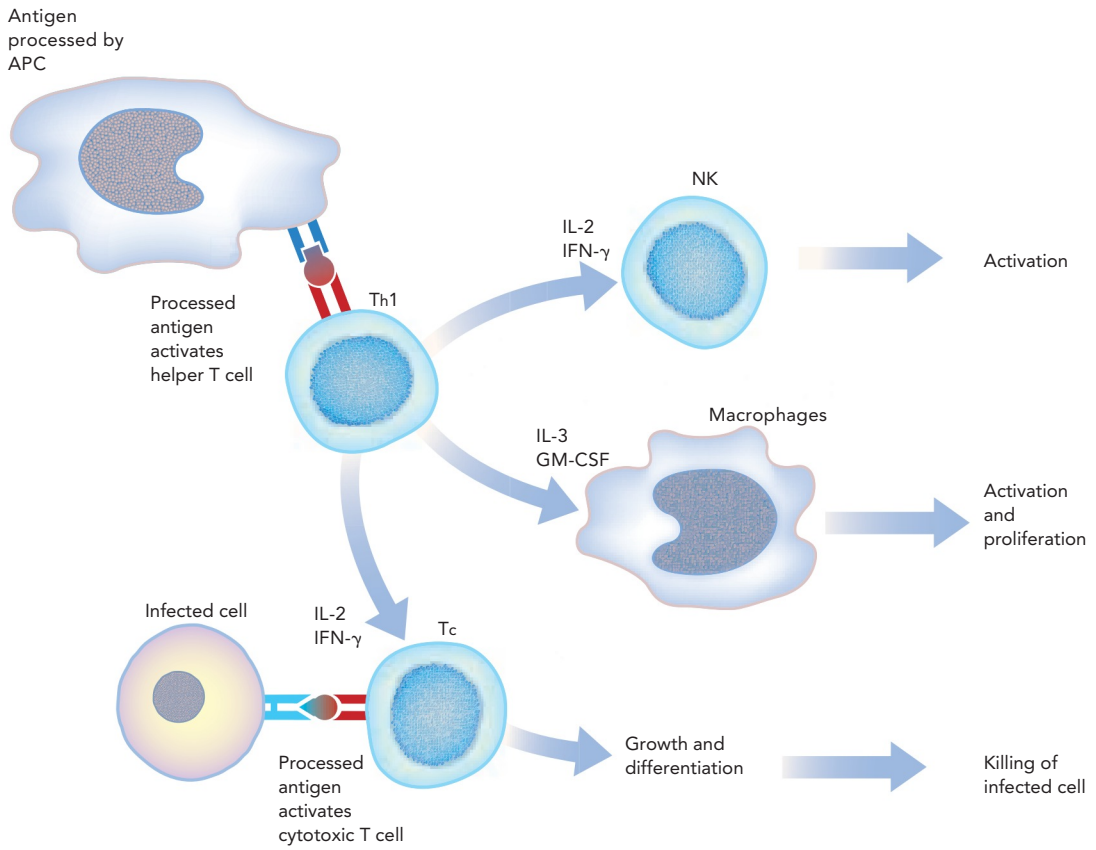


FIGURE 9.35

Cell-mediated immunity

T cells are activated by antigen-presenting cells and/or infected cells and can perform a variety of functions, depending on their type.

TABLE 9.5 Major cells involved in cell-mediated immunity

CELL	FUNCTIONS
Type 1 T helper cell (Th1)	Enhances function of cytotoxic T cells and phagocytes
Type 2 T helper cell (Th2)	Activates eosinophils and mast cells to attack large parasites
Follicular T helper cell (fTh)	Promotes the secretion of antibodies by B cells (plasma cells)
Cytotoxic T lymphocyte (Tc)	Destroys target cells: virus-infected cells, tumour cells
T regulatory cell (Treg)	Blocks activity of T cells and antigen-presenting cells to prevent autoimmune reactions
Natural killer T cell (NKT)	Cell-mediated immunity to tumours and infectious microorganisms; prevention of autoimmunity
Dendritic cell	Innate system cell that has a major role in activating T cells
Activated macrophage	Innate system cell that activates T cells Phagocytic activity to remove microbes
Natural killer (NK) cell	Destroys target cells; enhanced when activated by Th cells

Passive immunity is the immunity resulting from the transfer of pre-made antibodies (or immune cells) from an immune person (or animal) to a non-immune person. The recipient becomes immune, but only for a short time (up to several months) until the transferred antibodies (or cells) are degraded in the body. The immunity is short-lived because the immune system of the recipient is not stimulated.

Passive immunity can also be naturally or artificially acquired. Passive immunity is conferred naturally on a foetus as the mother's antibodies pass across the placenta into the foetal circulation. Antibodies are also transferred to an infant in colostrum and breast milk. These antibodies protect the foetus and then the young infant for several months after birth against the wide variety of microbes to

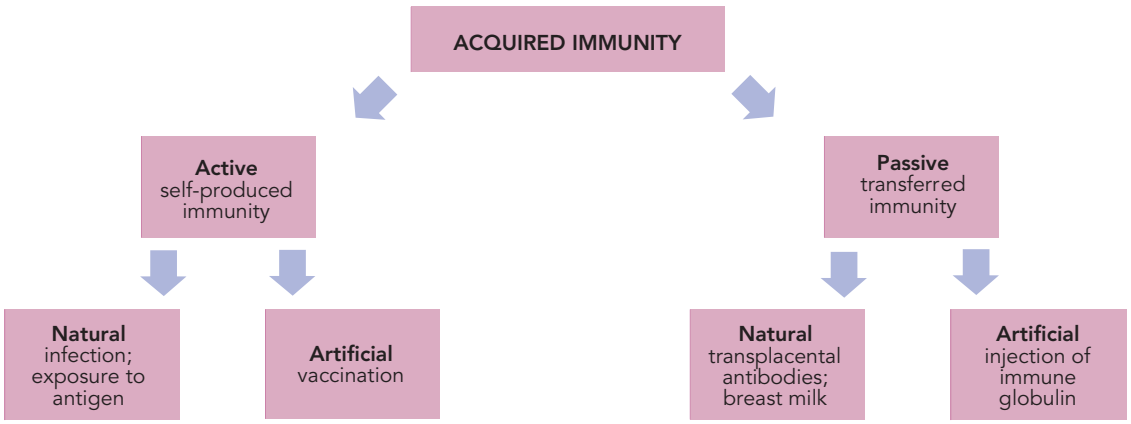


FIGURE 9.36

The different types of acquired (specific) immunity

which the mother is immune. In the meantime, the baby's own immune system is maturing.

Passive immunity is artificially conferred when a person is injected with antibodies from an immune human or, rarely, from an immune animal. **Normal immunoglobulin** is an immunoglobulin preparation extracted from the pooled serum of large numbers of blood donors. Each batch of this serum contains a mixture of antibodies that offers protection against a broad range of common infectious diseases. It is generally used to prevent diseases such as rubella and hepatitis A in people who have recently been exposed to these viruses. It is also sometimes used to replace antibodies in immunodeficient patients.

Specific immunoglobulin (**SIG**), on the other hand, is a specific antibody preparation obtained from the serum of people who are convalescing and in a hyperimmune state after an infection. Specific immunoglobulins are usually prepared against such diseases as pertussis, tetanus and hepatitis B. For example, a health worker who is accidentally exposed to hepatitis B virus by a needlestick injury is given hepatitis B immunoglobulin in an attempt to prevent establishment of infection. These preparations are different from normal immunoglobulin in that they contain higher titres of specific antibodies. The injected antibodies provide immediate protection, but their effect is short-lived.

Immunisation

Immunisation (also termed **vaccination**) is a procedure that aims to induce specific immunity in a person by artificial means. It involves exposing a person to material that is antigenic (able to stimulate the immune system) but not pathogenic (without causing disease). The discovery of vaccination just over 200 years ago by Edward Jenner (see Figure 9.37) was one of the most important developments in modern medicine. Vaccination has profoundly reduced the prevalence and impact of a number of infectious diseases that were once common and often deadly, arguably preventing more deaths than any other medical intervention. It has led to the eradication of smallpox, and the near-eradication

FIGURE 9.37

Jenner inoculating a child

Source: The Granger Collection, New York.

of polio, from the world. Vaccines for viral diseases are particularly important because of the lack of effective treatment for many of these diseases. However, decades of effort have failed to develop effective vaccines against a number of global pandemic diseases such as human immunodeficiency virus (HIV) infection, hepatitis C, dengue fever and malaria. And despite being used for many years, some vaccines, such as the BCG for tuberculosis, are recognised as having low efficacy.

Types of vaccines

In natural, acquired immunity, an infectious agent stimulates specific B and T lymphocytes and creates clones of memory cells which can give lifelong immunity. In immunisation, the objective is to generate the same response with a modified version of the microbe or its components. A safe and effective vaccine should mimic the natural protective response but not cause the infection; it should have long-lasting

effects and be easy to administer. Most vaccine preparations comprise one of the following:

- killed, whole bacterial cells or inactivated viruses
- live, attenuated bacteria or viruses
- inactivated bacterial toxins
- parts of bacterial cells or viruses.

The common types of vaccines in use are listed in Table 9.6.

Killed vaccines are prepared by cultivating large numbers of the required bacterium or virus and treating them with formalin, radiation or some other agent that kills them but does not destroy their immunogenicity. The vaccine contains whole, killed organisms. The vaccines for hepatitis A and inactivated polio vaccines are of this type. Because the microbe does not multiply in the host, killed vaccines often require several boosters to provide long-term protection.

A number of vaccines are prepared from live, attenuated microbes. **Attenuation** is any process that substantially reduces or (preferably) eliminates the virulence of microorganisms (i.e. their ability to cause disease) while still keeping them alive. It can be achieved by growing the organism in unusual conditions or by manipulating its genes in a way that eliminates its virulence factors. For example, the vaccine for tuberculosis (the BCG—Bacille Calmette et Guerin) was developed by Albert Calmette and Alphonse Guerin after more than ten years of subculturing the agent of bovine tuberculosis on an artificial culture medium. Vaccines for measles, mumps and rubella, and the oral polio (Sabin) vaccine, contain active, non-virulent (attenuated) viruses.

TABLE 9.6 Types of vaccines in common use

TYPE OF VACCINE	DISEASE
Killed bacteria	Cholera Typhoid
Inactivated viruses	Influenza Rabies Polio (IPV—inactivated polio virus) Hepatitis A
Live, attenuated bacteria	Tuberculosis
Toxoids (inactivated toxins)	Tetanus Diphtheria
Attenuated virus	Measles Polio (Sabin) Mumps Rubella Smallpox Rotavirus gastroenteritis Chickenpox
Microbial subunit	Haemophilus infections Pneumococcal infections Meningococcal infections Hepatitis B (surface antigen) Whooping cough Human papilloma virus infection

The advantages of attenuated vaccines are that viable microorganisms can multiply and produce mild or subclinical infection, which are more likely to induce strong antibody and cellular responses and long-lasting immunity. Many attenuated vaccines provide lifelong immunity without the need for boosters. The major disadvantages of using live vaccines are the inherent risk that the organism might mutate back to a virulent strain (see the section on polio in Chapter 20) and the possibility that they may cause full-blown disease in people whose immune system is compromised (see next section). However, live vaccines, where available, are more often preferred to killed types because of their greater efficacy.

For protection against diseases that are due primarily to the actions of a bacterial exotoxin (see Chapter 10), immunity to the toxin is often more useful than immunity to the whole bacterium. A type of vaccine called a **toxoid** consists of a bacterial exotoxin that has been inactivated so that it no longer causes disease, but is still antigenic. Toxoids elicit the production of *antitoxins*—antibodies that, in the event of real infection, neutralise the toxin and prevent it from causing disease. Examples of toxoids are the highly effective vaccines for diphtheria and tetanus.

If the exact antigenic determinants that stimulate good immunity are known, it may be possible to produce a vaccine based on that particular part of a microorganism. These are called **subunit vaccines**. Examples of subunit antigens are the capsular polysaccharides of *Haemophilus influenzae* (Hib vaccine), pneumococcus and meningococcus, and the surface antigen of hepatitis B virus and influenza viruses.

Multiple doses of toxoid and subunit vaccines are generally required to induce a long-lasting immunity. Also, highly purified antigens tend to be weak immunogens, so in order to induce a strong response, toxoid and subunit vaccines usually contain substances called **adjuvants**, which enhance the magnitude and quality of the immune response. Very few adjuvants have been licensed for use, and there are significant concerns regarding their toxicity or side effects. Furthermore, the mechanism of action of most adjuvants remains unclear, adding to the concerns about their use in vaccines.

The immunogenic components of some subunit vaccines are prepared by genetic engineering techniques (i.e. recombinant DNA technology). In this process, the gene for the synthesis of the protective antigenic determinant (epitope) is introduced into a bacterium or yeast cell. These microbes are grown in large numbers and the antigen is harvested for use in the vaccine. The hepatitis B vaccine is a subunit vaccine prepared in this way. Subunit and toxoid vaccines are non-living and thus need several boosters to produce long-term, effective immunity. These types of vaccines tend to be purer, and hence much safer, than those in which the antigen is extracted from the microorganism.

A recent innovation in vaccine design is the development of DNA vaccines. This involves the use of DNA in the vaccine, rather than the protein antigen. The DNA is inserted

into a plasmid, which is then injected into the recipient. The recipient's cells take up the plasmid and produce the antigen from the DNA by protein synthesis. The recipient's immune system then responds to the antigen. DNA vaccines are an interesting development, but are still largely experimental.

An additional, major advantage of immunisation is the establishment of herd immunity in the population. **Herd immunity** is based on the principle that individuals who are immune to an infectious disease will not be carriers of the organism, reducing the overall occurrence of that microbe and, therefore, the number of susceptible people who will encounter it. In other words, mass immunisation confers indirect protection on the whole population, including the non-immune. Herd immunity helps to avert epidemics. This is discussed more fully in Chapter 14.

Side effects of vaccines

Before vaccines are licensed, they must undergo stringent trials in experimental animals and humans. However, most vaccines can cause minor adverse effects. The most common of these are local hypersensitivity reactions (redness and swelling) at the injection site, pain and fever. For certain vaccines there are rare, but more severe complications; for example:

- encephalitis following measles vaccine (although the incidence of this is much lower than following infection)
- acute neurological complications following pertussis vaccine (although the incidence of this is much lower than following infection)
- vaccine-associated paralytic poliomyelitis associated with the live, attenuated polio vaccine
- systemic allergic reactions (anaphylaxis) following several vaccines, such as measles, mumps, rubella and influenza vaccine.

Health professionals involved in giving vaccinations should be aware of the possible risks, but also aware that the risk of contracting the disease could be far more serious (see Chapter 14). Extreme caution must be exercised in giving live vaccines (a) to the immunocompromised, because even low-virulence organisms can produce disease in such people, and (b) to pregnant women, because of possible risk to the foetus. Health professionals should also be aware that the effectiveness of vaccines can be lower in certain population groups. People with poor or weakened immune systems, such as infants, the elderly and people who are immunocompromised, may acquire inadequate immunity from certain vaccines.

Detailed, current information about vaccines and their use in Australia can be found at the *Australian Immunisation Handbook* website: <www.immunise.health.gov.au/internet/immunise/publishing.nsf/Content/Handbook-home>.

Tumour immunology

Cancer is one of the leading causes of death in industrialised countries. The term **tumour** refers to a swelling due to a new growth of cells. Development of a tumour involves a series of genetic changes in a group of cells, usually over a period of years, which ultimately lead to the cells replicating in an uncontrolled manner. Tumour cells are genetically unstable so, during tumour growth, genetic variants of the original cells usually develop. The term *cancer* refers to a tumour that is **malignant**, meaning that it has certain properties, which include a rapid growth rate, a lack of cell differentiation and normal tissue organisation, a capability for continual growth without inhibition from surrounding tissues, and a tendency to metastasise (spread to other tissues). A malignant tumour will eventually kill the host. A **benign tumour** is not capable of indefinite growth and does not usually kill the host.

The transformation of a normal cell into a tumour cell can be caused by a wide range of factors that are termed **carcinogens** (cancer-promoting factors):

- virus infection—for example, papilloma viruses in cervical cancer; hepatitis B and C viruses in liver cancer
- bacterial infection—for example, *Helicobacter pylori* infection and stomach cancer
- radiation—for example, UV radiation in skin cancer; X-rays
- chemicals—for example, cigarette smoke.

Tumours can also result from spontaneous mutation in a normal cell. Furthermore, specific genes called **oncogenes** have been identified in normal cells which, when activated in certain conditions, lead to malignant transformation of the cell.

There is substantial evidence that the immune system has a key role in preventing tumour development. That is, it is generally accepted that tumour cells are usually recognised by the immune system and eliminated as they arise. Thus, if a tumour does develop, it means that the tumour cells have

CASE HISTORY 9.2

Tetanus immunisation

Katherine, a 56-year-old retiree, suffered a deep cut to her hand while gardening, from a piece of broken glass buried in the soil. The medical clinic doctor noted that she had migrated to Australia from Indonesia 30 years ago and doesn't recall receiving any immunisations as a child. The doctor dressed the wound and gave her two injections: tetanus immunoglobulin and tetanus vaccine.

Questions

1. What are the reasons for the two separate injections?
2. Will Katherine be immune to tetanus after receiving these injections? Explain.
3. What does the tetanus vaccine contain?
4. Why was Katherine not given antibiotics?

somehow avoided or escaped from these body defences. The term **immune surveillance** was coined by MacFarlane Burnet in the 1960s to suggest that the immune system does seek out and destroy tumour cells.

Destruction of tumour cells by the immune system necessarily depends on the cells being recognised as foreign. In the development of tumour cells, alterations occur to the cellular DNA and to the regulation of the genes. This may lead to the development of new antigens on the tumour cells or to alterations to existing antigens that are found on the normal cells. The new antigens that are unique to the tumour cells are called **tumour-specific antigens**, while the antigens that are expressed differently in tumour and normal cells are called **tumour-associated antigens**. The immune system can, theoretically at least, respond to both types of antigens. Many of the innate and acquired defences that we have discussed in this chapter are thought to be involved in tumour cell destruction. In particular, phagocytes, NK cells, NKT cells and cytotoxic T cells (see Figure 9.38), and specific antibody in conjunction with complement have the capacity to eliminate cells that are considered foreign.

With all these defences, how is it that some tumours escape immune surveillance? There are several potential ways by which tumours are thought to escape the body's defences:

- Some tumours appear to lack tumour-specific or tumour-associated antigens and hence are not recognised as foreign.
- Some tumours have antigens that are only weakly immunogenic, and thus induce a poor immune response.
- Tumours tend to be genetically unstable and some can lose or change their antigens over time, making the immune response to the original antigens ineffective.
- Some tumour cells replicate so quickly that the immune system is overwhelmed by the amount of antigen it is exposed to.
- Some tumours have been shown to secrete immunosuppressive molecules or enzymes (e.g. transforming growth factor β—TFGβ). Many human tumours produce an immunosuppressive enzyme called indolamine-2,3-dioxygenase (IDO).
- Some tumours are thought to activate Treg lymphocytes, which then block the development of an immune response to the tumour antigens by secreting immunosuppressive cytokines, such as IL-10.
- Some tumours surround themselves with a layer of collagen or fibrin, normal body proteins that make the tumours invisible to the immune system.
- Some tumours appear to shed their antigens, which then interact with and block antibodies and T cells from reacting with the tumour cells.

The incidence of cancer is increasing, particularly in developed countries. Currently, the standard treatments for cancer are surgery, radiotherapy and chemotherapy, or a combination

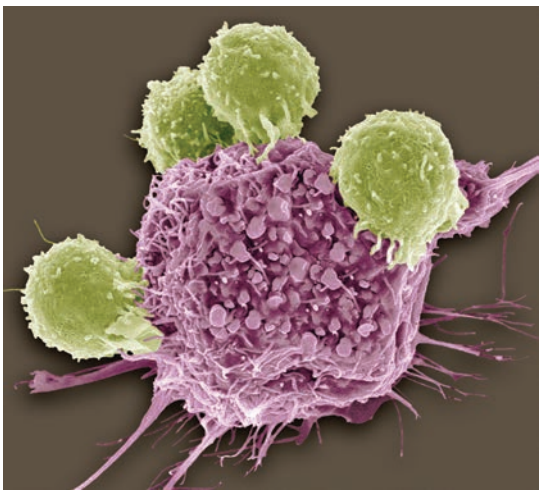


FIGURE 9.38

T lymphocytes (green) attached to a cancer cell

of these approaches. However, these methods often fail to achieve complete tumour remission and are associated with some unpleasant and serious side effects. In recent years, much attention has been given to the use of immunotherapy (therapy utilising the immune system) in the treatment of cancer. A large number of techniques are currently under investigation. Passive immunotherapy involves the use of therapeutic substances such as cytokines, which are designed to enhance the immune response to tumours, or pre-made antibodies to directly attack cancer cells. Active immunotherapy, which focuses on the use of vaccination to treat or prevent cancer, is prompted by the outstanding achievements in controlling many infectious diseases by this approach. A number of immunotherapeutic techniques for cancer treatment have been approved for clinical use, such as the monoclonal antibodies rituximab and trastuzumab, which are used for non-Hodgkin's lymphoma and breast cancer, respectively.

DISORDERS OF THE IMMUNE SYSTEM

There are certain situations where the immune system fails or functions inappropriately. When it fails, the individual becomes more susceptible to infectious diseases and sometimes cancer, and when it functions inappropriately it can cause disease and sometimes damage to the body. These disorders of the immune system can be classified as immunodeficiency, hypersensitivity or autoimmune disease, although some conditions are a combination of two or more of these.

Immunodeficiency

Immunodeficiency includes both inborn and acquired conditions in which the production or function of lymphocytes, phagocytes or complement is abnormal. These are the key components of the immune system, and a person with a defect in one or more of them is said to be **immunocompromised**, or **immunodeficient** if seriously immunocompromised. **Primary immunodeficiency** is generally due to genetic or developmental defects in the immune system, which are usually present at birth. **Secondary immunodeficiency**

results from damage to otherwise normal components and may be due to infection, cancer, malnutrition, or the use of drugs and other therapies that suppress the immune system.

Primary immunodeficiency

Selected primary immunodeficiencies are listed in Table 9.7. The two most serious primary immunodeficiency diseases are: (a) congenital thymic hypoplasia or aplasia (or DiGeorge syndrome), caused by a failure of the thymus to develop fully, resulting in a deficiency in T cells; and (b) severe combined immunodeficiency disease (SCID), in which lymphocyte precursors in the bone marrow fail to develop properly, resulting in a marked deficiency in both B and T cells. Since T cells are required for the proper functioning of both humoral and cell-mediated immunity, individuals afflicted with either condition have little or no protection against infection. Even normally minor infections can be fatal.

X-linked (Bruton's) agammaglobulinaemia is a primary immunodeficiency caused by incomplete maturation of B lymphocytes. Children (usually boys) with this disorder have very low B cell numbers and immunoglobulin levels, and suffer repeatedly from infections such as pneumonia, otitis media, meningitis and septicaemia.

Different types of immunodeficiency will result in increased susceptibility to different types of pathogenic microorganisms. In general, a T cell deficiency will mainly increase susceptibility to viruses, intracellular bacteria and other intracellular parasites such as the malaria protozoan and fungi, since it is the T cell that attacks infected host cells. A selective deficiency in B cells or antibodies, on the other hand, will greatly increase a person's susceptibility to most bacterial infections and, to a lesser extent, viruses. A deficiency in both cell types, as occurs in SCID, will make the individual highly susceptible to all pathogenic microbes and even to organisms with low virulence.

Complement mainly attacks foreign cells in extracellular fluids, so a deficiency in any one of the complement proteins will increase the risk of bacterial infections. A defect in macrophage activity will greatly impair defences against bacteria, and defence against viruses will also be affected, but to a lesser extent.

TABLE 9.7 Selected primary immunodeficiency diseases

DISEASE	NATURE OF DEFECT
Defects in B lymphocytes	
■ X-linked agammaglobulinaemia	Pre-B cells do not mature—no circulating B cells
■ common variable hypogammaglobulinaemia	B cells fail to differentiate into plasma cells, resulting in deficiencies in IgG and IgA
■ selective IgA deficiency	IgA is not synthesised
Defects in T lymphocytes	
■ congenital thymic aplasia (DiGeorge syndrome)	Improper development of the thymus gland—few T cells
Defects in both T and B lymphocytes	
■ severe combined immunodeficiency	Stem cell defect resulting in T and B cell deficiency
■ bare lymphocyte syndrome	Deficient expression of MHC molecules
Defects in phagocytes	
■ chronic granulomatous disease	Phagocyte enzyme deficiency
Defects in complement	
■ complement defect	Deficiency in any component of complement

Secondary immunodeficiency

There are numerous potential causes of secondary immunodeficiency. Any condition or agent that causes a suppression or malfunction of the bone marrow or lymphoid organs may cause a secondary immunodeficiency. For example, in leukaemia, a massive number of cancer cells are produced in the bone marrow, preventing the normal production of white cells. In Hodgkin's disease, the immune system is suppressed because of the growth of tumour cells in the lymph nodes.

Treatments for certain diseases can also cause immunodeficiency. For example, the cytotoxic drugs and radiation therapy used in the treatment of cancer suppress white cell production in the bone marrow. Corticosteroids, used to reduce inflammation in certain diseases, can reduce the number of leukocytes in the blood. Immunosuppressive drugs are used to prevent rejection of organ transplants, but they also make the patient more susceptible to infection.

The most obvious and devastating of the secondary immunodeficiencies is the **acquired immunodeficiency syndrome** (**AIDS**), caused by the human immunodeficiency virus (HIV). The HIV severely damages the immune system by infecting and destroying T helper cells. (This process is described more fully in Chapter 19.) A large variety of other microbes, including the measles virus, mumps virus, *Mycobacterium leprae* (leprosy) and the protozoa that cause malaria, can cause minor suppression of the immune system.

The factors that commonly lead to secondary immunodeficiency are summarised in Table 9.8. In addition to these secondary immunodeficiency diseases, innate defences can be impaired in certain situations. These are not generally regarded as immunodeficiency disorders because the defences affected are not strictly part of the immune system, but nevertheless they increase the risk of infection. For example, broad spectrum antibiotics can temporarily lower the body's innate defences by reducing the normal flora of the body. When the skin barrier is disrupted by burns, surgical wounds or other trauma, infection becomes more likely. A urinary catheter predisposes a person to urinary tract infection because of the loss of the flushing action of urine over the urethral surface, and impairment of mucociliary activity in the airways as a result of cigarette smoking is an important factor in some types of lower respiratory infection.

TABLE 9.8 Examples of factors that cause secondary immunodeficiency

FACTOR	EFFECT ON BODY DEFENCES
Some tumours	
■ leukaemia	Production of dysfunctional bone marrow cells
■ Hodgkin's disease	Disease of lymph nodes
Tumour therapy	
■ chemotherapy (cytotoxic drugs)	Suppression of white cell production
■ radiotherapy	Suppression of white cell production
Malnutrition	
■ protein deficiency	Poor lymphoid organ development, low antibody and complement concentrations, low lymphocyte numbers
Immunosuppressive therapy	
■ in transplant patients	Suppression of lymphocyte function
Anti-inflammatory therapy	
■ corticosteroids	Reduction of white cell production, impairment of lymphocyte function, and antibody and cytokine production
Infection	
■ human immunodeficiency virus	Destruction of T helper lymphocytes

Hypersensitivity

Hypersensitivity is an excessive, undesirable reaction of the immune system to an antigen it has previously encountered. There are different types of hypersensitivity reactions, which can be differentiated by the mechanisms involved. The British immunologists PHILLIP GELL and ROBIN COOMBS categorised them into four types. The first three types are due primarily to the action of antibodies, and the fourth type is due to the action of T cells. Some clinical conditions are actually a combination of more than one type.

Type I: Immediate hypersensitivity

Immediate hypersensitivity, or allergic reaction, such as hay fever and hives, affects around 25 per cent of people and appears to be increasing in incidence in Australia and other countries with a Western lifestyle. **Allergy** occurs within 15 minutes to several hours after a susceptible person comes into contact with the foreign antigen to which they are sensitised. The term **allergen** is used to distinguish the type of antigen that has the potential to elicit this type of reaction. Typical allergens include pollens, dust mites, fungal spores, certain foods, and some antibiotics and other drugs (see Figure 9.39).

A predisposition for allergies, termed **atopy**, is associated mainly with the production of IgE antibodies to the allergens; that is, allergic people produce IgE antibodies after exposure

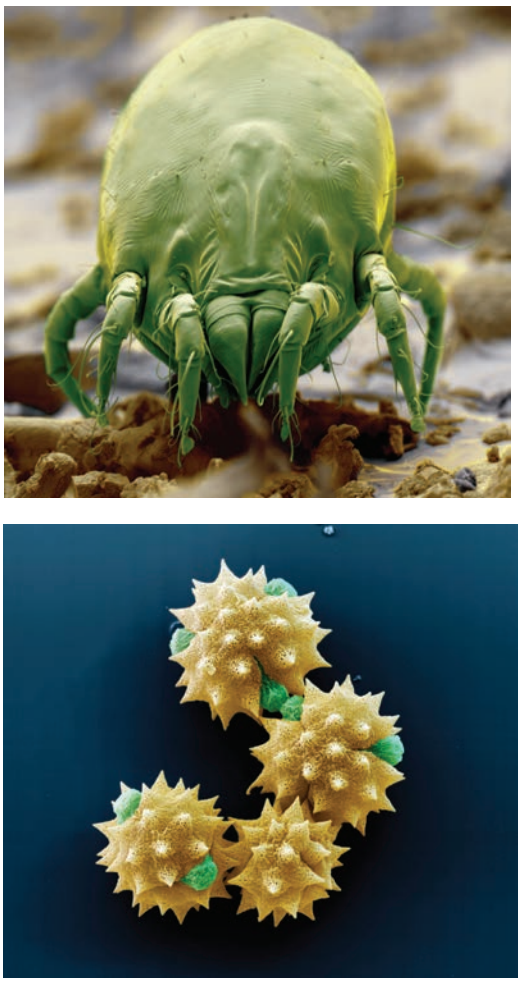


FIGURE 9.39

Common allergens

(a) Dust mite; (b) daisy pollen.

to allergens, whereas non-allergic people produce another class of antibody (mainly IgG) to the same antigens. The precise reasons why only some people produce IgE antibodies to these antigens are not fully understood. This predisposition for allergy is at least partly due to genetic factors, since a person with two parents who suffer from allergies has approximately a 60 per cent chance of suffering from them, a person with one allergic parent has approximately a 30 per cent chance, and a person who has no allergic parents has approximately a 15 per cent chance of suffering from allergies. The most common clinical manifestations of allergy are allergic rhinitis, allergic asthma, wheal and flare reaction (area of swelling and redness in the skin), food allergy, allergic eczema and anaphylaxis.

There is also some evidence that environmental factors may play a role. It is argued by some scientists that childhood exposure to a large variety of antigens is protective and that such people are less likely to develop allergies. The 'hygiene hypothesis', first proposed by DAVID STRACHAN in 1989, suggests that modern lifestyles in industrialised countries have led to a decrease of infectious burden and this is largely responsible for the increasing incidence in allergies

(and autoimmune disease) in Western populations (see Figure 9.40). Indeed, the incidence of allergy is significantly lower in developing countries, and a lower incidence has also been reported in rural areas of developed countries.

The basis of immediate (type I) hypersensitivity is the production of the IgE class of antibodies after initial exposure to the allergen. It has recently been recognised that type 2 T helper cells (Th2) release cytokines, such as IL-4, IL-5 and IL-13, that induce the formation of IgE by plasma cells (derived from B lymphocytes). In non-allergic people, Th1 cells predominate, and they induce IgG production, an antibody class that is not involved in allergy. In addition, Treg lymphocytes, which suppress allergic reactions in a number of ways, appear to have impaired function in allergic people. The IgE molecules thus produced have a special affinity for mast cells and basophils and attach to these cells via their Fc fragments. These sensitised cells may remain in the tissues for years. On subsequent exposure to the allergen, the allergen molecules cross-link the IgE antibodies attached to mast cells or basophils. This stimulates the cells to degranulate, releasing histamine and other inflammatory chemicals (e.g. kinins and proteases) that rapidly induce an inflammatory response (Figure 9.41). Activation of these cells also induces them to synthesise other powerful inflammatory mediators, particularly the prostaglandins and leukotrienes.

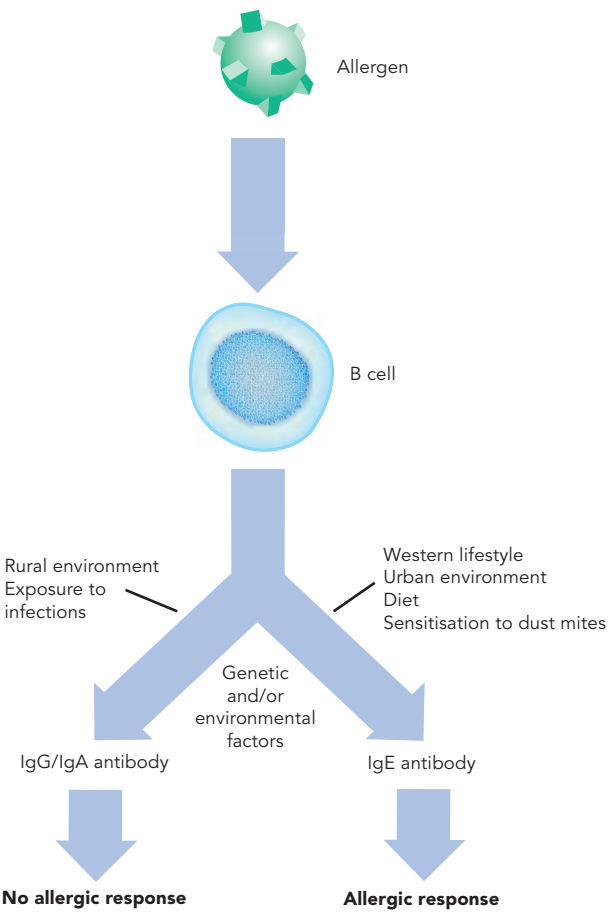


FIGURE 9.40

The basis of an allergic response

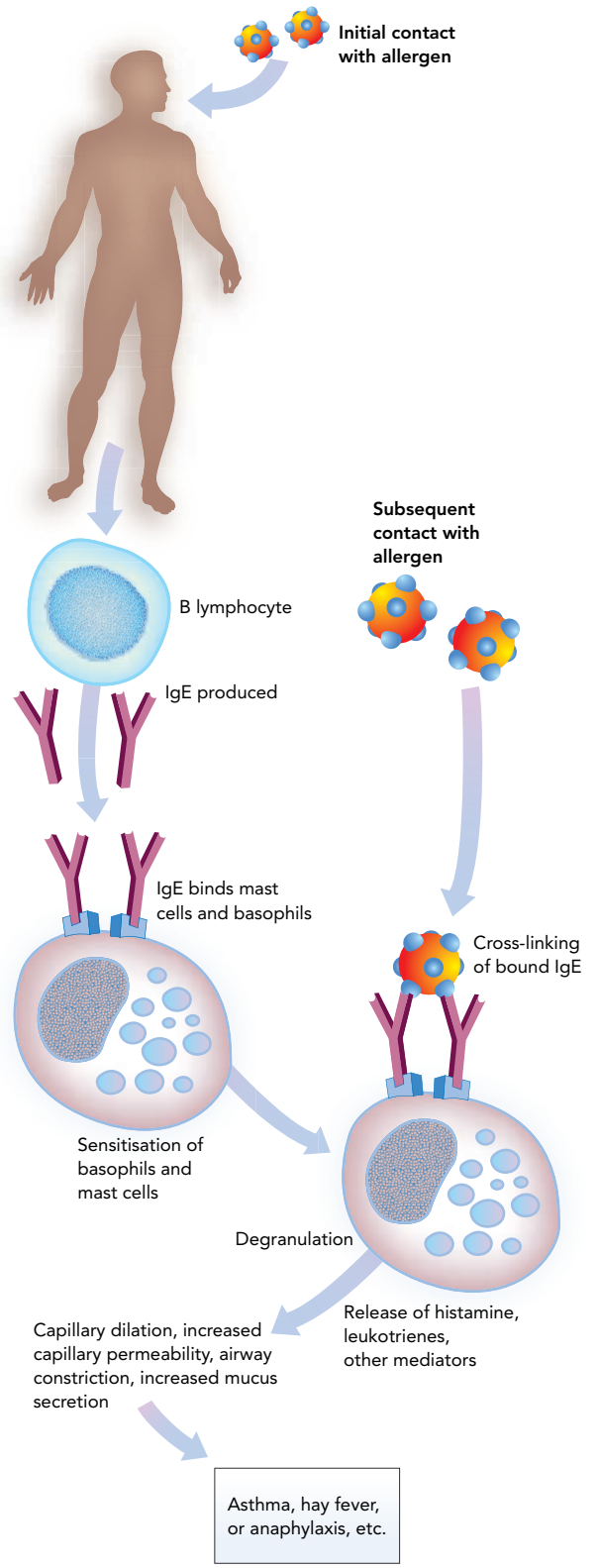


FIGURE 9.41

The mechanisms of allergy

Following first contact with an allergen, B lymphocytes are stimulated to produce IgE antibodies. These antibodies bind to mast cells and basophils, via their Fc region, thereby sensitising those cells. On subsequent contact with the allergen, it is bound to the sensitised cells, causing release of various inflammatory mediators which cause allergic reactions.

Eosinophils also play an important role in allergic reactions. They accumulate in tissues where allergic reactions are occurring and contribute to the process by secreting additional inflammatory mediators, especially leukotrienes.

Most allergic reactions are local, occurring typically where the allergen enters the body. Thus, most involve the skin, the respiratory passages or the gastrointestinal tract, because of binding of allergen to local mast cells. Because the mediators cause dilation and increased permeability of blood vessels, local reactions are usually characterised by redness and swelling. Other familiar signs and symptoms, such as a runny nose, watery eyes and itching, reddened skin (hives), are related to inflammation in the specific tissues affected. When the allergen is airborne and inhaled, symptoms of asthma may appear as a result of bronchiolar smooth muscle contraction, which constricts the bronchioles, restricting air flow. Excessive mucus production further constricts the airways. When the allergen is ingested (e.g. in food), gastrointestinal hypersensitivity may occur (cramping, vomiting or diarrhoea) and sometimes a skin rash. Table 9.9 details some common types of allergic reactions.

Fortunately, systemic allergic reactions, referred to as **anaphylaxis**, are far less common than local allergy. Anaphylaxis typically occurs when the allergen enters the blood and circulates through the body, as may happen with bee stings. It may also follow ingestion of a foreign substance (food allergy), or injection of a medication, such as penicillin, in a susceptible individual. Food allergy is the most common trigger for anaphylaxis in children, whereas medications and insect stings are more common triggers in adults. Anyone who has had any adverse reaction to penicillin, such as hives or throat or chest constriction, should not be given the drug again.

The mechanism of anaphylaxis is essentially the same as that of local responses but, when mast cells and basophils are degranulated throughout the body, the generalised inflammatory response can be life-threatening. The sudden vasodilation may cause lowered blood pressure, circulatory collapse and physiological shock and, together with constriction of bronchiolar smooth muscle, can cause death within minutes. Other common manifestations involve the skin (e.g. generalised hives, swollen lips) and the gastrointestinal tract (e.g. abdominal pain, vomiting). This serious form is called **anaphylactic shock**.

Type II: Cytotoxic hypersensitivity

Cytotoxic hypersensitivity results from the binding of antibodies to antigens on the surface of a cell, followed by complement activation and lysis of the target cell. The most common form is when a cell of the person's own body is

CASE HISTORY 9.3

Allergy

Michael, a 25-year-old advertising executive, has suffered from hay fever for many years, especially during the spring and summer months. His pharmacist friend, Sarah, suggests that he use Beconase nasal spray (active ingredient fluticasone propionate—a glucocorticoid steroid) as a preventative.

Questions

1. What is the likely basis for Michael's hay fever?
2. What is the rationale for the use of Beconase to prevent this condition?
3. What are the similarities and differences between hay fever and penicillin allergy?
4. If Michael prefers not to take the medication, how else might he prevent the condition?

TABLE 9.9 Common allergic reactions mediated by IgE

SYNDROME	TYPICAL ALLERGENS	ROUTE OF ENTRY	CLINICAL MANIFESTATIONS
Wheal and flare	Insect bites	Subcutaneous	Redness; oedema; itching
Hay fever (allergic rhinitis)	Pollens Dust-mite faeces	Inhaled	Oedema of nasal mucosa Irritation of nasal mucosa
Allergic asthma (extrinsic asthma)	Pollens Dust-mites	Inhaled	Bronchial constriction; increased mucus production; inflammation of airways
Food allergy	Shellfish Milk Eggs Peanuts	Ingested	Vomiting; diarrhoea; urticaria (hives); anaphylaxis
Allergic eczema (atopic dermatitis)	Pollens Moulds Dust mites	Through the skin	Dry, reddened, itchy skin
Systemic anaphylaxis	Penicillin Bee venom	Intravenous (directly or following rapid absorption)	Oedema; circulatory collapse; death

mistakenly recognised as foreign—that is, an autoimmune disease. In addition, complement activation leads to the formation of the anaphylatoxins C3a and C5a (see page 194), which stimulate mast cells to degranulate, leading to an inflammatory response. Examples of this type of hypersensitivity are autoimmune haemolytic anaemia and Goodpasture's syndrome, in which autoantibodies and complement cause lysis of red blood cells and cells of the glomerular basement membrane, respectively.

Cytotoxic (type II) reaction is a major cause of damage in many autoimmune diseases (see later section).

Type III: Immune complex hypersensitivity

Immune complex hypersensitivity results when soluble antigen–antibody complexes are formed and are not cleared quickly from the body. Normally, immune complexes are engulfed and destroyed by phagocytes, but if the complexes evade phagocytosis and persist they may be deposited in the basement membranes in certain sites, such as the kidneys, lungs, joints, and blood vessels of the skin. The complexes activate complement at the site of deposition and an inflammatory reaction occurs that can damage local tissues (Figure 9.42). The antigens in the immune complexes may be exogenous (e.g. from chronic bacterial or viral infections) or endogenous as a result of autoimmunity (see later section).

Acute glomerulonephritis is an example of an immune complex disease in which the complexes are deposited on the basement membrane of kidney glomeruli. Certain skin rashes, such as in systemic lupus erythematosus, result from the deposition of immune complexes in the basement membrane of surface blood vessels. Rheumatoid arthritis is due to a combination of immune complex hypersensitivity and autoimmune disease.

Type IV: Delayed hypersensitivity

Delayed hypersensitivity reactions are mediated by T cells and take 24 hours or more to appear after the introduction of antigen—hence the term 'delayed'. The mechanisms involved are basically that of a cell-mediated immune response. Th1 lymphocytes secrete cytokines which activate cytotoxic T cells, moncytes and macrophages. The cytotoxic T cells directly damage the tissues, while a massive infiltration of monocytes and macrophages occurs, which causes inflammatory reactions such as eczema, oedema and granuloma formation. This reaction represents a normal cell-mediated response in which host tissue cells are unfortunately damaged.

The *Mantoux test* for assessing immunity to tuberculosis depends on a delayed hypersensitivity reaction. When protein antigens of the tuberculosis bacterium are inoculated intradermally (just under the skin), a small, hard inflammatory reaction occurs two to three days later if the person has been previously sensitised to the antigen. Contact dermatitis is another example of delayed type hypersensitivity. Contact dermatitis may occur when a hapten (incomplete antigen) present in a chemical product binds to a body protein (usually a skin protein) and then induces a cell-mediated response, manifested as a delayed hypersensitivity reaction. Chemicals containing haptens that may induce contact dermatitis include some cosmetics, metals, soaps and dyes.

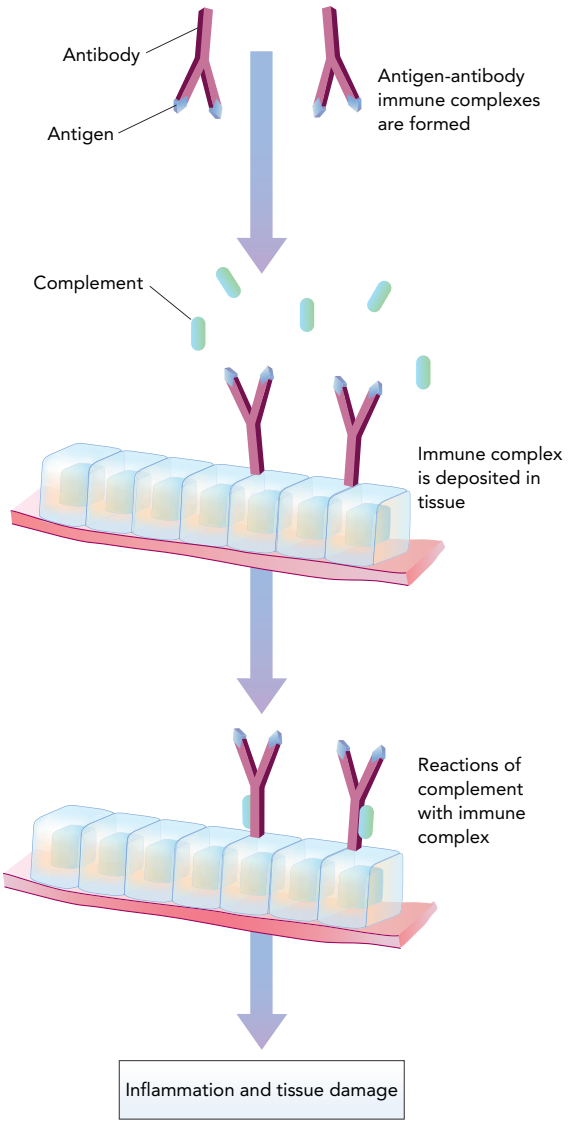


FIGURE 9.42

The mechanism of immune complex hypersensitivity

Immune complexes that are not rapidly removed by phagocytes are deposited in tissues where complement activation causes inflammation and tissue damage.

Autoimmune diseases

As described earlier in this chapter, human cells comprise numerous substances called HLA antigens that are potentially immunogenic. However, the immune system does not normally respond to these self-antigens. The non-responsiveness to self-antigens is called **tolerance**. The mechanisms involved in the induction and maintenance of tolerance are not fully understood, but clonal deletion (see page 204) is considered to play a major role. Also, a recently recognised subset of T lymphocytes, the Treg cells, have

been shown to play a significant role in tolerance by blocking immune reactions to self-antigens.

Nevertheless, the immune system occasionally loses its tolerance to one or more self-antigens. When this happens, the body produces antibodies (**autoantibodies**) and/or sensitised T cells against its own tissues. However, it has been shown that self-reactive lymphocytes are actually part of the normal cell repertoire and that only in certain situations do some of these cause damage. If the damage is significant and disease results, it is called an **autoimmune disease** (see Table 9.10). The immune system has extraordinary potential for preventing reaction against self-antigens, and autoimmunity is in fact relatively rare.

There are more than 70 known autoimmune diseases, and around 5 per cent of people in developed countries are afflicted by one of these conditions. Autoimmune diseases are very diverse in terms of targeted tissues affected, symptoms, age of onset, and responsiveness to therapy. In most cases, it is not known what precipitates autoimmunity but genetic factors, environmental factors, sex, age and some infections are known to influence its incidence. In some disorders, the body's own cells are thought to appear 'foreign' because they are distorted or altered in some way. A common feature appears to be the involvement of both humoral and cell-mediated immune responses in the tissue injury.

The self-antigens that are the targets of attack have not been clearly identified in many autoimmune diseases, although the targets and mechanisms of a few conditions have been clarified to a large extent. **Autoimmune haemolytic anaemia** is a condition in which red cells are destroyed by autoantibodies in conjunction with complement. In this disease, an alteration to the surface composition of red cells causes the immune system to recognise them as foreign and produce antibodies against them. The factor that causes the surface of the red cells to be altered is often unknown, although some viruses and drugs have been shown to be involved in some instances.

In **rheumatoid arthritis**, an altered antibody molecule (an IgG) is thought to be the basis of the disease. First, the IgG to some unknown antigen is formed within a joint. Then, also for unknown reasons, this IgG becomes altered and eventually appears sufficiently foreign to stimulate an immune response in the form of antibodies (usually IgM). This anti-IgG antibody can be found in serum and is called the **rheumatoid factor**. The immune complexes formed by the binding of IgM to the altered IgG antibodies activate complement, leading to chronic inflammation and joint damage. This disorder is thus a combination of autoimmune disease and immune complex hypersensitivity.

Antibodies made against a foreign antigen sometimes cross-react with a self-antigen that has a similar structure and composition. For instance, antibodies produced during a streptococcal throat infection have been found to cross-react with components of heart muscle, causing inflammation and damage to the heart muscle and valves. This rare autoimmune sequela of streptococcal sore throat is called **rheumatic fever** (see Chapter 19). It affects only a small percentage of people, those with the genetic makeup to produce the cross-reacting antibodies.

TABLE 9.10 Examples of autoimmune diseases

DISEASE	TARGET OF THE AUTOANTIBODY	OUTCOME
Autoimmune haemolytic anaemia	Red blood cells	Red blood cell destruction resulting in anaemia
Idiopathic thrombocytopenic purpura	Platelets	Platelet destruction resulting in bruising and haemorrhage
Grave's disease	TSH receptor on thyroid cells	Hyperthyroidism
Rheumatic fever	Cardiac muscle	Damage to heart muscle and valves
Myasthenia gravis	Acetylcholine receptor at the neuromuscular junction	Block of the receptor resulting in muscle weakness
Goodpasture's syndrome	Basement membrane in kidney and lungs	Kidney failure and lung disease
Systemic lupus erythematosus	Nucleic acid, red blood cells, platelets, etc.	Many symptoms possible, including facial rash, photosensitivity, arthritis, pericarditis
Insulin-dependent diabetes mellitus	Pancreatic beta cells	Inadequate insulin production causing excessive blood glucose levels
Rheumatoid arthritis	Antibodies, connective tissue	Precipitation of immune complexes in joints resulting in inflammation of joints

SUMMARY

- The ability of the body to prevent infection is called resistance.
- Lack of resistance, or vulnerability to an infection, is called susceptibility.
- The body's resistance to infection depends on innate defences (the body's first and second lines of defence) and acquired defences (the body's third line of defence).
- The acquired defences and some of the innate defences comprise the body's immune system.

INNATE IMMUNITY

- The function of the innate defences is to prevent the entry of pathogens into the body, or to destroy them quickly if they do manage to enter.
- The body's first lines of defence against invasion by microorganisms are the skin and the mucous membranes.
- The normal microbial flora of the body suppress the growth of potential pathogens by various means and may be considered part of the first line of defence.
- Phagocytes are body cells that actively ingest and digest foreign particles and remove cellular debris from the tissues.
- The major types of phagocytes in the body are the macrophages, dendritic cells and the neutrophils.
- Phagocytes are attracted to sites of damaged tissue or microbial invasion by chemicals released in a process called chemotaxis.
- Natural killer (NK) cells are a unique group of cells that are capable of destroying cancer cells and virus-infected cells.
- NK cells kill the target cell by insertion of perforins into the cell membrane, creating a pore through which toxic substances can be injected into the target cell.
- The acute inflammatory response is a second line of defence that is the body's response to tissue injury.
- The major function of inflammation is to clear the injured site of cellular debris and foreign material, thereby preparing the area for repair processes.
- The four main signs of acute inflammation are redness, heat, swelling and pain.
- The chemicals responsible for the physiological events that occur in inflammation are called the chemical mediators of inflammation.
- The most important mediators of inflammation are histamine, kinins, prostaglandins, leukotrienes, tumour necrosis factor and complement.
- Aspirin, indomethacin and other non-steroidal anti-inflammatory agents act by inhibiting the synthesis of prostaglandins.
- Steroidal anti-inflammatory drugs block both prostaglandin and leukotriene synthesis.
- Wound repair is a process which aims at the removal of microorganisms and tissue debris and a return of the tissue to normal structure and function.
- Chronic inflammation occurs if the acute inflammatory response is unsuccessful in eliminating the organisms or foreign material from the tissues.
- Fever represents a systemic response to invading microorganisms and often accompanies severe or systemic inflammation.
- The complement system is a complex system of plasma proteins that, when activated, enhance phagocytosis, produce inflammation and directly lyse foreign cells.
- Antimicrobial peptides are molecules containing fewer than 100 amino acid residues, which protect against a broad range of microorganisms. They include the cathelicidins, defensins and histatins.
- Cytokines are chemical substances that act as messengers between cells in the immune system.
- The major groups of cytokines are interleukins, interferons, colony stimulating factors, tumour necrosis factors and chemokines.

ACQUIRED IMMUNE SYSTEM

- The acquired immune system consists of a variety of cells, especially lymphocytes and macrophages, and various lymphoid organs.
- When a microbe is able to persist in the tissues, the body reacts by producing an acquired (specific) immune response.
- The immune response is able to recognise foreign substances, is specific and has memory.
- There are two major populations of lymphocytes: the B lymphocytes (B cells) and T lymphocytes (T cells).
- B cells are mainly responsible for humoral immunity and T cells provide cell-mediated immunity.
- On the surface of mature lymphocytes there are receptors of a single type, which enable the cells to recognise and bind to a specific antigen.
- T cells that possess the CD4 marker (CD4+ cells) are primarily T helper cells; T cells with the CD8 marker (CD8+ cells) are cytotoxic T cells.
- Different types of T helper cells exist. They have a central role in helping cells to produce an immune response.
- Cytotoxic T cells bind directly to and kill target cells. They attack virus- or bacteria-infected cells and tumour cells.
- Antigen-presenting cells play a critical role in activating lymphocytes by presenting processed antigen to them.
- The major antigen-presenting cells are macrophages and dendritic cells.
- Antigens are present on the surfaces of microorganisms and human and animal cells.
- The major histocompatibility complex (MHC) codes for class I and class II MHC proteins which are vital for cell-to-cell interactions in immune reactions.
- Macrophages and dendritic cells are the first cells to recognise antigen, and they engulf and process antigen for presentation to lymphocytes.
- Each mature B lymphocyte carries molecules of specific antibody on its surface and is activated when an antigen binds to this receptor.
- Following activation, B cells proliferate and then differentiate into plasma cells or memory B cells.

- Antibodies are secreted by the plasma cells into body fluids.
- The primary immune response is the response of the acquired immune system the first time it is exposed to a particular antigen.
- A secondary immune response occurs if a person is exposed to the same antigen for a second time; this response is faster, stronger and longer-lasting.
- Five major immunoglobulin classes exist in humans: IgD, IgM, IgG, IgA and IgE.
- Microorganisms or other particles, when coated with opsonising antibody, become more readily recognised and ingested by phagocytes.
- Antibody attachment, followed by complement fixation, can lead to lysis of foreign cells such as bacteria and cancer cells.
- Neutralisation is a process in which antibodies bind to and block specific attachment sites on viruses or bacterial toxins.
- The cell-mediated immune response involves the activation, differentiation and actions of different types of T cells.
- When T helper cells are activated by exposure to specific antigen, they secrete a variety of different cytokines.
- Cytotoxic T cells directly attack and kill target cells, especially virus-infected cells.
- Active immunity is developed when the acquired immune system responds to microorganisms or other foreign substances entering the body.
- Active immunity is naturally acquired during microbial infections, and artificially acquired as a result of vaccination.
- Passive immunity results from the transfer of pre-made antibodies (or immune cells) from an immune person (or animal) to a non-immune person
- Most vaccine preparations comprise one of the following: killed, whole microorganisms; live, attenuated microorganisms; inactivated bacterial toxins (toxoids); parts of bacterial cells or viruses.
- Herd immunity is based on the principle that individuals who are immune to an infectious disease will not be carriers of the organism, thus reducing the occurrence of that microbe and the number of susceptible people who will encounter it.

DISORDERS OF THE IMMUNE SYSTEM

- Immunodeficiency includes both inborn and acquired conditions in which the production or function of lymphocytes, phagocytes or complement is abnormal.
- Primary immunodeficiency is due to improper foetal development of one or more of these components.
- Any agent that causes a suppression or malfunction of the bone marrow or lymphoid organs may cause a secondary immunodeficiency.
- Hypersensitivity is an overreaction of the immune system to an antigen it has previously encountered.
- An immediate hypersensitivity (type I immediate hypersensitivity), or allergy, occurs within minutes to several hours after contact with the foreign antigen to which the person is allergic.
- IgE molecules have a special affinity for mast cells and basophils.
- Allergen molecules cross-link the IgE antibodies, stimulating mast cells to release histamine and other inflammatory chemicals.
- Anaphylactic reactions occur when the allergen enters the blood and circulates through the body.
- Type II cytotoxic hypersensitivity results from the binding of antibodies to antigens on the surface of a cell, followed by complement activation and lysis of the target cell.
- Type III immune complex hypersensitivity results when antigen–antibody complexes are deposited on the basement membranes of tissues; the complexes activate complement and an inflammatory reaction occurs.
- Type IV delayed type hypersensitivity reactions are mediated by T cells and take a day or more to appear after contact with the antigen.
- When the body produces autoantibodies and sensitised T lymphocytes against its own tissues, disease can result, called an autoimmune disease.

STUDY QUESTIONS

1. What are the major differences between the non-specific and specific defences of the body?
2. What factors make intact skin such an effective barrier against infection?
3. What is the mucociliary escalator, and what is its function?
4. What are the major phagocytic cells of the body, and what are their functions?
5. Describe the process of phagocytosis.
6. What are the major events in acute inflammation, and what are their functions?
7. What are chemical mediators of inflammation?
8. What is pus, and what is its significance?
9. Explain the physiological basis of fever.
10. What is complement, and what are its functions?
11. Describe the types and activities of interferon.
12. Define the terms: immune system, acquired immune system and immune response.
13. What are the three important characteristics of the acquired immune system?
14. What are the major differences between humoral immunity and cell-mediated immunity?
15. List the primary and secondary lymphoid organs in humans.
16. Describe the functions of plasma cells.
17. Describe the functions of memory cells.
18. Draw a graph showing the major differences between a primary and secondary immune response.

19. Draw a basic antibody molecule showing its antigen-binding sites.
20. What is an antitoxin?
21. List the major types of T lymphocytes and describe their functions.
22. What is meant by the term 'immunodeficiency'?
23. What is meant by the term 'hypersensitivity'?

TEST YOUR UNDERSTANDING

1. Why are regular booster doses needed for some vaccines but not for others?
2. Explain the principle behind the clonal selection theory.
3. A 25-year-old nurse pricks herself with a needle after using it to collect blood from a patient with hepatitis B. She has not been vaccinated against hepatitis B. What prophylactic treatment should she receive, and why?
4. Describe four conditions that would directly reduce a person's innate immunity and the effects each of these conditions would have.
5. What is opsonisation? Why is opsonisation important in the defence against microorganisms such as *Streptococcus pneumoniae*?

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CHAPTER

10

Pathogenic mechanisms and evasion strategies of microorganisms

CHAPTER FOCUS

- How do microorganisms cause disease?
- What substances do microorganisms produce that enable them to cause disease?
- How do microorganisms damage human cells?
- How do pathogenic microorganisms evade the defence systems of the body?

INTRODUCTION

In order to cause disease, pathogenic microorganisms have to possess properties that enable them to bypass or overcome the host's defences. In Chapter 9 we examined the ways by which the human body protects itself against disease. Ultimately, health or disease is determined by the effectiveness of the body's resistance versus the pathogenic mechanisms of the microorganism (see Figure 10.1).

As described in Chapter 7, the ability of an organism to cause disease is referred to as its **pathogenicity**. The degree of pathogenicity of a microorganism, or its relative ability to cause disease, is termed its **virulence**. For example, the rotavirus is regarded as being highly virulent, because it readily causes gastroenteritis in children, whereas *Lactobacillus* spp. does not cause disease and is thus non-virulent (or avirulent). The factors that contribute to an organism's ability to cause disease are called **virulence factors**. The virulence factors of many organisms have not been fully identified, but for some they are well defined. We examine some of these factors in this chapter.

Some microorganisms (called true pathogens) are able to cause disease in normal hosts, whereas others (called opportunistic pathogens) can only cause disease in hosts whose defences have been compromised in some way. The true pathogens have attributes or virulence factors that enable them to cause disease, despite the presence of normal defences in the host. It should also be recognised that disease is a complex process which can be due to the activities of the microorganism alone, to the response of the body to infection, or to a combination of both. For example, *Clostridium tetani* causes tetanus by releasing a substance that has toxic effects on nerve cells. The symptoms of rheumatic fever, on the other hand, are essentially due to a damaging immune response initiated by *Streptococcus pyogenes*. The pathophysiology of bacterial meningitis is at least partly due to focal tissue damage caused by the bacteria and the body's inflammatory response to the infection.

Most microorganisms enter the human body by way of a mucous membrane or a break in the skin. In order to establish themselves, most microbes must first adhere to specific tissues within the host. Once inside the body, pathogenic microorganisms may cause damage to the host, and hence disease, in a variety of ways. This usually involves the invasion of tissues and cells and/or the release of substances that cause cell damage or cellular dysfunction. Some microbes have to enter cells to damage them, whereas others can cause damage from outside the cell.

A recently identified attribute of bacterial cells is their ability to communicate with each other, via chemical signals, in order to coordinate their attack on the host. This form of cell-to-cell communication, which is termed **quorum sensing**, is based on the population of bacteria gaining a critical number, after which genes for certain virulence factors are activated, enabling the bacteria to more successfully invade and damage the host. Quorum sensing is involved in the regulation of some important virulence factors that are discussed in this chapter—namely, biofilm production and toxin synthesis and secretion.

ADHERENCE

Adherence is the process by which microorganisms attach to host tissues. Some non-pathogens (e.g. some normal flora organisms) adhere to tissues in a specific way. Adherence is also a critical step in the disease process of many pathogenic organisms, particularly those that enter the body via mucous membranes. If, after entering the body, the microorganism does not adhere and is quickly swept away (e.g. as a result of coughing, sneezing or urination), it will be unable to cause infection. Often adherence involves a specific interaction between surface components of a microorganism and surface receptors on host cells. For example, fibronectin, a protein found on the surface of many human cells, is the receptor to which *Streptococcus pyogenes* and some other Gram-positive bacteria attach when colonising the upper respiratory tract.

In some cases, this specific interaction helps to explain why a certain organism can only infect a particular host species and even a particular cell type in that species. This is especially true for viruses, which tend to infect a single type of cell or, at most, a limited number of cell types.

The substances or structures on the surfaces of microorganisms that enable them to attach to cell surfaces are called **adhesins**. Many microorganisms have chemical groups on their surface, such as specific glycoproteins or lipoproteins, which enable them to attach to specific chemicals (receptors) on the surface of host cells. For example, *Streptococcus pyogenes* attaches to host cells by way of its adhesion molecule called protein F.

The adhesins of some bacteria are specific attachment structures called *pili*, or *fimbriae*—hair-like appendages extending from the cell surface (see Figure 10.2). The tip of the pilus (sing.) attaches to specific molecules on the host cell surface (see Figure 10.3a). Strains of *Escherichia coli* that cause urinary tract infections have attachment pili that enable them to establish infection in the bladder and kidneys. The piliated strains are much more commonly associated with urinary tract infections than the non-piliated strains; the latter tend to be flushed out more easily by urination. Other strains of *E. coli* that cause diarrhoea adhere specifically to epithelial cells of the small intestine by way of pili (different from those for the urinary tract).

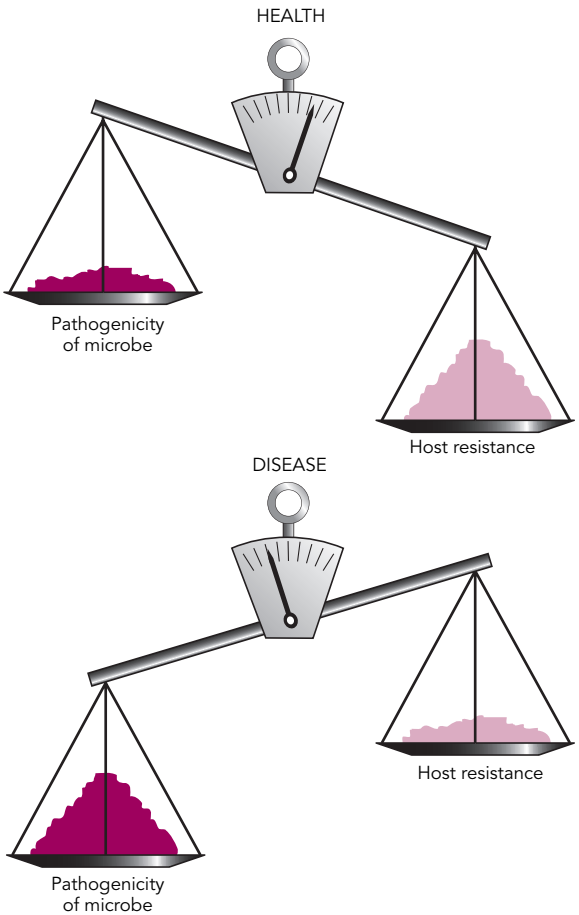

FIGURE 10.1

The balance between disease and health

When the resistance of the host is greater than the pathogenicity of the microbe, the host remains healthy; when the pathogenicity of the microbe is greater than the resistance of the host, disease occurs.

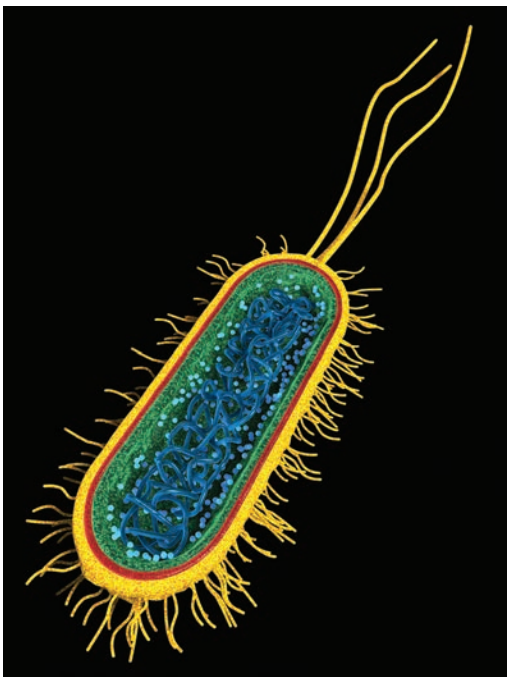

FIGURE 10.2

Computer-generated image of *E. coli* showing numerous pili (short yellow appendages)

Source: Pasieka/Science Photo Library.

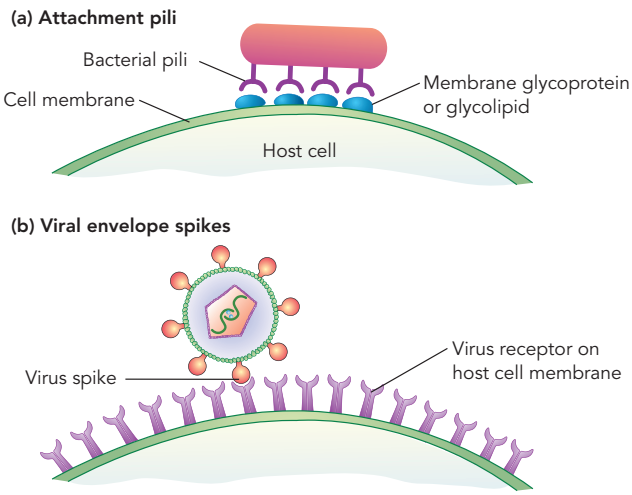

FIGURE 10.3

Mechanisms of adherence of pathogenic microorganisms

(a) Attachment pili; (b) viral envelope spikes.

Similarly, pathogenic strains of *Neisseria gonorrhoeae*, the cause of gonorrhoea, have pili which allow them to attach to epithelial cells in the urogenital tract, whereas non-piliated strains of this organism rarely cause disease.

Adherence is also important in the pathogenesis of bacterial meningitis. To cause meningitis, bacteria must enter the central nervous system from the bloodstream, and to do this they have to cross the highly specialised capillaries that comprise the blood–brain barrier. It is thought that polysaccharide capsules of the bacteria may play a critical role in their attachment to the endothelial cells of the capillaries. Indeed, pathogenic strains of three important causes of acute bacterial meningitis—*Neisseria meningitidis*, *Haemophilus influenzae* and *Streptococcus pneumoniae*—are all encapsulated.

Viruses attach to specific membrane receptors on the cells they infect. Some have specialised attachment structures such as tail fibres (as in bacteriophages) or envelope spikes (see Figure 10.3b and Chapter 5). Generally, the presence of a particular receptor on the membrane of a cell is the key determinant of whether or not a particular virus can infect that cell. For example, the human immunodeficiency virus (HIV) preferentially infects cells that possess a glycoprotein called the CD4 receptor on their surfaces (see Chapter 19). This receptor is found mainly on T helper lymphocytes, which are therefore the major targets for this virus. Many parasitic helminths (worms) fasten to host tissues by way of suckers or hooks (see Chapter 6). Similarly, the protozoan *Giardia intestinalis* attaches to epithelial cells lining the intestine via an adhesive disc.

Biofilms

Some bacteria adhere to surfaces by secreting a sticky, gelatinous substance called a **glycocalyx** (or slime layer) (see Figure 10.4). The glycocalyx is usually a polymer of polysaccharides, proteins, nucleic acids and lipids. Microorganisms often produce a glycocalyx (also referred to as an extracellular polymeric substance) to form multilayered communities, called **biofilms**, which enable the community to adhere to surfaces. Biofilms may contain a single type of microorganism, but usually there is more than one species involved (sometimes bacteria and fungi together).

Apart from enabling microorganisms to adhere strongly to a surface and be difficult to remove, a biofilm provides a number of other important benefits to the microbes embedded in it. Due to their close proximity, cell-to-cell interactions including communication, or quorum sensing (see previous section), are facilitated. This allows the microbes to coordinate their activities in a variety of ways, such as sharing of nutrients or activation of virulence factors to produce disease. Also because of the close proximity of cells, biofilms provide an ideal opportunity for plasmid exchange between bacteria. As is discussed in Chapter 4, plasmids often contain genes for antibiotic resistance, so biofilms may facilitate the development of multiple drug resistance. Furthermore, the microorganisms are less susceptible to antibiotic therapy and disinfectants because of the diffusion barrier created by the polymer matrix. Also, the bacteria in biofilm infections are protected from host defences because the glycocalyx forms a physical barrier that defence cells have difficulty in penetrating. The moist, polymeric matrix also protects the community against desiccation.

Biofilms are now recognised as playing a critical role in many types of infections. They are particularly important in infections related to implanted medical devices, such as intravascular catheters, endotracheal tubes, urinary catheters and orthopaedic implants. The biofilm enables microbial cells to attach to these devices, facilitating their infection of associated tissues and posing real problems for treatment. Biofilms are also important in chronic wound infections, enabling the microorganisms to persist in the wound despite routine wound care practice and antibiotic therapy. Thus, procedures for biofilm-based wound care, aimed at disrupting the biofilm, have recently been developed. Dental plaque begins as a bacterial biofilm. *Streptococcus mutans* attaches to the tooth surface in a biofilm,and, by colonising this site and producing acids, contributes to plaque formation. Figure 10.5 shows a biofilm on the bristle of a used toothbrush.

TOXINS

Toxins are substances produced by some microorganisms that interfere with the normal functioning of host cells or tissues. They do this either by directly damaging cells or tissues, or by altering normal cellular processes. Not all microorganisms produce toxins, but the production and release of toxins are, for some, the most important determinants of their ability to damage tissues and thus cause disease. A loss of their ability to produce toxins results in their loss of pathogenicity. We know most about bacterial toxins, although we fully understand the action of only some of them. Some species of protozoa, fungi and helminths also produce toxins. Viruses do not produce toxins but cause cell damage by other means.

Bacterial toxins are classically divided into two broad groups:

1. **Exotoxins** are toxins secreted into the environment in which the bacteria are growing (e.g. host tissues, food, soil, water); their general characteristics are listed in Table 10.1.
2. **Endotoxins** are toxins that are part of the outer membrane of Gram-negative bacteria; they are not actively secreted but are released when the bacterial cell is damaged or dies.

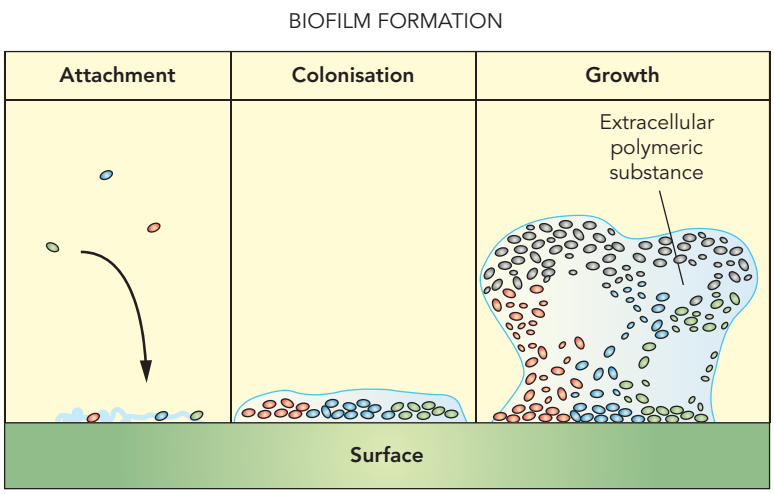

FIGURE 10.4

Biofilm formation

Source: Center for Biofilm Engineering, Peg Dirckx, <www.biofilm.montana.edu/resources/images/multicellularextracellular/biofilm-formation-3-steps.html>, Center for Biofilm Engineering, 2003.

FIGURE 10.5

Electron micrograph of a biofilm on a toothbrush bristle

Source: Steve Gschmeissner/Science Photo Library.

TABLE 10.1 **General characteristics of exotoxins**

1. Produced by some Gram-positive and Gram-negative bacteria.
2. Usually are proteins.
3. Secreted or released from the bacterial cell.
4. Each has a specific effect on host cells.
5. Moderate to high toxicity.
6. Elicit an antibody response in the host.
7. Many can be toxoided.

Exotoxins

Exotoxins are heat-labile proteins that are usually released as the organism grows, although in some spore-forming bacteria, like the clostridia, toxin release occurs as a by-product of spore formation. While many different forms of toxins exist, a general structure to which many conform is the AB toxin structure. In this *two-subunit* structure the B subunit is responsible for initial binding of the toxin to the target cell and the entry of the A subunit into the cell. The A subunit is responsible for cell damage by inhibition or alteration of a cellular function.

Some bacteria produce a single exotoxin, and the major manifestations of the disease are due to the action of that toxin. Others produce many toxins, which have a number of different effects on the host. The more common exotoxin-producing bacteria are listed in Table 10.2. Some exotoxins are extremely potent and include some of the strongest poisons known. One gram of botulinum toxin, for example, is sufficient to kill 10 million people, and less than 1 kilogram would be enough to kill the entire population of the world! The tetanus and *Shigella* toxins are also very potent.

Most bacteria secrete their toxins while growing in the host. But some, like *Clostridium botulinum* and *Staphylococcus aureus*, secrete their toxins into food and the toxin is ingested when a person eats the contaminated food. To produce disease, these organisms do not need to grow inside the host. In fact, the bacteria do not survive well in the body when ingested. Since the toxin is responsible for disease, and not the growth of the organism itself, this type of disease is often referred to as an **intoxication**, rather than infection.

Bacterial exotoxins vary greatly in their degree of specificity. Some affect a wide range of cells and tissues. Others have specific targets and are sometimes named according to the tissues they primarily affect. Two of the major types are the **neurotoxins**, which act predominantly on nervous tissue, and the **enterotoxins**, which act specifically on the small intestine. The term **toxaemia** refers to the presence of toxins in the bloodstream. In the following sections, we describe the major types of exotoxins, grouped according to their mode of action.

Toxins that break down cells

A large number of exotoxins act by damaging host cell membranes, resulting in lysis and death of the cells. Some of these toxins are phospholipases, like the α-toxin of *Clostridium perfringens*, a cause of gas gangrene. Phospholipase enzymes lyse cells by hydrolysing phospholipids in mammalian cell membranes. Other lytic toxins damage host cell membranes by inserting themselves into the cell membrane, creating pores or channels. The membrane becomes more permeable to water and, because of the higher solute concentration of the cytoplasm compared to the environment, water rushes into the cell, causing it to swell and rupture. The α-toxin of *Staphylococcus aureus* and the streptolysin-O of *Streptococcus pyogenes* are examples of pore-forming toxins.

Many phospholipase and pore-forming toxins damage red blood cells and are therefore referred to as **haemolysins**, although they can break down other cells as well. When bacteria that secrete haemolysins are grown on culture media containing blood (e.g. sheep blood agar), distinct zones of red cell clearing can be seen around the bacterial colonies. The soluble exotoxins diffuse into the agar and cause destruction of red cells around the colony (see Figure 10.6). This property is useful in the identification of some bacteria.

Some bacteria, such as *S. aureus* and *S. pyogenes*, secrete toxins called **leukocidins** that kill phagocytic leukocytes (neutrophils and macrophages). These leukocidins induce the release of the lysosomal enzymes into the cytosol of the white cell itself. These highly destructive enzymes are normally packaged up safely within the lysosomal membrane but, once released into the cytosol, cause lethal damage to the cell by breaking down cellular components. Destruction of phagocytic white cells in this way increases the ability of leukocidin-producing organisms to survive and persist

TABLE 10.2 Examples of diseases caused by exotoxins

ORGANISM AND DISEASE	EXOTOXIN	ACTION OF TOXIN	SYMPTOMS PRODUCED
Bacillus anthracis (anthrax)	cytotoxin	Increases vascular permeability	Pulmonary oedema
Bordetella pertussis (whooping cough)	pertussis toxin	Damages respiratory mucosal cells	Paroxysmal cough
Clostridium botulinum (botulism)	neurotoxin	Blocks acetylcholine release at neuromuscular junctions	Flaccid paralysis
Clostridium difficile (antibiotic associated diarrhoea)	enterotoxin cytotoxin	Causes loss of fluid and electrolytes from intestinal cells Destruction of intestinal mucosa	Diarrhoea, pseudomembranous colitis
Clostridium perfringens (gas gangrene, food poisoning)	α-toxin enterotoxin	Lysis of various cell types Causes excessive loss of fluid and electrolytes from intestinal cells	Tissue destruction, gas gangrene Diarrhoea
Clostridium tetani (tetanus)	tetanus toxin	Interferes with transmission in inhibitory interneurones	Severe muscle spasm
Corynebacterium diphtheria (diphtheria)	diphtheria toxin	Damages many cell types by stopping protein synthesis	Ulceration of throat Heart and nerve tissue damage
Entamoeba histolytica (amoebic dysentery)	enterotoxin	Lysis of epithelial cells lining the colon	Bloody, mucoid diarrhoea
Escherichia coli (gastroenteritis)	enterotoxin	Causes loss of fluid and electrolytes from intestinal cells	Diarrhoea
Listeria monocytogenes (listeriosis)	listeriolysin	Lysis of many cell types	Fever, muscle aches, diarrhoea, shock
Pseudomonas aeruginosa (various infections)	exotoxin A	Inhibits protein synthesis in cells	Burns, wound infections, urinary tract infections, septicaemia
Shigella dysenteriae (dysentery)	shiga toxin	Inhibits protein synthesis in many cell types	Bloody diarrhoea
Staphylococcus aureus (various infections)	α-toxin leukocidin enterotoxin exfoliatin TSST-1	Lysis of red and white cells Kills white cells Stimulates intestinal cells Unclear Stimulates T cells to release cytokines	Infection and abscess Infection and abscess Vomiting, diarrhoea Scalded skin syndrome Toxic shock syndrome
Streptococcus pyogenes (scarlet fever and other infections)	pyrogenic exotoxin streptolysin O and S	Vasodilation of skin capillaries Lysis of red and white cells	Diffuse rash and fever Tissue damage
Vibrio cholera (cholera)	cholera toxin	Causes severe loss of fluid and electrolytes from intestinal cells	Profuse diarrhoea

longer in the body. In addition, release of the lysosomal enzymes from dead phagocytes can cause further damage to the surrounding tissue.

Shiga toxins (Stx1 and Stx2) are potent cytotoxins with an AB subunit structure, produced by *Shigella dysenteriae*, *Shigella flexneri* and some strains of *Escherichia coli*. They inhibit protein synthesis and cause cell death in intestinal cells, resulting in dysentery (inflammatory diarrhoea with blood and pus). Shiga toxins can also attack glomerular endothelial cells in the kidney, resulting in haemolytic uraemic syndrome (HUS), a serious, sometimes fatal disease characterised by progressive renal failure. Haemolytic uraemic syndrome (HUS) is the most common cause of acute renal failure in children.

Toxins that enhance microbial spread or survival in tissues

Many bacteria secrete enzymes that break down tissues. These enzymes increase the *invasiveness* of bacteria by breaking down substances that would otherwise impede their spread through tissues. **Hyaluronidases** are produced by many bacteria, including staphylococci, streptococci and clostridia. These enzymes break down hyaluronic acid, a component of the ground substance of connective tissue, making the tissue less viscous and easier to penetrate. **Collagenases** are enzymes that break down collagen, the tough fibres that give strength and a supporting network to connective tissues. *C. perfringens* and some helminths are examples of

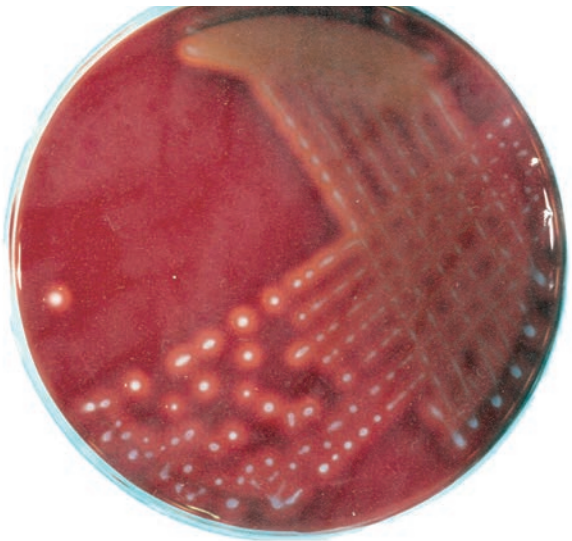

FIGURE 10.6

Bacterial haemolysis

Clearing or complete haemolysis of blood in the agar caused by colonies of Streptococcus pyogenes.

Source: G. Jayachandran, Sydney Medical School, University of Sydney.

organisms that secrete collagenases. *Pseudomonas aeruginosa* secretes **elastases**, which break down elastin in the extracellular matrix of lung tissue. These enzymes are thought to be critical for the organism's ability to invade and spread through the lungs. Some bacteria, such as some strains of *S. pyogenes*, cause so much tissue destruction with proteases and other enzymes that they are sometimes referred to as 'flesh eating bacteria'.

Skin is a highly protective coating against microbial infection, due mainly to the presence of the relatively indigestible protein called *keratin*. However, the fungi that cause tinea (ringworm) are able to infect skin because they secrete a keratin-degrading enzyme, or **keratinase**. Some organisms (e.g. *Vibrio cholerae*) secrete a **mucinase** which digests a glycoprotein in mucus, thereby enhancing their ability to colonise mucous membranes.

The body's defence against local infection sometimes involves the deposition of a layer of fibrin around the infected site. This is designed to wall off the infection, preventing the spread of microorganisms into adjacent tissue. **Kinases** are a group of enzymes produced by certain bacteria that help them to dissolve fibrin clots and hence break through this barrier. **Streptokinase** is produced by some streptococci, including *S. pyogenes*, and **staphylokinase** is produced by *S. aureus*. Purified streptokinase is in fact used therapeutically as a thrombolytic agent that breaks down blood clots in patients with thromboses.

Coagulase is an enzyme produced by some staphylococci that has almost the opposite effect to kinases. This substance coagulates fibrinogen to form a deposit of fibrin material around the bacterial cells, and this is thought to offer them some protection against host phagocytic cells. The fibrin matrix may also account for the highly localised nature of *S. aureus* infections. The pathogenicity of staphylococci depends largely on their ability to produce coagulase. Some coagulase-negative staphylococci are pathogenic, but generally they are less frequently associated with disease than the coagulase-positive species.

CASE HISTORY 10.1

Staphylococcus aureus cellulitis

A previously healthy 5-year-old girl was diagnosed as having chickenpox by her family GP. Three days later her mother, who is a part-time physiotherapist at the local hospital, took her to the Emergency Department of the hospital after her left upper arm had become swollen, tender and red. Purulent skin lesions were not present. The girl was admitted to the hospital with a diagnosis of cellulitis and received intravenous clindamycin, but her symptoms did not improve. A skin biopsy was taken and sent to the microbiology laboratory, and ultimately grew methicillin-resistant *S. aureus* (MRSA). She was then treated successfully with vancomycin.

Questions

1. What attributes of *S. aureus* enable it to infect deeper tissues?
2. What attributes of *S. aureus* enable it to evade host defence mechanisms?
3. Why was clindamycin ineffective in this case?
4. How could this girl have acquired the *S. aureus* cellulitis?

Toxins that interfere with cellular functions

There are several toxins that enter host cells and alter the cell's metabolism in some way. The toxin of *Corynebacterium diphtheriae* is one of the best understood of these. The toxin first binds to a receptor on the host cell membrane and then enters the cell by an endocytotic process. The toxin blocks protein synthesis by inactivating elongation factor 2, an enzyme necessary for the growth of the polypeptide chain in translation. The cell dies because it can no longer synthesise proteins. The pathogenicity of *C. diphtheriae* is entirely due to this toxin; strains that do not have the gene for toxin production are non-pathogenic.

Although *C. diphtheriae* remains localised in the throat during the infection, the secreted toxin is absorbed and enters the bloodstream. The actions of the toxin in the throat cause considerable damage to mucosal cells, resulting in a membrane (called a pseudomembrane) over the mucosal surface, consisting of fibrin, bacteria and inflammatory cells. When the larynx is involved, a life-threatening obstruction of the airway can occur (see Chapter 17). The action of diphtheria toxin on other organs, especially the heart, can result in irregular heartbeat, coma and death.

The exotoxin A of *Pseudomonas aeruginosa* appears to have a similar action on protein synthesis to that of diphtheria toxin.

The enterotoxin of *Vibrio cholerae*, the causative agent of cholera, alters the regulatory control in cells, rather than directly damaging them. Cholera toxin binds specifically to intestinal epithelial cells. It then enters the cell and causes increased production of cyclic AMP, the mediator of a number of regulatory systems in cells. The increased levels of cyclic AMP cause uncontrolled secretion of chloride ions from the epithelial cells. This change in ionic balance results in a massive outflow of water from the cells into the lumen of the intestine, manifested as a profuse diarrhoea (see Figure 10.7).

Cholera victims can lose so much water that they may die from extreme dehydration if their fluids and electrolytes are not replaced. The toxins of enteropathogenic *Escherichia coli* and *Salmonella* are believed to have a similar action.

Toxins that affect the nervous system

The neurotoxins of the anaerobic bacteria *Clostridium tetani* and *Clostridium botulinum* are two of the most potent toxins known. *Clostridium tetani* usually grows in deep, necrotic puncture wounds where anaerobic conditions exist. The bacterium itself does not spread far from the initial site of infection, but the exotoxin that it secretes travels to the central nervous system to cause severe neuromuscular dysfunction.

C. tetani is a good example of an organism that produces only a single toxin. The toxin, called *tetanospasmin*, binds to the surface of nerve cells, is internalised and then transported to the spinal cord, where it enters central inhibitory neurons. It interferes specifically with neurotransmitter release from the inhibitory neurons, creating an imbalance in the excitatory and inhibitory transmissions to motor neurons. This causes an excessive stimulation of muscles, resulting in an uncontrolled, rigid muscle contraction, or spastic paralysis. Different muscles may be affected, such as the powerful masseter muscles of the jaw, resulting in the classic condition of tetanus known as *lockjaw*. Often, back muscles are affected, causing arching of the back and sometimes even crushing of spinal processes in severe cases (see Figure 20.9, page 524 in Chapter 20). Severe contraction of respiratory muscles may lead to asphyxiation and death.

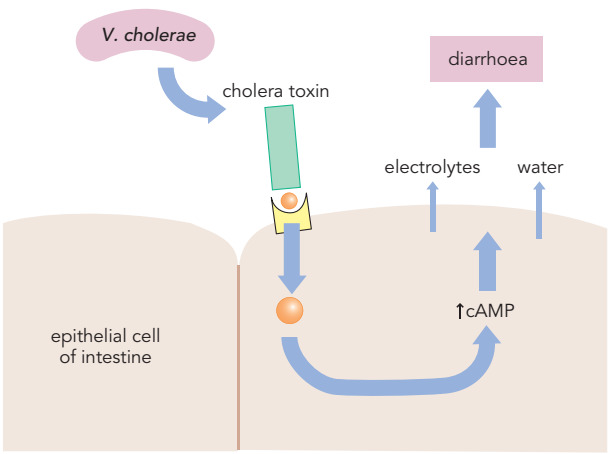

FIGURE 10.7

The mode of action of cholera toxin

The toxin increases cyclic AMP production in epithelial cells of the intestine. This results in an outflow of water and ions from these cells, producing diarrhoea.

Botulism occurs when the preformed toxin of *C. botulinum* is ingested in contaminated food. The organism itself normally causes no problem if ingested because it is unable to compete successfully with the normal gut flora. Botulism in most people is therefore an intoxication, caused by toxins produced outside the body, rather than an infectious disease. The botulinum toxin is resistant to gastrointestinal digestion. After ingestion it is absorbed in the stomach and small intestine and ultimately localises at neuromuscular junctions.

Infant botulism is different in that growth of the clostridia and production of botulinum toxin occur in the colon. In this disease the clostridia are able to grow and produce toxin because the infant colon has a poorly developed microbial flora which is unable to inhibit the pathogen.

In contrast to tetanus toxin, botulinum toxin acts on peripheral nerve endings. At neuromuscular junctions the toxin binds to presynaptic terminal membranes and inhibits the release of acetylcholine, preventing transmission of impulses from the nerve cell to the muscle cell, and hence markedly reducing muscle contraction (see Figure 10.8). This flaccid paralysis in botulism can lead to the death of the patient, usually due to dysfunction of the respiratory muscles and respiratory failure.

Some strains of *Staphylococcus aureus* can produce enterotoxins in foods they contaminate. After the toxins are ingested they are absorbed in the intestine and then stimulate neural receptors that activate the vomiting centre in the central nervous system. Projectile vomiting can result.

Toxins that act as superantigens

Some microorganisms produce toxins that non-specifically and indiscriminately activate large numbers of T cells.

These substances are called **superantigens**. The activation of large numbers of T cells is believed to result in a massive release of cytokines (including interleukin-1 and tumour necrosis factor) that can cause injury to host tissues and septic shock, as well as suppressing normal immune responses. Enterotoxins of some strains of *S. aureus*, the toxic shock syndrome toxin-1 (TSST-1) of other strains of *S. aureus* and some exotoxins of *Streptococcus pyogenes* are examples of superantigens.

Toxoids and antitoxins

Exotoxins are proteins and, as such, are usually antigenic; that is, they are capable of stimulating the production of antibodies by the immune system (see Chapter 9). An **antitoxin** is a specific antibody produced against a toxin; it is able to bind to the toxin and neutralise it, thereby preventing it from binding to its target cell or tissue. Exotoxins are thus effective immunogens, but of course cannot be used in their natural form in vaccines. Fortunately, many exotoxins can

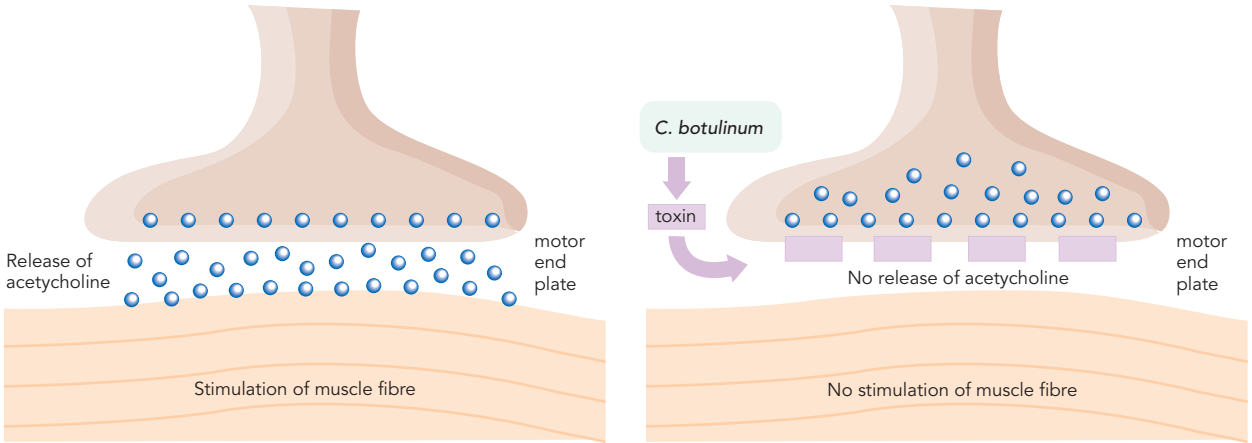

FIGURE 10.8

The mode of action of botulinum toxin

The toxin blocks the release of acetylcholine at the neuromuscular junction, causing a flaccid paralysis.

be modified by heat or chemicals, such as formaldehyde or phenol, to remove their toxicity but still retain their ability to elicit an immune response.

Toxins modified in this way are called **toxoids** and are used in vaccines to stimulate immunity without causing disease. Toxoids are particularly useful in vaccines when the toxin is the major (or only) virulence factor of the organism. Toxoids for diphtheria and tetanus are used in the combined diphtheria-pertussis-tetanus (DPT) vaccine.

The treatment of diseases caused by exotoxin-producing bacteria sometimes involves the use of antitoxins to neutralise the toxin already in the body. Antitoxins are usually derived from the serum of immune humans or animals. They are particularly important for diseases such as tetanus and botulism, which can have a rapidly fatal outcome. Injection of specific antitoxin (also called **specific immunoglobulin**) is aimed at immediate neutralisation of the toxin before it is able to bind to target cells. Without antitoxin, patients may die because their immune system does not have sufficient time to make the required antibodies.

Endotoxins

Endotoxins are the lipopolysaccharides (LPS) of the cell walls of Gram-negative bacteria. As described in Chapter 4, the outer membrane of the Gram-negative cell wall contains LPS. The lipid A portion of LPS is the actively toxic component. Being a part of the bacterial cell wall, endotoxins only exert their effect once the cell wall breaks down. During infection, small amounts of LPS may be sloughed off as cells grow or may be released as older bacterial cells die. Death of bacteria and release of endotoxin can be greatly accelerated once phagocytes begin to digest them or once antibiotics that kill the bacteria are administered. Thus, in some cases, antibiotics may initially increase LPS release and thus cause a temporary worsening of symptoms.

Other major differences between exotoxins and endotoxins are that the latter are generally less toxic, relatively heat-stable and cannot be made into toxoids (see previous section). Although endotoxins can induce an immune response, they cannot be treated in a similar way to exotoxins to render them non-toxic while still preserving their ability to induce antibody formation.

Endotoxin, regardless of its source, produces similar signs and symptoms in the host. It causes a variety of host responses, many of which involve some part of the immune system. Fever is one of the more common symptoms produced. Endotoxin causes fever by stimulating the release of endogenous pyrogens from mononuclear phagocytes. Currently, the best known of these pyrogens are the cytokines

CASE HISTORY 10.2

Diphtheria

A 55-year-old woman saw her local doctor about a sore throat and cough. Based on her symptoms, the presence of a greyish membrane over her pharynx, and the fact that she had recently returned from a holiday in Eastern Europe, the doctor suspected that she had diphtheria and took a swab for laboratory diagnosis. The doctor gave her a dose of diphtheria antitoxin and a prescription for a course of penicillin.

Questions

1. How is the clinical diagnosis of diphtheria confirmed? (See Chapter 17.)
2. What is the rationale for giving the woman the antitoxin?
3. Would a dose of diphtheria toxoid have been just as effective as the antitoxin?
4. What is the rationale for giving the woman the penicillin?

interleukin-1 (IL-1) and tumour necrosis factor (TNF) (see Chapter 9). These act on the temperature-regulating centre in the hypothalamus to reset the body temperature set-point (or thermostat) to a higher level, resulting in an increase in body temperature. Other possible responses of the host to endotoxin include muscle weakness, generalised aches, diarrhoea and malaise.

Large amounts of endotoxin may be released during systemic infection caused by Gram-negative bacteria such as *E. coli*, *Neisseria meningitidis* and *Pseudomonas aeruginosa*. Severe infection with one of these bacteria can result in **septic** (or **endotoxic**) **shock**, which is frequently fatal. In this situation, endotoxin is believed to induce the release of cytokines from a variety of cells, including monocytes, macrophages and lymphocytes. The systemic release of large amounts of cytokines causes damage to blood capillaries and alters their permeability characteristics. Large amounts of fluid leak into the tissues, causing a severe drop in blood pressure, resulting in shock. The complement system is activated (see Chapter 9), causing further inflammation.

Endotoxin may also cause a non-specific activation of blood-coagulation mechanisms, resulting in the blockage of small vessels by thrombi in organs such as the kidney, brain and lungs. Tissue necrosis in the blood-deprived areas of these organs (infarction) may then occur. This potentially fatal disorder is referred to as **disseminated intravascular coagulation**. This is an important contributor to the fatal outcomes of some infections, such as meningococcal disease.

INVASION OF CELLS

Viruses typically cause disease by invading and damaging host cells. As explained in Chapter 5, viruses are obligate intracellular parasites and are able to replicate only inside their specific target cells. Once inside the cell a virus can cause changes to the cell, called **cytopathic effects** (**CPE**). Different viruses cause different CPE in cells, and some of these can be observed by microscopic examination of the viruses grown in tissue culture (see Figure 10.9). Some viruses kill the cells they infect and are said to be *cytocidal*. They can do this by:

- diverting the cell's metabolic processes and stopping the synthesis of proteins or other macromolecules
- causing the release of lysosomal enzymes into the cytoplasm of the cell
- inserting viral proteins into the membrane of the cell during the release phase, resulting in rupture of the membrane and cell lysis.

Even if the virus does not directly kill the cell, it takes control of its regulatory mechanisms and produces viral proteins and nucleic acids, usually causing the cell to become dysfunctional. Some non-cytocidal viruses cause CPE characterised by the formation of inclusion bodies within the cell, such as the Negri bodies found in the brain cells of humans and animals infected with the rabies virus. These inclusion bodies are granules of unassembled viral components or deposits of viral remnants.

Other viruses cause host cells to fuse with each other, producing multi-nucleated 'giant cells' (e.g. measles virus and respiratory syncytial virus). Eventually, the accumulated damage to the cell results in its death. However, some viruses, like the herpes simplex viruses, do not kill the cell; instead they remain dormant inside the cell for long periods, sometimes for life. These highly adapted parasites cause minimal damage to the nerve cells they infect, enabling them to remain there for years. Sometimes the viruses are reactivated many years later and cause symptomatic disease (e.g. the herpes zoster virus and shingles).

Another important outcome of some viral infections is transformation of normal cells into cancer cells. This is discussed in the following section.

A few other organisms, apart from viruses, also invade cells and damage them. For example, chlamydia are bacteria that are obligate intracellular parasites. They enter epithelial cells and multiply quickly, lysing these cells within a couple of days. Rickettsia are also obligate intracellular bacteria

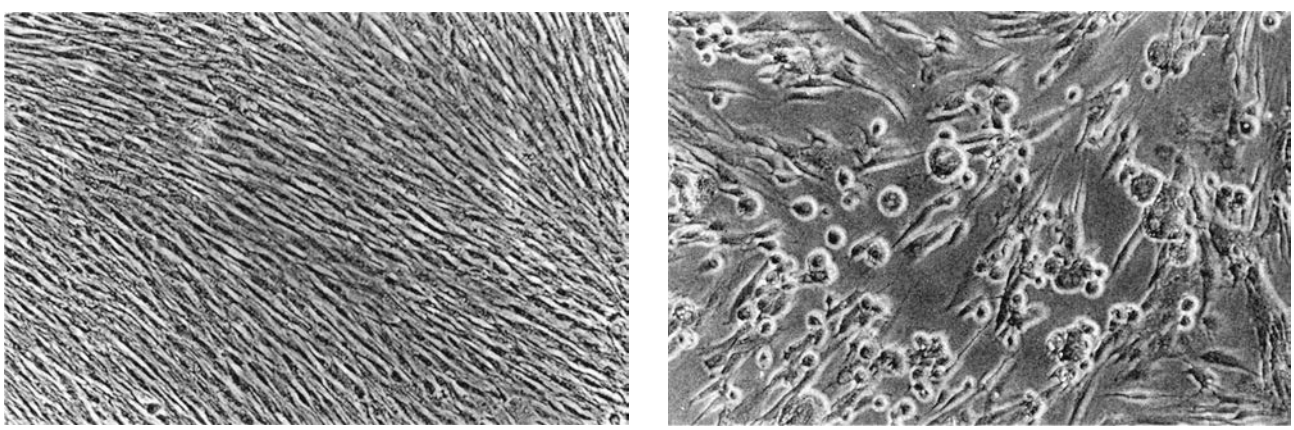

FIGURE 10.9

Cytopathic effects caused by viruses

(left) Uninfected fibroblasts; and (right) the same cells after being infected with herpes virus.

Source: Dr Penny Bishop.

which can invade many types of cells, but especially vascular endothelium. They grow slowly in these cells, eventually breaking them open to release large numbers of organisms. *Plasmodium* spp., the causative agents of malaria, typically go through cycles of red blood cell invasion, multiplication, lysis of the red cells with release of large numbers of the merozoite form into the bloodstream, and then infection of many more red cells.

CELLULAR TRANSFORMATION INTO TUMOUR CELLS

Some viruses cause disease by transforming host cells into tumour cells. Transformed cells differ markedly from normal cells in a number of ways. Typically, they have an altered morphology and do not have an ordered pattern of growth. Instead, they divide in an unregulated manner and form random aggregations of dysfunctional cells. Some human papillomaviruses (e.g. HPV 1, 2, 3 and 10) cause cutaneous warts, which are benign tumours. Other HPV viruses are believed to be involved in malignant tumours, such as types 16, 18, 31 and 33 in cervical cancer. Hepatitis B and C viruses can cause hepatocellular carcinoma.

Transformation of cells is brought about by the incorporation of viral nucleic acid into the host cell genome, which is then replicated along with the cell's DNA. The tumour-causing viruses and their relationship to oncogenes are described in Chapter 5.

OTHER MECHANISMS OF PATHOGENESIS

Microorganisms can cause disease in a variety of other ways. Sometimes, the clinical features of a disease arise from the physiological responses of the host to infection. For example, if the invading organism causes the host's immune response to be 'overreactive', tissue damage may occur. This over-reactivity of the immune system is referred to as **hypersensitivity**, and is the basis of many diseases. For example, many pathogenic helminths release waste products during infection that induce allergic reactions. The bases of hypersensitivity reactions are described in Chapter 9. Examples of hypersensitivity reactions induced by microorganisms are listed in Table 10.3.

Another way in which microorganisms can cause damage is by inducing autoimmunity. If microbes can confuse the immune system so that self-tissues appear foreign to it, then the immune system may attack those tissues and produce an autoimmune disease. Some strains of *Streptococcus pyogenes* have antigens that cross-react with heart muscle and can cause a type of autoimmune disease called rheumatic fever (see Chapter 19).

Microorganisms may also cause disease by physical means. For example, a mass of *Ascaris* worms in the intestinal tract may cause obstruction. The larvae of a number of pathogenic helminths cause damage by blocking the flow of blood in small vessels, thus reducing the amount of oxygen and other nutrients to the adjacent tissues. In some diseases, other narrow, tubular structures such as lymph vessels may be obstructed, such as occurs in elephantiasis, caused by the nematode *Wuchereira*.

TABLE 10.3 Hypersensitivity reactions induced by microorganisms

TYPE OF HYPERSENSITIVITY INDUCED	EXAMPLES
Allergy/anaphylaxis	Some helminths, e.g. *Ascaris*
Cytotoxic hypersensitivity	Hepatitis B
Immune complex disease	Malaria
	Acute glomerulonephritis (*Streptococcus pyogenes*)
	Syphilis
	Allergic alveolitis
Cell-mediated hypersensitivity	Tuberculosis
	Leprosy
	Tertiary syphilis
	Viral rashes

Clostridium perfringens, the major cause of gas gangrene, produces gas in the tissues where it is growing. The gas causes compression of the tissue and blood vessels in the area. The resultant lack of oxygen in the site allows further growth of this anaerobic bacterium, causing more tissue necrosis. These physical effects may then be aggravated by the body's inflammatory response to infection—in particular, the exudation of fluid from the blood into the injured tissue. This fluid may compress the tissue further, causing more damage.

EVASION STRATEGIES

When a microorganism invades the body it usually faces a hostile response from the host. The human body has developed numerous mechanisms by which it defends itself against microbial invasion and these are mobilised when organisms gain entry. However, many microbes are known to possess special structures or attributes that protect them from the host's defences. Although these microbial countermeasures do not directly cause tissue damage, some are largely responsible for the organism's pathogenicity, or capacity to cause disease.

Escape from phagocytes

Phagocytes are vital components of the body's defence system. They are responsible for scouring the body's tissues, searching for foreign substances and microbes, which they engulf and destroy. Their critical role in defence is described in Chapter 9. Many microorganisms have developed ways of escaping phagocytosis by blocking or avoiding some part of the phagocytic process. Indeed, the large variety of ways that microbes have developed to avoid being phagocytosed reflects the importance of phagocytosis in defending the body.

You may recall from Chapter 9 that phagocytosis involves:

1. attraction (chemotaxis) of phagocytes to the site of infection
2. binding of the phagocyte to the microbe, followed by ingestion and containment of it within a membrane-bound vesicle called a phagosome
3. fusion of lysosomes with the phagosome to form a phagolysosome
4. destruction of the microbe by a variety of toxic substances and degradative lysosomal enzymes.

Avoidance of, or interference with, one of these steps can allow a microbe to escape destruction by these cells.

A few bacteria, such as *Bordetella pertussis*, the cause of whooping cough, produce factors that inhibit leucocyte chemotaxis. Some organisms possess capsules or a surface slime layer (see Chapter 4) that prevent the phagocytes from binding to and ingesting them. *Streptococcus pneumoniae* appears to lack any major virulence factor other than its capsule, but this enables it to avoid being ingested by alveolar macrophages, and thus to survive and multiply in the lungs. Mutant strains of the organism that do not have a capsule are unable to cause disease.

Other organisms that rely on an antiphagocytic capsule for pathogenicity include *Haemophilus influenzae*, *Neisseria meningitidis*, *Yersinia pestis* and *Cryptococcus neoformans*, the yeast that causes meningitis.

Instead of a capsule, *Streptococcus pyogenes* has a cell wall component, termed an M-protein, which helps it to avoid ingestion by phagocytes by interfering with complement-mediated opsonisation (see Chapter 9).

Some microorganisms allow themselves to be ingested by phagocytes but have developed ways of surviving inside them. Not only does this allow them to avoid destruction, but the phagocytes provide them with a place to hide and multiply and may even help them to spread through the body. Some organisms, such as *Listeria monocytogenes* and *Mycobacterium leprae*, appear to avoid being killed by exiting the phagosome and entering the cytoplasm of the phagocyte. This is a privileged site since lysosomes do not normally release their enzymes and toxins into the cytoplasm.

Other organisms, such as *Mycobacterium tuberculosis* and *Legionella pneumophila*, avoid intracellular digestion by inhibiting the fusion of lysosomes with the phagosome. The protozoan, *Toxoplasma gondii*, actively attaches to macrophages, inducing its own phagocytosis. It then prevents lysosomal fusion and multiplies within the cell, eventually killing it. *Staphylococcus aureus* resists intracellular killing by producing the enzyme **catalase**, which breaks down hydrogen peroxide, one of the substances phagocytes produce to kill microbes.

Still other organisms, such as the protozoa *Leishmania*, are innately resistant to lysosomal enzymes and can survive in the phagolysosome. These organisms probably have resistant cell surface structures and/or secrete substances that inhibit the lysosomal enzymes.

The most aggressive strategy is to kill the phagocyte. As stated earlier in this chapter, some bacteria, such as strains of staphylococci and streptococci, secrete toxins called leukocidins, which kill phagocytes as well as other cells.

Concealment

Some microorganisms evade the host defences by hiding, usually inside cells or in other so-called privileged sites, where defence cells do not normally circulate. All pathogenic viruses and some bacteria penetrate and grow inside of host cells. If a microorganism can remain inside a cell, without any sign of its antigens on the surface of the cell, it will be invisible to defence cells. Even if specific immune responses are induced before the organism invades the cells, once inside, the antibodies or activated immune cells would be ineffective against these hidden pathogens. Fortunately, in most cases, antigens of the microbe are displayed on the cell surface after it infects the cell. This allows components of the immune system (especially cytotoxic T cells and natural killer cells—see Chapter 9) to recognise the infected cells and destroy them, thereby exposing the microbes to other immune factors.

CASE HISTORY 10.3

Shingles

A 67-year-old man develops a painful rash on his face which his doctor diagnoses as shingles. The doctor advises him that this infection is related to the man's bout of chickenpox when he was a child.

Questions

1. What virus causes chickenpox?
2. What virus causes shingles?
3. How could the man's shingles be related to the chickenpox he had as a child?

In latent viral infections, however, the viruses inhibit the display of their antigens on the infected cell surface. The herpes simplex and varicella zoster viruses can remain in this way in sensory neurons for years, sometimes for a lifetime, despite the presence of specific immunity against them. Retroviruses, like the human immunodeficiency virus (HIV), can also remain hidden in lymphoid cells or macrophages by first having their RNA transcribed into DNA and then having this integrated into the cell's DNA. Again, as long as there is no display of viral antigens on the surface of the cell, the virus remains undetected by the immune system.

The cytomegalovirus protects itself from natural killer cells by displaying an imitation of host self-recognition antigens on the surface of the host cells it infects. Malaria parasites spend some of their time inside liver cells (see Chapter 19). During this latent period they avoid presenting a target for the immune system.

Other privileged sites for microorganisms include those areas of the body where complement, antibodies and lymphocyte activity are low. A good example is the central nervous system (CNS). Complement and antibody levels are very low in cerebrospinal fluid (CSF), and there are few white cells. Even though white cells may enter the CNS during infection, their activity appears to be limited by the lack of opsonic antibodies and complement proteins. Other such privileged sites include the joints and testes.

Some microorganisms are able to create their own privileged sites. Hydatid disease is caused by *Echinococcus granulosis*, a tiny tapeworm that can infect the liver, lung or brain. This parasite evades host defences by forming and residing within thin-walled capsules that resemble small bladders (see Figure 10.10). The worms can survive inside these fluid-filled cysts, even if there are protective antibodies in the blood of the patient. A connective tissue coating of host origin around the cyst prevents it from being attacked by host defences.

The blood fluke, *Schistosoma*, conceals itself by becoming coated with plasma glycoproteins and glycolipids. These host substances are not recognised as foreign and the worm escapes detection. *Toxoplasma gondii* forms cysts within macrophages of the nervous system and lungs. Within these cysts the protozoan avoids stimulating the immune system.

FIGURE 10.10

Hydatid cysts

Hydatid cysts are thin, fluid-filled sacs produced by the tapeworm Echinococcus granulosis. *The worms hide from the body's defences by residing inside cysts.*

Source: Audio Visual Services, Royal Prince Alfred Hospital, Sydney.

Antigenic variation

Antigenic variation is a phenomenon in which some bacteria, viruses and protozoa repeatedly or progressively change their cell surface components, or antigens. If the microbe changes the antigens to which immune responses have been developed, it will be unaffected by any immunity directed at those original antigens. Microorganisms contain numerous antigens, but immunity to only some of these is protective. That is, the strategy of changing antigens to escape host defences depends on changes being made to critical antigens against which protective immune responses are directed. Antigenic variation can occur during the course of infection, enabling the organism to persist longer, or it may occur as the organism spreads through the community, allowing it to reinfect the same individual over time (see Figure 10.11). The ways in which microbial antigens can be changed are antigenic drift, antigenic shift and gene switching.

Antigenic drift is the term used for repeated minor mutations in the genes that code for antigens of microorganisms. The antigens are sufficiently critical, and the mutations change them sufficiently, to reduce the effectiveness of prior immunological memory, allowing the organism to cause infection again, despite immunity in the host to a prior infection. The influenza virus is the classic example of this. The tendency for influenza to occur repeatedly in a population and in an individual is due partly to the ability of influenza viruses to undergo antigenic drift. The influenza viruses have two surface glycoprotein antigens (the haemagglutinin and

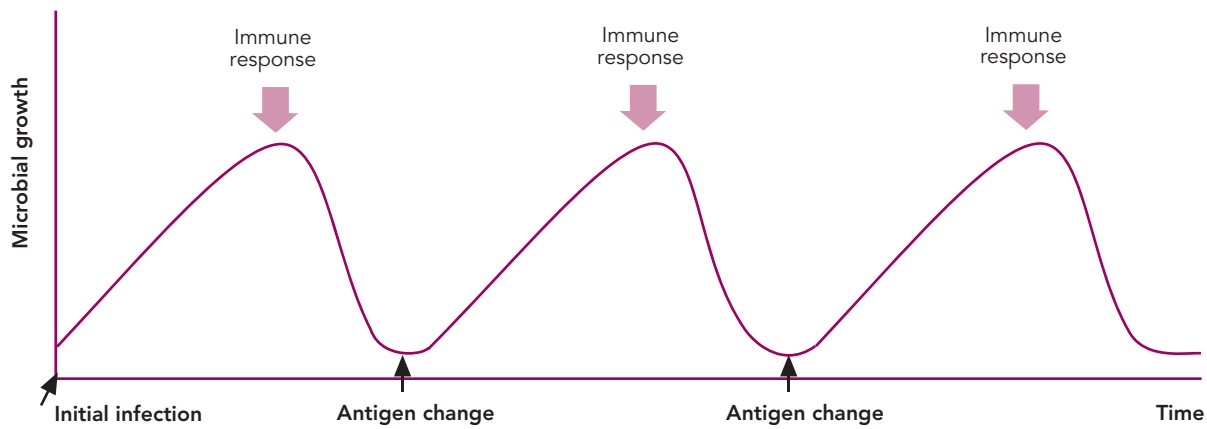

FIGURE 10.11

Antigenic variation

By repeatedly changing its critical antigens, an organism is not affected by prior immune responses to it, thereby evading the host's immune system.

neuraminidase 'spikes'). Antigenic drift occurs when minor mutations in DNA result in small changes in the amino acid sequence of these antigens. If the changes occur in areas where antibodies bind to the antigen, the virus is not susceptible to antibodies produced in previous infections. The cold viruses (rhinoviruses) have a similar ability. We can thus have the flu or a cold year after year, partly because of antigenic changes to the viruses that cause them.

The HIV is also capable of antigenic drift while it is infecting a person. This is thought to occur partly because the infection persists for so long, and partly because the viral reverse transcriptase is prone to making mistakes in transcribing RNA into DNA. This antigenic drift may be part of the reason why the immune system is so ineffective in controlling this infection.

Antigenic shift is displayed by the influenza A virus. When two different strains of this virus infect the same cell, a recombination of the genes that code for the surface glycoproteins can occur. This results in a new strain, different from previous strains, which can spread through a population because of the lack of protective antibodies in the population. Major pandemics of influenza in 1957, 1968 and 1977 are thought to have been due to the sudden appearance of a new strain of influenza A, as a result of antigenic shift. In 2009–10, an H1N1 influenza (swine flu) pandemic occurred, affecting more than 214 countries and causing over 18 000 deaths. This 'new' virus is thought to be the result of a re-assortment of genes from swine influenza viruses, avian influenza viruses and human influenza viruses (see Chapter 17). Since 2002 there has been widespread concern about another possible influenza pandemic, due to the highly pathogenic avian influenza virus H5N1. See Chapter 17 for a detailed description of this virus and the threat it still holds for humans worldwide.

Another form of antigenic variation results from the ability of some microorganisms to switch the genes (gene switching) that code for certain surface antigens. The best-known example of this occurs in the protozoa that cause African sleeping sickness, the *Trypanosoma*. These organisms are covered with a thick coat made of proteins called variable surface glycoproteins. These protozoa carry genes for hundreds of distinct surface glycoproteins, but only one is expressed at any given time. Periodic switching of these genes (e.g. at weekly intervals) during the course of infection results in new antigenic forms being produced during infection. By the time antibodies are made to a given antigenic type, the gene may have been switched and a new antigenic type has appeared.

Some bacteria are also capable of switching their surface antigens, in a process called phase variation. Bacteria such as *Neisseria* spp., *Haemophilus influenzae* and *Helicobacter pylori* are able to frequently and reversibly switch on and off expression of cell surface components. Outer membrane proteins, pili and capsules can be varied, increasing the antigenic variability and, hence, virulence of the bacterium.

Immunosuppression

A number of microorganisms cause a depletion or reduction in the defences of the host. As a consequence, the immune response to the antigens of the microbe (and sometimes other unrelated antigens) may be depressed, resulting in little or no antibody production or cellular immunity (see Chapter 9) to these antigens. The human immunodeficiency virus (HIV) is the most dramatic of microbial immunosuppressors, in fact deriving its name from this activity.

The HIV suppresses the immune system in a number of ways. Much of it is believed to be due to infection, killing and depletion of T4 lymphocytes, which play a central role in immune responses. This greatly reduces the host's ability to produce adequate amounts of antibody, activated T cells or cytokines (see Chapter 9). However, the virus is also believed to cause damage to the immune system in other ways, including:

- prevention of T4 cells responding to antigens by blocking the cell receptor sites
- infection of lymph nodes, leading to their damage or destruction
- infection of other protective cells bearing the CD4 receptor, particularly macrophages and glial cells in the brain
- the initiation of autoimmune responses to T4 cells and other cells bearing the CD4 receptor.

The end result of this immunosuppression is that patients in the terminal stages of HIV infection are incapable of mounting an immune response to many infections and cancers that are normally readily dealt with by the immune system. AIDS patients may then die from one of these secondary diseases.

Other microbes do not have such a dramatic and devastating effect on the immune system, but nevertheless can cause some degree of immunosuppression. For example, *Staphylococcus aureus* seems to interfere with immune defences by secreting toxins that are potent T cell mitogens (stimulators). These cause an uncontrolled and excessive activation of T lymphocytes, thus diverting the immune system to unproductive activity. Other microbes, such as the Epstein-Barr virus, may cause a similar diversion of the immune system by polyclonal activation of B lymphocytes, resulting in the production of large amounts of irrelevant antibodies.

Some organisms release substances that interfere with immunologically important molecules. For instance, many pathogenic strains of *Neisseria gonorrhoeae*, *Streptococcus pneumoniae* and *Haemophilus influenzae* secrete a protease that cleaves the IgA molecule, the major antibody type in mucosal secretions. This presumably facilitates the persistence of these bacteria on mucous membranes even if specific antibodies of this class are produced. Strains of *Pseudomonas* secrete enzymes that inactivate components of the complement system. This is thought to inhibit the critical role that complement plays in opsonising (enhancing the phagocytosis of) foreign particles (see Chapter 9).

Non-induction of an immune response

There is an intriguing group of diseases called *transmissible spongiform encephalopathies*, or *prion* diseases, that result from an accumulation of an abnormal form of a protein in

brain cells (see Chapter 20). They are neurodegenerative diseases and are nearly always fatal. One of the many unusual features of these diseases is that they do not induce any perceptible inflammation or immune response. These abnormal proteins can therefore persist and induce the production of more abnormal proteins in the body without any intervention of the immune system.

The almost 100 per cent mortality is presumably due to the non-induction of an immune response and hence the inability of the immune system to remove the proteins from the body. Fortunately, there are only a small number of these diseases (that we know of) and they have a very low incidence throughout the world.

It appears that many microorganisms have mechanisms that inhibit the defence systems of the host in some way. Even a temporary suppression of host factors can give the microbe enough advantage to enable its establishment and growth in the body.

SUMMARY

- The ability of an organism to cause disease is referred to as its pathogenicity.
- The factors that contribute to an organism's ability to cause disease are called virulence factors.

ADHERENCE

- Adherence is an important step in the pathogenicity of many organisms.
- The substances or structures on the surfaces of microorganisms that enable them to attach to cell surfaces are called adhesins.
- Viruses attach to specific membrane receptors on the cells they infect.
- Microorganisms often produce a glycocalyx to form multilayered communities, called biofilms.
- Biofilms play a critical role in many types of infections related to implanted medical devices, such as intravascular catheters and orthopaedic implants.

TOXINS

- Toxins are substances produced by some microorganisms that interfere with the normal functioning of host cells or tissues.
- Exotoxins are heat-labile proteins that are released as the organism grows.
- A number of exotoxins act by damaging host cell membranes, resulting in lysis and death of the cells.
- An antitoxin is an antibody that is able to bind to a toxin and neutralise it.
- Many exotoxins can be chemically modified to remove their toxicity, while still retaining their ability to elicit an immune response—called toxoids.
- Endotoxins are the lipopolysaccharides (LPS) of the cell walls of Gram-negative bacteria.
- Endotoxin, regardless of its source, produces similar signs and symptoms in the host.
- Fever is one of the more common symptoms induced by endotoxin.
- Endotoxic (septic) shock is caused by the release of large amounts of endotoxin.

INVASION OF CELLS

- Viruses typically cause disease by invading and damaging host cells.
- Viruses cause changes to the cell that are called cytopathic effects (CPE).

CELLULAR TRANSFORMATION INTO TUMOUR CELLS

- Some viruses cause disease by transforming host cells into tumour cells.
- Some viruses are believed to be involved in malignant tumours, such as some HPV types in cervical cancer, and hepatitis B and C viruses in hepatocellular carcinoma.

OTHER MECHANISMS OF PATHOGENESIS

- Superantigens produced by some microbes indiscriminately activate T cells to release cytokines that cause tissue injury.
- Some microorganisms cause tissue damage by physical means (e.g. a mass of worms in the intestinal tract may cause obstruction).

EVASION STRATEGIES

- Some organisms possess capsules or a surface slime layer which prevents phagocytes from ingesting them.
- Some microorganisms have developed ways of surviving inside phagocytes.
- Some microorganisms evade the host defences by hiding inside cells, or in 'privileged sites' where defence cells do not normally circulate.
- Antigenic variation is a phenomenon in which microorganisms change their cell surface antigens, enabling them to evade the immune system.
- Several microorganisms cause a depletion or reduction in the host's defences, resulting in little or no immunity to their antigens.
- The group of diseases called transmissible spongiform encephalopathies (e.g. Creutzfeldt-Jakob disease) does not induce an immune response.

STUDY QUESTIONS

1. What are virulence factors?
2. By what routes do most microorganisms enter the body?
3. What is a biofilm?
4. How do viruses attach to the cells they infect?
5. Define the terms 'endotoxin', 'enterotoxin' and 'neurotoxin'.
6. What are leukocidins?
7. What is coagulase, and how does it increase the pathogenicity of staphylococci?
8. Describe how the tetanus and botulinum toxins cause disease.
9. Define the terms 'toxoid' and 'antitoxin'.
10. What is septic shock?
11. How do viruses kill host cells?
12. Describe the ways by which microorganisms hide from the immune system.
13. What is meant by the terms 'antigenic variation' and 'antigenic drift'?
14. What is thought to be the main way in which the human immunodeficiency virus causes immunosuppression in the host?
15. How could an IgA protease assist microorganisms to cause disease?

TEST YOUR UNDERSTANDING

1. Describe the differences between exotoxins and endotoxins.
2. Describe the advantages that biofilms provide to microorganisms.
3. In a patient with tetanus, why is the administration of specific immune globulin more important than antibiotics?
4. Explain why the administration of antibiotics to a patient with an infection caused by Gram-negative bacteria can initially cause a worsening of symptoms.

FURTHER READING

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UNIT
3

Prevention and control of infectious diseases

CHAPTER

11

Principles of sterilisation and disinfection

CHAPTER FOCUS

- What are the basic principles used for the effective control of microbial growth?
- What are the main methods used to destroy or inactivate microorganisms?
- Why are thorough cleaning procedures an important part of disinfection and sterilisation procedures?
- What determines the method of sterilisation to be used?
- How are chemical disinfectants used?

INTRODUCTION

In previous chapters we have described the relationships that humans have with microorganisms and the factors that determine whether the relationship results in a state of health or disease. We have looked at ways that pathogens are spread from one person or site to another and how they cause disease. In this unit we examine the ways that have been developed over many years to prevent and control infectious diseases. Given that infectious diseases are still a major concern for humans, it is obvious that these microbial control methods are only partially successful. The success of a given method, even if highly effective, can be easily compromised by poor implementation or practice. Therefore, healthcare professionals should not only be aware of the established methods for microbial control, but also have a good understanding of their limitations and of the factors that are critical for their success.

Infection control involves the application of procedures, processes and antimicrobial agents that eliminate microorganisms and/or prevent their transmission to a susceptible person. The most appropriate methods of control for a given situation depend not only on the type of microorganisms that are present, but also on their location. Different methods are applicable for the home, a ward in a hospital, an operating room, a radiography department, a dental clinic and an aged-care facility. Infection control procedures for the prevention of transmission of microorganisms in healthcare facilities are described in detail in Chapter 13. In this chapter we focus on the methods used for eliminating microorganisms or reducing their numbers on medical equipment and in the healthcare environment. The three main methods used are:

- cleaning
- disinfection
- sterilisation.

In the home or community environment where people are generally healthy, it is usually sufficient to use cleaning agents, and occasionally chemical disinfectants, to reduce the numbers of microorganisms to an acceptable level—that is, a level where there is a low risk of infection. In a healthcare setting, however, much higher standards of cleaning and disinfection are needed because of the type of microorganisms that are present and the susceptibility of the patients to infection. Some procedures performed in healthcare warrant the use of equipment that is totally free of microorganisms (i.e. sterile).

TRADITIONAL METHODS OF CONTROL

Preservation of food

Humans have always needed to devise ways to prevent food spoilage and to preserve food for the winter months. Methods such as drying or curing (smoking) of meat and fish have been used for centuries. In areas where salt was available, meat and fish were salted in large barrels; other foods were pickled in vinegar. Vegetable seeds, such as beans and lentils, were dried. We now know that these methods create conditions of low water content or low pH, which are unfavourable for the growth of bacteria.

The use of heat to destroy bacteria and preserve food arose from the work of Louis Pasteur in the 19th century (see Chapter 1). Pasteur (see Figure 11.1) showed that food spoilage was due to microorganisms already present in the food and that these could be destroyed by the application of heat. Provided the food was in a container that prevented the re-entry of microbes, the food would remain unspoiled. The technique he developed, which now bears his name (**pasteurisation**), does not kill all microbes but it does kill most of the types that produce organic acids (mainly the lactic acid bacteria), which cause souring of wine and milk. When applied to milk, the method also destroys certain pathogens, such as those responsible for tuberculosis. *Mycobacterium bovis* causes tuberculosis in dairy cattle and can be transmitted to humans in unpasteurised milk.

FIGURE 11.1

Louis Pasteur—the father of 'pasteurisation'

The use of heat to destroy microorganisms is still one of the most efficient and reliable methods available today.

Source: © Bettmann/Corbis.

Prevention of disease

For centuries, people have been aware of the seriousness of infectious diseases (see Chapter 1) and have tried many methods to cure them or prevent their spread. During the plague that swept through Europe in the Middle Ages, people burnt the bodies and clothes of those who had died and burnt sulfur and aromatic plants in their houses to try to 'purify the air'.

The mid-19th century saw the first attempts at disinfection, or the use of chemicals to destroy microorganisms. Joseph Lister, a Scottish surgeon, was one of the first to use chemicals to reduce the occurrence of fatal gangrene infections in surgical patients (see Chapters 1 and 13). Ignaz Semmelweis, a Hungarian physician working in Vienna, introduced handwashing in a solution of chlorinated lime for all nurses and physicians to reduce the incidence of childbed (puerperal) fever (see Chapters 1 and 13). The methods used by Lister and Semmelweis involved very crude, corrosive chemicals. Florence Nightingale advocated cleanliness and hygiene and introduced measures into the field hospitals in the Crimea that dramatically reduced the number of infections.

Different methods of control or destruction of microorganisms are required for different situations. In the community, good hygiene practices, such as keeping homes and buildings clean and well ventilated, the provision of a safe, clean water supply and the correct disposal of waste and sewage, will create a healthy environment relatively free of pathogenic microorganisms. In today's society there is a strong focus on household cleaners and disinfectants—often with little understanding of the correct use and application of the chemicals involved. Furthermore, there is a risk that overuse of antimicrobial agents in domestic settings could contribute to the emergence of resistant strains of microorganisms.

GENERAL PRINCIPLES OF MICROBIAL REMOVAL

Two main approaches for the prevention of infection in a healthcare setting are: (1) removal of microorganisms from the healthcare environment; and (2) removal of organisms from patient care equipment. In general, removal of organisms from the environment may require a cleaning or a disinfection process, whereas removal of organisms from equipment may require cleaning, disinfection or sterilisation, depending on the nature and use of the equipment. **Cleaning** is the mechanical removal of material (visible or not) from the surfaces of objects. The material may be organic (e.g. blood) or inorganic (e.g. a dried solution). **Disinfection** is a process that aims to destroy or remove most or all of the pathogenic microorganisms present on an object. Bacterial endospores and some viruses may remain after disinfection. **Sterilisation** is a process that completely destroys or removes all microorganisms, including bacterial endospores and viruses, from an object. These and other terms that are commonly used to describe the destruction or removal of microorganisms are listed in Table 11.1.

TABLE 11.1 Terms used in the destruction or control of microorganisms

TERM	DEFINITION
Antiseptic	A type of chemical disinfectant, suitable for use on skin or living tissue, used to kill or remove harmful microorganisms without damaging the tissue.
Biocide	A chemical agent that is capable of killing microorganisms.
Cleaning	The mechanical removal, usually with detergent and water, of material from the surface of an object.
Decontamination	The removal of possibly harmful microorganisms from an object by cleaning or disinfecting.
Disinfectant	A chemical substance normally used for the disinfection of inanimate objects.
Disinfection	The destruction or removal of all or most pathogenic microorganisms. Usually kills vegetative bacteria, but not endospores or some viruses.
Germicide	A chemical agent that is capable of killing microorganisms (germs).
Pasteurisation	A disinfection method developed to preserve milk and other liquids without altering their taste or quality. Uses heat to destroy some, but not all, microorganisms.
Sanitisation	Thorough cleaning of an object or utensil to remove most microorganisms. Does not imply use of a disinfectant.
Sterile	Free of all living organisms.
Sterilisation	The complete destruction or removal of all microorganisms, including endospores and viruses.
Sterility assurance	The probability of a viable microorganism being present on an item after sterilisation.

Rate of death

When a microbial population is exposed to a biocide (a chemical that kills microorganisms), the rate of death of the cells follows a particular time course. In general, each cell must come into actual contact with the biocidal agent in order for it to be killed. Thus, in order to be effective, the biocidal agent must have sufficient time to penetrate throughout the whole population of cells. Under specified conditions, a certain number of microbes will be killed in a certain period of time; for example, 90 per cent of the microbial population may be contacted and killed in one minute. After these cells are killed, it will take another minute for the biocidal agent to come into contact with 90 per cent of the

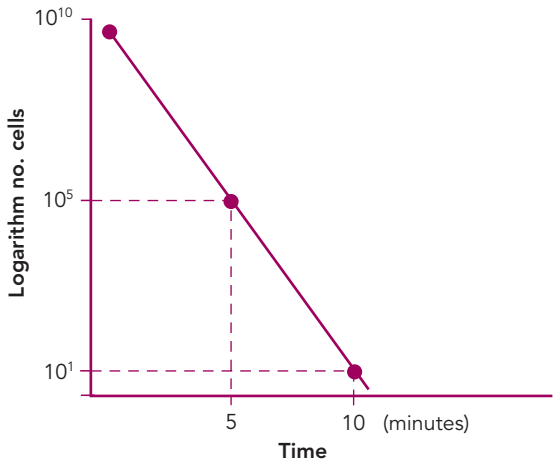

FIGURE 11.2

Killing curve: logarithmic plot of the rate of microbial death versus time

remaining live cells. So, 90 per cent will be killed in the next minute, and so on. This is called an *exponential killing curve* (see Figure 11.2). If the rate of death is plotted logarithmically, it is a straight line.

Obviously, the more microorganisms that are present initially, the longer it will take to destroy them all. It is essential, therefore, that any process is continued for a sufficient period of time to ensure the death of all microbial cells. Most procedures are standardised, and manufacturers (and hospitals) recommend that articles are treated for a specified period of time in a given process, to ensure that all organisms present are destroyed. Hospital-based sterilisation processes are designed to achieve a level of safety in the order of a one-in-a-million chance of an organism surviving the process. Failure to follow instructions and to allow the article to be in contact with the biocidal agent for the correct period of time may jeopardise the efficiency of the procedure.

Several other factors can affect the rate of killing of microbes by heat or chemicals. A major factor is the type of microorganisms that are present. Some chemicals have a different type of killing action for different types of microbes (e.g. Gram-negative versus Gram-positive bacteria). Some bacteria, such as the pseudomonads and the mycobacteria, are naturally resistant to some chemical disinfectants. The cysts of the protozoan *Giardia* are resistant to chlorine, which is often used for water purification.

Mycobacterium tuberculosis is killed by relatively mild heat treatment (the temperature used in pasteurisation is 63°C for 30 minutes). On the other hand, bacterial endospores are able to survive for several hours at 100°C, so a much higher temperature of 121°C is required for guaranteed killing.

Concentration of biocidal agent

For chemical inactivation of microorganisms to take place, there has to be a sufficient number of molecules of the chemical present to interact with each microbial cell. Thus, any chemically based sterilisation process, disinfectant or antiseptic solution must be used at an adequate concentration to ensure that all microbes are killed. The manufacturer's instructions usually specify the optimum concentration to be used to ensure that the chemical will be effective. A weaker or stronger solution may compromise the effectiveness of the treatment. For example, ethanol works best as a disinfectant at a concentration of between 60 and 90 per cent. It is less effective outside of this range.

In summary, a disinfection or sterilisation process relies on three critical factors to be effective:

- an appropriate biocidal agent
- effective contact of the agent with all surfaces to be treated
- sufficient exposure (in terms of the length of time of exposure and the concentration of the biocidal agent).

SELECTION OF METHOD FOR REMOVAL OF MICROORGANISMS

Risk level

In healthcare facilities an important mode of transmission of pathogenic microorganisms to a susceptible person is via equipment, which includes medical and surgical instruments and patient care items and furnishings. Not all of these items need to be sterile prior to use, so decisions often need to be made regarding the type of decontamination necessary for equipment in a given situation. An approach for determining the appropriate method for removing microorganisms from equipment was initially suggested by Spaulding in 1968. It has since been refined and modified a number of times, but such a scheme is often utilised by healthcare organisations and infection control professionals when planning disinfection and sterilisation strategies. The scheme identifies the minimum level of cleaning, disinfection and sterilisation necessary, dependent on the risk associated with the body site where the equipment will come into contact with the patient (see Table 11.2 and Chapter 13).

The highest risk (critical) is associated with any object or material that enters sterile tissue or the vascular system. Critical items contaminated with any microorganism pose a high risk of infection. Items in this category include surgical instruments, surgical implants, urinary and intravascular catheters, probes used in sterile body cavities, and solutions for intravenous (IV) infusion. Any item of equipment that falls into the high-risk category must be sterile, and the highest level of aseptic technique must be followed when using the item. Ideally, items in this category are single-use items purchased as sterile; however, some items of equipment must be reused, and hence must be sterilised between uses.

Items of equipment that come into contact with a patient's non-intact skin or mucous membranes, and are not meant to breach them, are regarded as semi-critical items. These items should be free of all microorganisms, although small numbers of bacterial endospores are permissible. This category includes equipment such as flexible endoscopes,

TABLE 11.2 Guidelines for sterilisation and disinfection of equipment

LEVEL OF RISK	USE OF ITEM	PROCESS	EXAMPLES
Critical	Equipment that penetrates into sterile tissue, body cavity or bloodstream	Sterilisation	Surgical instruments, needles (syringes), IV and urinary catheters, implants, solutions for IV administration
Semi-critical	Equipment that comes into contact with patients' non-intact skin or mucous membranes	Single use, or sterilisation or high-level chemical disinfection	Endoscopes, cystoscopes, respiratory equipment
Non-critical	Contact with intact skin	Cleaning (plus low–intermediate-level disinfection if decontamination is required)	X-ray machines, bedpans, thermometers, blood pressure cuffs, patient furniture

certain respiratory equipment, and cystoscopes. These items should be used once only (i.e. 'single-use' equipment) or be sterilised; or, if this is not possible, they should receive high-level disinfection with chemical disinfectants between uses. Chemicals that provide high-level disinfection include gluteraldehyde, hydrogen peroxide, and peracetic acid with hydrogen peroxide (see later in this chapter).

A non-critical item is one that only comes into contact with intact skin. X-ray equipment, bedpans, blood pressure cuffs, trolleys, beds and other furniture carry a low risk of cross-infection and appropriate cleaning is often sufficient. If decontamination is required, a low- or intermediate-level disinfectant may be used after cleaning. Intermediate-level disinfectants (e.g. some chlorine products and phenolics) kill all vegetative bacteria, most viruses and fungi, but not bacterial spores. Low-level disinfectants (e.g. some chlorine products and phenolics, quarternary ammonium compounds, and alcohol) kill vegetative bacteria, some viruses and fungi, but not mycobacteria or bacterial endospores.

It should be noted, however, that a non-critical item that is used on damaged skin becomes a semi-critical item. Therefore, it is important to assess the risk category of the item carefully to determine the appropriate method to be used.

Single-use equipment

Many items are labelled 'single use only' and these *must not be reprocessed*. Any single-use article or instrument that has penetrated the skin, mucous membrane or tissue must be discarded immediately after use.

Factors impacting on the effectiveness of methods

Apart from the risk level associated with the use of a particular item, a number of other factors are important determinants when choosing a method for removal of microbes from an object. The most important of these is the nature of the object to be sterilised. Heat (see later sections) is the most reliable and efficient method for sterilisation and disinfection, but there are many items (e.g. pharmaceutical solutions, delicate surgical/medical instruments) that are damaged by the high temperatures necessary in these processes. Such items will need to be treated by other methods. Some items, such as endoscopes, have a structure and shape that makes it difficult to get a sterilant or disinfectant to all parts of the instrument.

Another important factor is the susceptibility of the microorganisms present to the procedure. This is most relevant when chemical disinfectants are being used, since some microbes are resistant to certain disinfectants. For example, bacterial endospores are resistant to 70 per cent alcohol, so this commonly used disinfectant is not appropriate for an area contaminated with *Clostridium difficile* (a spore former). This issue is discussed in detail in the later section on disinfectants. Although heat is a highly efficient method, prions are resistant to normal sterilising temperatures (see Spotlight box: Prions).

Some materials, such as organic matter (see next section) and bacterial biofilms (see Chapter 10), can also interfere with microorganism removal methods.

CLEANING

Cleaning of instruments and equipment

One of the most important procedures for the removal of microorganisms from inanimate objects is *cleaning*. Bacteria thrive in moist organic matter such as tissue, blood and other body fluids. Thorough cleaning of an item is essential before a disinfecting or sterilising procedure is applied to it. The item must be freed of organic material, grease and dirt, as these can provide a protective barrier for the microorganisms and markedly reduce the effectiveness of the procedure. For example, organic matter reacts with some chemical disinfectants, making the disinfectant less effective. Some disinfectants are inactivated completely by organic material. Therefore, if an item cannot be cleaned, it cannot be disinfected or sterilised either.

Washing of equipment with hot water and detergent does not kill all bacteria, but it does reduce their numbers to a level where disinfection and sterilisation procedures are more likely to be effective. Ultrasonic cleaning (by automated cleaners) is useful for complicated instruments with parts that are difficult to reach with standard cleaning techniques. This method involves placing the equipment in a solution of detergent and water which is subjected to high-frequency sound waves. The action of the sound waves dislodges dirt, blood and tissue from inaccessible parts of the

Prions

SPOTLIGHT ON

Prions are infectious proteins that are highly resistant to hydrolysis by proteolytic enzymes (in the digestive tract) and to conventional methods of sterilisation and disinfection. They are the cause of a range of rare but fatal neurodegenerative disorders called transmissible spongiform encephalopathies (TSEs)*. Among these, 'classic' Creutzfeldt-Jakob disease (CJD) is the most common. CJD manifests itself in a range of neurological symptoms. A definitive diagnosis depends on post-mortem examination of brain tissue, which exhibits distinctive spongiform degeneration, astrocytosis and accumulation of amyloid filaments. About 85 per cent of CJD cases occur sporadically; about 10 per cent of cases occur in people with a family disorder; and a small percentage are acquired.

Iatrogenic (medically acquired) cases of CJD have occurred in Australia in patients who have been treated with contaminated hormone preparations (prepared from pooled pituitary glands from cadavers). Small numbers of people have been infected via contaminated dura mater or corneal transplants.

A new form, called variant CJD (vCJD), appeared in Britain in the 1990s, linked to the consumption of beef products derived from cattle suffering from BSE (bovine spongiform encephalopathy), or 'mad cow disease'. To date, there have been a total of 176 deaths in the UK definitely or probably due to vCJD. The outbreak peaked in 2003, with 18 deaths in that year. So far, no cases of vCJD have been detected in Australia, but the government has banned blood donations from people who spent more than six months in Britain between 1980 and 1996.

Prions are not susceptible to routine methods of disinfection and sterilisation, so special procedures are necessary when handling equipment that has been used on a patient suspected of having a prion-related disease. Where possible, single-use equipment should be used, and after use it should be placed in an appropriate waste container for incineration or other approved method of waste destruction. If an item must be processed for reuse, cleaning with an anionic detergent followed by steam sterilisation at 134°C for three minutes is necessary. Items that cannot be processed in this way should be destroyed by incineration. Chemical disinfection is not recommended. More detailed guidelines can be found on the Department of Health and Ageing website: <www.health.gov.au/internet/main/publishing.nsf/Content/icg-guidelines-index.htm>.

* See Chapter 20 for a discussion of transmissible spongiform encephalopathies.

instrument. Shared, non-critical clinical equipment (see the previous section on 'Risk level') should be cleaned between patient uses with a detergent solution only, except where risk assessment indicates a more stringent process is necessary.

Detailed procedures for cleaning of reusable instruments and equipment can be found in the Australian Standards AS/NZS 4187 and AS/NZS 4815. Standard Precautions, including use of appropriate personal protective equipment for the task, should always be followed (see Chapter 13).

Environmental cleaning

Microorganisms can be transmitted from the hospital environment to patients by direct contact with contaminated items, or indirectly via the hands of healthcare workers. Therefore, the cleaning of wards, bathrooms, floors and furnishings is an important part of infection control (see Chapter 13). The routine cleaning of the physical environment in healthcare facilities is detailed in the *Australian Guidelines for the Prevention and Control of Infection in Healthcare*. The guidelines recommend a risk assessment approach, with different procedures for 'minimal touch surfaces' compared to 'frequently touched surfaces'. Minimally touched surfaces, such as walls, floors and blinds, should be cleaned with a detergent solution when they are visibly dusty or soiled. Frequently touched surfaces, such as bedrails, over-bed tables and light switches, should be cleaned with a detergent solution at least daily, and when visibly soiled and after known contamination. Allowing the cleaned surface to dry is an important part of the cleaning process, since most microorganisms do not survive well on clean, dry surfaces. Use of a disinfectant is not considered necessary for routine cleaning of surfaces, but is warranted if multi-resistant microorganisms (e.g. MRSA, *C. difficile*) are known or suspected to be present. Physical cleaning must be performed before the disinfection process. Figure 11.3 outlines the recommended processes for routine cleaning in healthcare settings. The cleaning of surfaces contaminated with blood or other body substances would usually involve cleaning followed by disinfection (see Chapter 13 for more detail).

The equipment used for cleaning should be kept dry when not in use to prevent the growth of bacteria. A damp, contaminated mop head can be responsible for spreading pathogens around the hospital.

A description of the correct cleaning procedures to be followed for the fixtures and equipment in a hospital is usually contained in the hospital's infection control manual.

STERILISATION

Sterilisation is the complete destruction or removal of all microorganisms, including spores and viruses, from an object. Sterilisation methods are designed to give a minimum sterility assurance of one-in-a-million; that is, no more than a one-in-a-million probability that the item remains

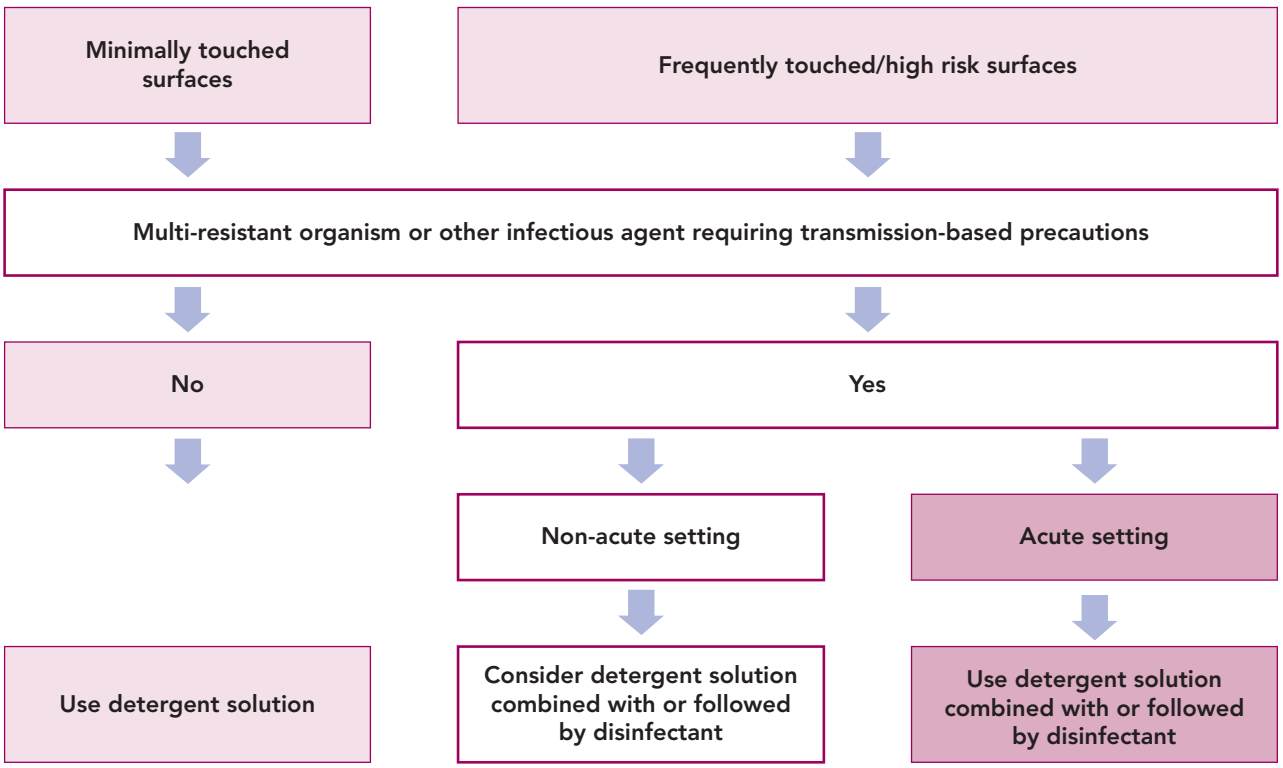

FIGURE 11.3

Processes for routine cleaning

Source: NHMRC, *Australian Guidelines for the Prevention and Control of Infection in Healthcare* (Canberra: Commonwealth of Australia, 2010). Available at: <www.nhmrc.gov.au/_files_nhmrc/publications/attachments/cd33_complete.pdf>.

contaminated after the sterilising procedure. Achievement of this level of sterility assurance relies on the initial reduction in the number of microbes by thorough cleaning. In practical terms, an object is considered to be either sterile or non-sterile—that is, sterility is an absolute state.

A variety of methods can be used to sterilise an object (Table 11.3). They include physical methods that make use of heat or radiation, mechanical methods such as filtration (used for gases and liquids), and chemical methods that use highly toxic chemicals, such as ethylene oxide, hydrogen peroxide and peracetic acid. The method employed depends on the nature of the object to be sterilised, the type of facility where it will be used and the use to which the object will be put.

Heat is the most widely used of all sterilisation methods. It is cheap, efficient and easily controlled. Dry heat kills microorganisms by inactivation of cell components by oxidation, whereas moist heat destroys microorganisms mainly by denaturation of essential cellular proteins. Microorganisms vary in their susceptibility to heat. Whereas most vegetative bacteria are quite susceptible to heat, the endospores of bacteria tend to be more resistant.

Moist heat sterilisation: steam under pressure

In the hospital setting, patients are protected from infection by the use of sterile equipment, instruments, dressings and IV solutions. To ensure these objects are sterile, they must be subjected to a reliable procedure that can be accurately monitored. Overall, the most practical method is moist heat sterilisation, which uses a machine called a steam steriliser or autoclave. The process is reliable, has known killing action, and is used in a way that provides a wide safety margin. It kills vegetative bacterial cells, as well as endospores and viruses.

Although boiling is used to kill microorganisms, it is not an accepted method of sterilisation. It is, however, a useful process for disinfection of certain materials (e.g. water). Some organisms can withstand the temperature of boiling water (100°C at normal atmospheric pressure) for long periods; therefore, to achieve sterility, it is necessary to use higher temperatures. These can be reached by increasing the pressure in the sterilising chamber so that the temperature at which the water boils (i.e. the temperature of the steam) is raised. The higher the pressure, the higher the temperature that can be reached.

Example:

Steam at 121°C
recommended holding time 15 minutes
Steam at 134°C
recommended holding time 3 minutes

For example, when steam from boiling water is placed under a pressure of 15 pounds per square inch (or 1

TABLE 11.3 Methods of sterilisation

METHOD	ADVANTAGES	DISADVANTAGES
Steam steriliser: steam under pressure at 121°C for 15 minutes	Reliable—kills all microbes and spores. Inexpensive to run.	Requires special equipment. Not suitable for heat-sensitive objects or solutions.
Dry heat 180°C for 1 hour, or 160°C for 2 hours	Inexpensive	Slow. Not suitable for heat-sensitive materials.
Incineration	Reliable—kills all microbes and spores.	Destruction of contaminated articles.
Ionising radiation (gamma)	Good for prepackaged materials.	Requires access to commercial cobalt-60 source.
Filtration	Useful for sterilising heat-sensitive solutions.	Awkward to handle. Difficult to monitor efficiency.
Ethylene oxide gas	Useful for heat-sensitive materials.	Requires special facility in sterilising department. Gas is toxic and flammable. Equipment must be well aired before use. Difficult to monitor efficiency.
Hydrogen peroxide, low-temperature 'plasma' sterilisation	Suitable for delicate instruments. Fast, non-toxic.	Requires special unit. Not suitable for cellulose, paper or fabric.
Peracetic acid	Low temperature. Kills most organisms.	Strong odour. High cost.

atmosphere), the temperature of the steam is increased to 121°C. A temperature of 121°C is sufficient to kill all microorganisms, including endospores, in 15 minutes. At 134°C (29 pounds per square inch) the time required for sterilisation is reduced to three minutes. It can be seen that time for sterilisation with heat is inversely proportional to the temperature used.

It should be noted that these times are measured from the point when steam penetrates to all parts of the object to be sterilised. This is especially important for thick or bulky items, which may require a longer penetration time. It is also important to note that prions (infectious proteins) are effectively destroyed at 134°C, but not at 121°C (see the Spotlight box in this chapter).

Steam sterilisers vary in size from small bench-top models, used in medical and dental surgeries, to large, fully automated commercial models used in hospitals and industry (see Figure 11.4).

A steam steriliser consists of a double-walled or jacketed chamber made of thick steel. Steam is circulated through the outer jacket and then supplied, under pressure, to the closed inner chamber (see Figure 11.5). Both the inner chamber and the airtight doors are constructed to withstand the pressures and temperatures generated.

For sterilisation to be effective, steam must be able to contact every part of the object to be sterilised (and thus every microorganism that is present). To ensure the correct temperature is reached and all surfaces of the items are contacted, all the air must be eliminated from the load and the chamber, and replaced with steam. The most effective method of air removal is achieved by the use of a mechanical pump to suck all the air out of the chamber. A steriliser that uses this type of air removal is often referred to as a 'prevacuum' steriliser. Older models of steam sterilisers may use passive methods of air removal, such as gravity, to remove the air from the chamber.

FIGURE 11.4 A laboratory autoclave

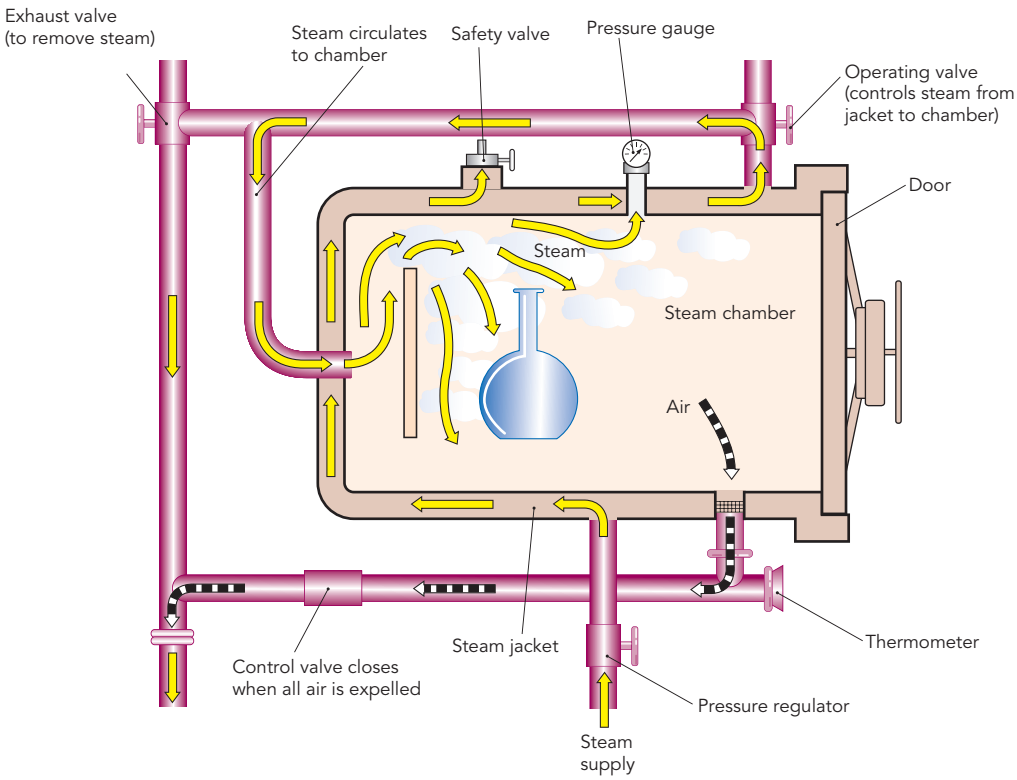

FIGURE 11.5

Diagram of steam steriliser (autoclave)

Steam enters and forces air out through an opening on the bottom of the chamber. When all air is expelled, the control valve is closed by the higher temperature of the steam. As the pressure is increased, the temperature of the steam is raised.

There are four main stages in the sterilising cycle.

1. Removal of air, admission of steam and heating of the chamber to the selected sterilising temperature.
2. Sterilisation time, which includes:
   (a) *penetration time*, the time taken for the least accessible part of the load to reach operating temperature
   (b) *holding time*, including a safety factor, which is the minimum time required for the whole load to be maintained at operating conditions in order to reach the level of sterility assurance required.
3. Removal of steam and drying of the load.
4. Restoration of atmospheric conditions by admission of filtered air.

Equipment to be sterilised must be wrapped in a suitable material such as paper or fabric, which allows penetration of steam and maintains sterility after removal from the steriliser. Special chemical indicators and tapes that change colour when the desired temperature is reached are placed in the middle of a wrapped package and should change colour evenly if the heat has penetrated uniformly. The load should be allowed to dry inside the steriliser before being exposed to the outside air. This is to prevent bacteria from passing through the wet wrappings and contaminating the inside of the package. Liquids should be placed in loosely stoppered flasks and allowed to cool to below 100°C before being removed from the chamber.

At the end of a sterilisation cycle, the process record must be checked to ensure that sterilisation parameters were achieved. Packages must be visually inspected for the correct colour change of the chemical indicator and for any damage to the packaging, and there should be no visible wetness. The wrapping of the package must remain intact, or the contents cannot be considered sterile. Sterilised equipment has a finite shelf life and staff should check the expiry date before use and reprocess the equipment if necessary (see Figure 11.6).

When properly used and maintained, a steam steriliser is a very efficient method of sterilisation. It should be regularly calibrated and maintained by trained personnel. The steriliser must be performance-tested and the sterilisation process routinely monitored, according to the requirements of AS/NZS 4187, to ensure that the steriliser is reaching the required temperatures and pressures for the required amount of time.

Dry heat sterilisation

To sterilise using dry heat, a temperature of 180°C for 60 minutes is required. Dry heat sterilisers are only suitable for sterilising equipment that is not harmed by long exposure

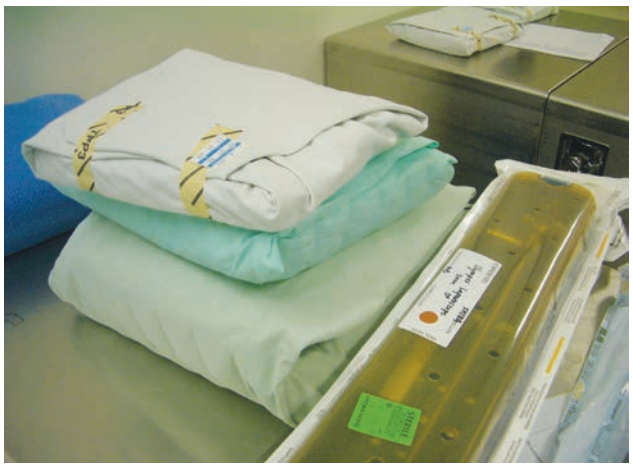

FIGURE 11.6

Sterile packages

Note labels and dates. Black stripes on tape show package has been steam sterilised.

to high temperatures. Glass and some metal objects can be sterilised in this way. Fabrics and wrapping materials cannot usually withstand these temperatures. Reusable equipment must be packaged in heatproof containers (metal or glass).

The time required to heat the oven, in addition to the sterilising time, makes this method less practical than a steam steriliser. The high temperature required is also a major limitation, although the dry atmosphere is preferable for some metal instruments as it does not cause corrosion.

Incineration

Incineration of contaminated material is another method of destroying the microorganisms present in biological materials, contaminated waste, soiled dressings and disposable equipment. In the field, infected animals may be incinerated, such as occurred in the UK in the 1990s with the epidemic of mad cow disease (see Chapter 20) and as is currently occurring with poultry suspected of avian influenza infection. It is also used to sterilise the platinum inoculating loops that are used to handle and transfer microorganisms in the laboratory.

Radiation

Ionising radiation damages or kills microorganisms by causing disruption to their DNA molecules. Gamma rays from a radioactive cobalt-60 source are used industrially to sterilise much of the disposable equipment used in hospitals, such as prepackaged dressing packs, needles, syringes, suture material, catheters and prostheses. Because gamma rays readily penetrate materials, such products can be packaged first, and then sterilised.

It is a useful method for heat-sensitive, organic material such as pharmaceutical products for injection—anaesthetics, therapeutic drugs, hormones, vaccines and antibiotics. However, not all materials can be sterilised by this method without causing changes to their composition. Because of the highly dangerous nature of the radiation, this type of sterilisation treatment is only performed in a specially designed commercial facility.

Microwave radiation consists of electromagnetic radiation that interacts with molecules such as water, producing heat. The microwaves do not directly kill microbes, but the heat that they produce exerts the killing action. However, the uneven distribution of heat produced means that microwaves are unsuitable for use as a controlled method of sterilisation.

Filtration

Filtration involves the removal of microorganisms from liquids or gases by passage through a filter with pores small enough to retain microorganisms. Filters are used to sterilise fluids that cannot be sterilised by other methods. Many of the aqueous solutions used for procedures such as surgical irrigation, peritoneal dialysis, intravenous therapy or total parenteral nutrition of hospitalised patients contain substances that are adversely affected by high temperatures. Some pharmaceutical preparations such as ophthalmic solutions, drugs and vitamins, and biological materials, such as serum, and some culture media used in microbiology laboratories are also sensitive to high temperatures. They can be sterilised by filtration through specially designed membrane filters (see Figure 11.7). These are made of nitrocellulose and manufactured in a range of specific pore sizes capable of retaining different-sized particles (Table 11.4).

It is more difficult to control sterility with filtration, as the equipment can be awkward to use and the efficiency of the filtration process is difficult to assess.

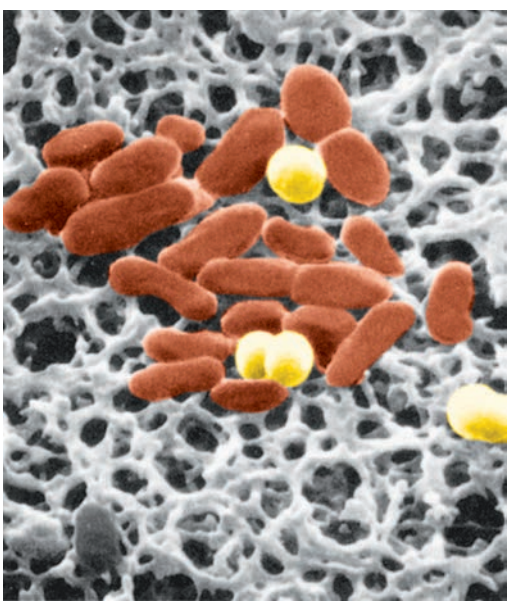

FIGURE 11.7

Membrane filters

Scanning electron micrograph showing rod-shaped Serratia marcescens *and round* Staphylococcus epidermidis *cells trapped on the surface of a 0.22-μm Millipore membrane filter. Membrane pore size may be selected so as to allow viruses, but not bacteria, to pass through, or to prevent both from passing.*

Source: Merck Pty Limited.

TABLE 11.4 Pore sizes of membrane filters

PORE SIZE	FILTRATION USE
Less than 0.025 mm	Required to ensure removal of all bacteria, viruses and mycoplasmas (ultrafilter).
0.22 mm	Allows passage of all viruses and molecules. Retains bacteria.
0.45 mm	Allows a few bacteria through. Retains yeasts, protozoa, blood cells.
1.2 mm	Allows viruses and most bacteria through. Retains yeasts.

Ethylene oxide gas sterilisation

Ethylene oxide gas is a useful sterilising agent for articles made of rubber or other heat-sensitive components. The gas is effective against all microorganisms, as it inactivates proteins by combining with sulfhydryl groups. However, it is a highly toxic, flammable gas that explodes when mixed with oxygen. It is also teratogenic, mutagenic and carcinogenic, and exposure causes irritation to the eyes and respiratory tract, and nausea and dizziness. For these reasons, the method is confined to industrial facilities or the central sterilising units of large hospitals. Careful control of the conditions is necessary, as time, temperature, humidity and gas concentration all affect the efficiency of the sterilisation.

Items sterilised in this way must be aerated with filtered air for up to 12 hours to remove residual gas. Staff handling ethylene oxide should be aware of its toxicity and of the occupational health and safety guidelines for its use. If articles are not adequately aerated, patients can suffer from hypersensitivity reactions when the article touches the skin.

The method is difficult to monitor, requiring the use of spore strips (specimens of endospores of *Bacillus subtilis*), which are exposed during the sterilising cycle. The time required for aeration, and for assessing whether the spores have been killed, makes this a rather slow method of sterilisation. It has good powers of penetration, however, and is most useful for sterilising delicate equipment that would be damaged by heat or liquids.

Low-temperature hydrogen peroxide plasma sterilisation

Hydrogen peroxide vapour is used as a low-temperature sterilant suitable for heat- and moisture-sensitive equipment (e.g. electronic equipment, fibreoptic cables, camera heads). It is a useful alternative to ethylene oxide and is suitable for use in hospital central sterilising departments.

Commercially available systems use hydrogen peroxide which is vaporised and exposed to radio waves, giving rise to a gaseous plasma (a mixture of high-energy species of hydrogen peroxide, H_2O_2). This mixture is a sterilant killing all microorganisms, including viruses and bacterial endospores. The products of the process are non-toxic (water and oxygen). The system uses low temperatures (around 45°C), the cycle is rapid (75 minutes) and no venting time is required.

The process is suitable for sterilising medical devices made from stainless steel and most plastics. The presence of organic material on instruments can compromise efficiency, and equipment with a narrow lumen may require the use of a booster to ensure penetration of the plasma into the tube.

Peracetic acid sterilisation

This process uses 0.2 per cent buffered peracetic acid solution at low temperatures (50–55°C) for around 30 minutes to sterilise instruments. It is mainly used as a replacement for glutaraldehyde for the processing of flexible endoscopes and other temperature-sensitive devices capable of being immersed in a liquid. Peracetic acid is an oxidising agent and can corrode instruments immersed in it. Commercial systems have been developed which are automated, eliminate the corrosive effects of the chemical, and have non-toxic by-products.

As this method of sterilisation uses a liquid chemical, items are not dry at the completion of the cycle and must be used immediately to ensure sterility is maintained. Peracetic acid is active against vegetative bacteria and endospores, fungi, viruses and mycobacteria.

DISINFECTION

Disinfection is a process that aims to kill or inactivate large numbers of microorganisms, but does not sterilise. Disinfecting methods must be able to reduce the number of microbes present to an 'acceptable level'. The definition of an 'acceptable level' in the clinical environment depends on the purpose for which the article is to be used. Current approaches make very clear distinctions between the level of disinfection expected for non-critical versus semi-critical patient-care equipment (see previous section on 'Risk level'). For example, the level of disinfection required for an item such as a bedpan is less than that expected for an item of respiratory equipment because of the different level of infection risk associated with the use of each item.

An article that has been *disinfected* has usually been treated in one of the ways described below. Methods include the use of heat, radiation and various chemical agents that destroy microorganisms (see Table 11.5).

As mentioned earlier, all articles to be disinfected must first be thoroughly cleaned. The presence of organic matter, grease or dirt interferes with the ability of the disinfecting agent to penetrate, contact and inactivate the microorganisms present.

Sanitisation is a poorly used term. It usually means that an article has been cleaned, although it is used in some contexts to imply that the article is 'germ-free'. It does not necessarily mean that an object has been exposed to a disinfecting procedure, and health workers should not assume that an article that is 'sanitised' has been disinfected.

TABLE 11.5 Methods of disinfection

METHOD	USE
Heat	
Controlled-temperature automated washers	Instruments
100°C boiling water	Food, water
63°C pasteurisation	Food, milk products
Radiation	
Short-wave UV	Surfaces, air spaces
Filtration	Air in safety cabinets, operating theatres, isolation units
Chemicals	Surfaces, equipment
	Decontamination of skin
	Hand hygiene
	Blood spills

Heat

Heat is a very useful method of disinfection as it penetrates readily and is easier to control than most chemical disinfectants. Boiling water (100°C for five minutes) kills most microorganisms except for bacterial endospores, the cysts of protozoa and some viruses. As such, it is an inexpensive and reliable method of decontaminating many items, such as tableware, baby bottles and drinking water. However, the simple use of boiling water as a method of disinfection is not acceptable in healthcare facilities. To be effective, the process must be controlled and so the old-fashioned boiling-water 'sterilisers' are considered obsolete.

Thermal disinfection in healthcare facilities is now achieved by the use of automated washer-disinfectors that meet stringent standards. These machines must be calibrated, maintained and routinely monitored to ensure that disinfection is being achieved. Standard AS/NZS 4187 specifies the correct time and temperature combinations required to achieve thermal disinfection of medical equipment.

It is important to remember that thermal disinfection is not a method of sterilisation and should not be used for instruments that breach the body's defences (see Table 11.2). Thermal disinfection can be used for equipment such as bedpans.

Many organisms are killed by exposure to temperatures below 100°C. A temperature of 63°C for 30 minutes is the standard method of *pasteurisation*. This method destroys most pathogens likely to be present in certain foods or milk, without causing deterioration in the quality of the food. Another method (flash pasteurisation) involves passing milk through a tube heated to 72°C, holding it there for 15 seconds and then cooling it quickly. Pasteurisation removes organisms such as *Mycobacterium bovis*, a potential cause of tuberculosis, from milk and related products. Various other heat treatments to preserve food have been developed. One involves the *ultra-high temperature (UHT) process*, in which the milk is heated to 140°C for two to four seconds and then rapidly cooled.

Ultraviolet radiation

Many microorganisms are killed or damaged by **ultraviolet radiation**—exposure to short-wave ultraviolet (UV) light. UV light is absorbed by the DNA of the microbial cell and causes the molecules of nucleic acid to break, interfering with DNA and cell replication. However, UV light does not penetrate well into liquids or through paper, or even through a layer of dust. Over short distances it is useful for killing bacteria on clean surfaces and for disinfecting limited air spaces such as microbiological safety cabinets. The amount of UV radiation received depends on the time of exposure (see Figure 11.8) and the distance the object is placed from the UV source The amount of UV radiation that an object is exposed to is inversely proportional to the square of the distance of the object from the source.

Sunlight is a significant source of UV light, and exposure of articles to sunshine significantly reduces their microbial population. Exposure of contaminated wounds or ulcers to sunshine is an old-fashioned but sometimes effective treatment. It also inhibits bacterial growth by drying the wound.

Filtration

Microorganisms can also be removed from air by the use of high-efficiency particulate air (HEPA) filters. A **HEPA filter** is defined as one that removes a minimum of 99.97 per cent of particles equal to, or larger than, 0.3 μm in diameter. It does not sterilise air, but significantly reduces the number of organisms present. Some laboratory work involves the use of hazardous materials, such as infectious microorganisms or contaminated objects, and these can be handled safely in

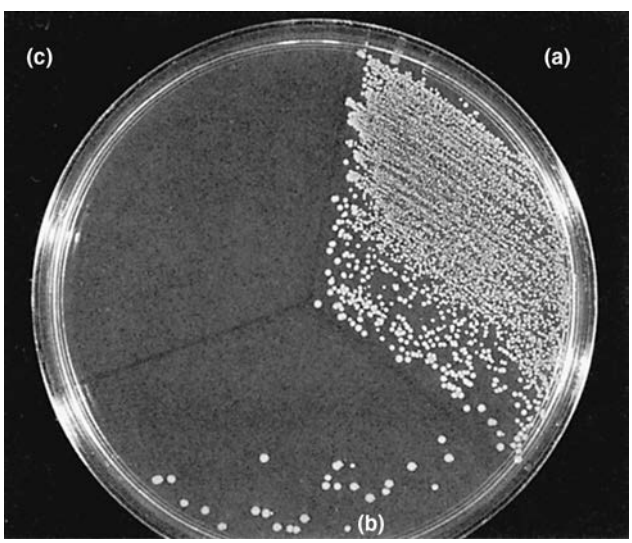

FIGURE 11.8

Effect of UV radiation on the growth of bacteria on a plate

Effect on bacterial growth of different times of exposure to UV light (280 nm, d = 15 cm): (a) control, no exposure; (b) one minute; (c) five minutes.

Source: © Christine Bishop.

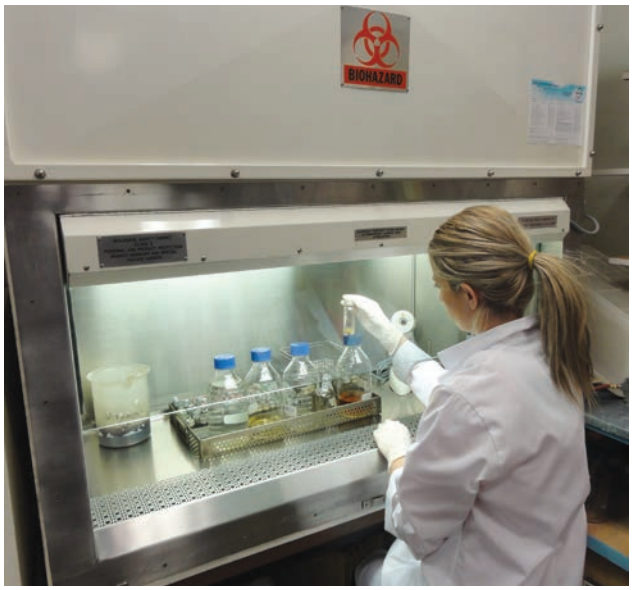

FIGURE 11.9

Biosafety class II cabinet

Source: Dr Penny Bishop.

specially designed laminar flow cabinets that provide protection from dangerous aerosols (Figure 11.9). Air is drawn into the cabinet away from the worker and filtered (HEPA) before leaving the cabinet.

HEPA filters are used in ventilation systems to clean the air entering operating theatres, burns wards, isolation units and high-dependency wards. They can also be used on exhaust systems in isolation units to trap pathogens that might be released from infectious patients.

Chemical disinfectants

A large number of chemicals are able to kill or inactivate microorganisms. However, not all of them can be safely used as disinfectants, usually because they are too toxic or corrosive. The widespread use of chemical disinfectants in hospitals for routine cleaning has been largely replaced by the use of neutral detergent and thorough cleaning practice. However, disinfectant chemicals are still used on contaminated articles or surfaces, to kill microorganisms present in accidental spills of potentially infected body fluids, and as antiseptics for disinfecting skin and tissue. Disinfectants are regulated by the Therapeutic Goods Administration (TGA). Each disinfectant is generally designed for specific purposes, so users should read the product label carefully to ensure that the correct product has been selected for the situation. The list of solutions that should be used for cleaning or disinfecting various areas under certain prescribed conditions is usually contained in the infection control manual for each hospital.

Chemicals classified as **disinfectants** are formulated for use on surfaces and equipment. These chemicals are usually too toxic or damaging for use on skin or tissue. **Antiseptics** are disinfectants for use on skin and tissue.

It is important that healthcare workers understand the processes involved in disinfection, and are aware of the importance of following the manufacturer's instructions regarding the correct concentration of chemicals and exposure times to achieve the desired result.

When using chemical disinfectants, care must be taken to ensure that every part of the article to be disinfected comes into contact with the disinfecting solution. The article must first be cleaned to remove dirt, grease, blood or any other organic material that might reduce the activity of the chemical. The solution must be used at the correct concentration to ensure there are sufficient molecules of the disinfectant present to kill all the microorganisms.

Some chemical disinfectants are unstable, especially in dilute solution, and so should always be freshly prepared. It is also essential that the articles are immersed for a sufficient period of time. (See Figure 11.2, which shows the killing curve for chemical disinfectants and sterilants.)

Not all chemical disinfectants are equally useful. A number of factors warrant consideration when selecting a disinfectant, including its cost, chemical stability, spectrum of activity, ability to penetrate, and residual activity on surfaces. The advantages and disadvantages of some disinfectants are listed in Table 11.6.

Some chemicals (e.g. alcohol) react very rapidly. Others, like the hypochlorites, also act rapidly but are corrosive at high concentrations. Some chemicals are more active against one type of microorganism than another.

Phenolic disinfectants

One of the earliest disinfectants used was the phenol, carbolic acid, introduced by Lister in the 19th century. Carbolic acid has a very powerful odour and is quite corrosive, so it has been replaced by derivatives of phenol with more acceptable properties. Phenols are active against a wide range of bacteria, including mycobacteria, but they do not kill endospores. They exert their effect by disruption of the cell membrane, denaturation of proteins and inactivation of enzymes, leading to cell death. Phenolic disinfectants are still widely used as they retain some activity in the presence of organic material and are inexpensive and stable. However, they are slightly corrosive and should not be used on skin or tissue. Their strong odour makes them unsuitable for use on surfaces that come into contact with food.

The most commonly used phenolic disinfectant is *o-cresol,* which is the main ingredient in a number of commercial brands of disinfectants. A 1–2 per cent solution of this 'clear' phenol is used for terminal cleaning of contaminated environments and high-risk areas and for decontamination of some equipment.

Triclosan is a chlorophenol used as a broad spectrum antibacterial. It is used as a preservative in the pharmaceutical industry and in hand and body wash preparations for laboratories and medical/surgical units. It has received a lot of publicity because of its inclusion in antimicrobial household products. There is no clear evidence that triclosan or other biocides are necessary in households, and there is some concern that their use may lead to the selection of resistant organisms.

TABLE 11.6 Chemical disinfectants

DISINFECTANT	USE	ADVANTAGES	DISADVANTAGES
Phenolics	Environmental surfaces	Economical; not affected by organic material	Unpleasant odour
Chlorine (as sodium hypochlorite)	Environmental uses: ■ decontamination of blood spills ■ terminal cleaning ■ treatment of swimming pools ■ purification of drinking water	Inexpensive; active against bacteria, viruses, spores, TB	High concentrations are corrosive; unstable, inactivated by organic matter
Chlorhexidine	Skin, handwash, surgical scrubs	Non-toxic; antibacterial; good residual activity	Not active against viruses, endospores
Povidone-iodine (iodophors)	Skin, preparation for surgery	Active against bacteria, viruses and endospores	Causes stains
Alcohol: 70 per cent ethyl or isopropyl alcohol	Skin decontamination before injections; alcohol wipes for equipment	Inexpensive; active against bacteria and fungi	Flammable
Quaternary ammonium compounds	Preservative in pharmaceutical preparations	Stable; non-toxic; effective against most microbes	Easily contaminated with *Pseudomonas*

Hexachlorophene is a phenol derivative that was once widely used as an antibacterial agent in soaps, lotions and surgical scrubs, and in various cosmetic and hygiene products. However, it has been shown to have toxic effects after excessive use, causing neurological damage, and so its use has been largely discontinued.

Halogens

The halogens—iodine and chlorine—are very effective antimicrobial agents. *Chlorine*, in the form of sodium hypochlorite (common bleach—NaOCl), is an effective disinfectant that is active against a range of microorganisms. Sodium hypochlorite is not recommended for routine disinfection in healthcare settings, but may be used in specific situations, especially where contamination with blood-borne or gastrointestinal viruses or *C. difficile* is known or suspected. It is relatively inexpensive and readily available for domestic use (swimming pools, bathroom cleaners, soaking baby's bottles and nappies). However, because it is an oxidising agent it is inactivated by any organic material that may be present. It is therefore important to clean articles thoroughly before treating them with hypochlorite solutions.

The hypochlorite compound breaks down gradually, especially in sunlight, so fresh solutions should be used. A number of commercial preparations are available and the concentration of available chlorine varies between products. It is therefore necessary to check the strength of the solution used (usually stated as a percentage of available chlorine) and to ensure that the correct dilution is made. If the solution is too weak, the disinfecting action will not occur. However, chlorine is corrosive and care must be taken that the solution used is not too strong, especially if it comes into contact with skin or with utensils used for babies.

Chlorine is used at a concentration of 10 000 ppm (parts per million) or 1 per cent for decontamination of spillages of blood or body fluids. It is sometimes replaced by sodium dichloroisocyanurate, a solid chlorine-releasing agent that is less corrosive. It comes in tablet or powder form and can be added as a solid to blood spills. It is advisable to wear gloves when handling these chemicals and to avoid inhaling the vapour.

For environmental decontamination, such as terminal cleaning of a room that has accommodated an infectious patient, or for the disinfection of contaminated equipment, chlorine is used at 1000 ppm or 0.1 per cent. Chlorine is widely used to purify drinking water and in swimming pools, at a concentration of 1–5 ppm. **Chloramines** are organic chlorine-containing compounds that release chlorine slowly over a period of time. They are used to purify household water supplies as they do not have the unpleasant taste and odour sometimes associated with the chlorination of water.

Alcohol

Ethyl alcohol and *isopropyl alcohol* are effective antibacterial chemicals. Alcohols affect microbial cells by dissolving the lipids in the bacterial cell wall and membranes. However, they are not effective against bacterial or fungal spores. They are most effective when mixed with water (usually 70 per cent alcohol/water), as the water helps the alcohol to penetrate the cell and denature the proteins. Alcohols are used mainly to disinfect clean surfaces such as trolleys and thermometers, as alcohol wipes, and as a skin disinfectant before injections are given. Alcohols leave no residue, once evaporated. The presence of organic material interferes with their action.

Glutaraldehyde

Glutaraldehyde is a non-corrosive chemical that can act as a sterilising agent when used at high concentrations and for sufficient exposure time. However, it is more commonly used at lower concentrations and for shorter periods of time as a high-level disinfectant. It appears to exert its effect by disrupting the structure of the microbial proteins. It is a broad spectrum antimicrobial chemical that can kill vegetative cells in a few minutes and endospores within a few hours.

Glutaraldehyde can be used on delicate heat-sensitive instruments such as flexible endoscopes. It can retain its potency even in the presence of organic material. However, it acts as a fixative that hardens organic material and thus creates a protective barrier for the microorganisms against contact with the agent. As with any disinfection or sterilisation process, equipment should be cleaned before immersion in glutaraldehyde solutions.

Glutaraldehyde is somewhat unstable, especially at alkaline pH and high temperatures, so solutions may retain their activity for only a limited time.

Glutaraldehyde is toxic and allergenic and equipment treated with it must be thoroughly rinsed in sterile water before use. There is increasing concern about its toxic properties—contact with glutaraldehyde solutions, aerosols and vapours can cause irritation to the skin, eyes and mucous membranes, as well as producing headaches and nausea. People working with glutaraldehyde can become sensitised to the chemical and develop allergic reactions. Gloves should always be worn when handling glutaraldehyde and it must be used only in an area with local exhaust ventilation, but preferably in a closed containment system such as an automated endoscope reprocessor.

It is not recommended for routine cleaning or disinfection. It should only be used when no other method of reprocessing is suitable.

Quaternary ammonium compounds (QUATS)

These compounds are cationic detergents with a strong bactericidal action, due to the positive charge on the molecule. They are also effective against many fungi, some protozoa and enveloped viruses, but they do not affect endospores. They are thought to act by damaging the cell membrane. The main advantage of QUATS is that they are relatively stable, non-toxic, odourless and tasteless. However, some organisms, such as certain species of *Pseudomonas*, are resistant and even thrive in aqueous QUAT solutions. Because of the ease of contamination with *Pseudomonas*, which is a serious opportunistic pathogen, QUATS are no longer widely used in hospitals.

Two of the most common QUATS in use are benzyl alkalonium chloride, which is used as a preservative in eye drops, and cetyl pyridinium chloride, which is used extensively in oral preparations such as antibacterial lozenges and mouthwashes.

Skin disinfectants—antiseptics

Specially formulated chemical disinfectants that are sufficiently mild to be used on skin, mucous membranes and exposed tissues are called *antiseptics*. Most antiseptics marketed in Australia are either registered medicines or listable medicines and require a registration number on the label. The label claims are important and should be followed. The TGA is responsible for regulating skin disinfectants and antiseptics.

Alcohol

Alcohol is commonly used as a skin disinfectant, having the major advantage of rapid evaporation, which removes the need for rinsing or drying. Alcohol handrubs are recommended in current healthcare infection control guidelines to improve hand hygiene compliance in staff and visitors (see Figure 11.10 and Chapter 13). Alcohol is sometimes used in combination with other antiseptics, such as chlorhexidine, to add some residual antimicrobial activity to the preparation. Alcohol wipes are used to disinfect skin prior to injection or blood collection.

Chlorhexidine

Chlorhexidine is a very effective antibacterial agent, widely used as an antiseptic for disinfection of skin and mucous

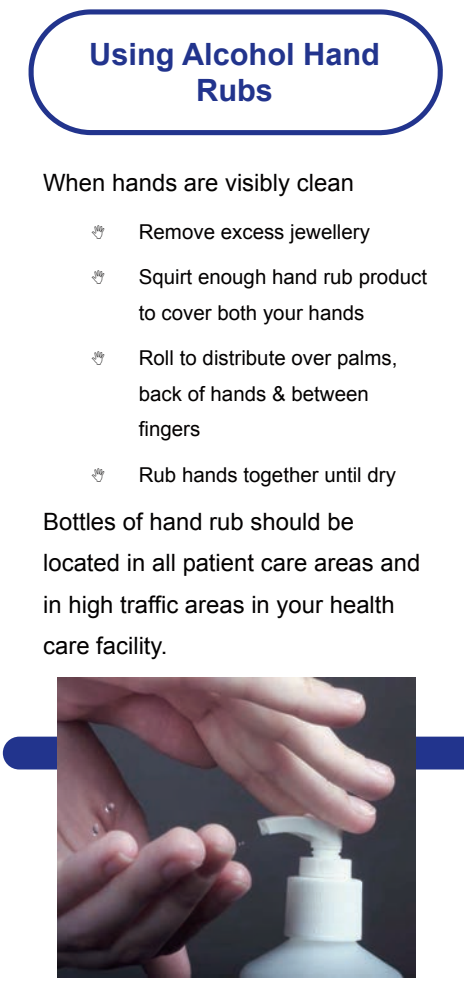

FIGURE 11.10

Hand hygiene poster for hospital visitors

Source: Hand Hygiene Australia, <www.hha.org.au>.

membranes. A number of commercial preparations are available containing chlorhexidine in combination with various soaps or detergent, as alcohol rubs or in skin lotions.

Chlorhexidine has an immediate, cumulative and residual action on many types of bacteria, by attacking the cell membrane. It does not affect mycobacteria or endospores, and *Pseudomonas* spp. are able to survive in aqueous solutions. It is effective against fungi and some viruses, including HIV. One of the main advantages of chlorhexidine is its low toxicity and its ability to persist on the skin for extended periods. This residual activity makes it useful in hospitals for hand disinfection and as a surgical scrub.

Povidone-iodine

Iodine is an effective antimicrobial substance, generally used in combination with povidone, a synthetic dispersing agent. The advantage of povidone-iodine is that the iodine is released slowly, producing good residual antiseptic activity on the skin. Povidone-iodine, when combined with detergent, is an effective antiseptic surgical scrub and is used for disinfecting wounds. It has a broad spectrum of antimicrobial activity, including endospores. For this reason it is widely used for skin disinfection prior to surgical incision.

The disadvantage is that it can produce hypersensitivity reactions and causes (temporary) discolouration of the skin. Some microorganisms, such as *Pseudomonas*, are able to grow in povidone-iodine solutions, if not freshly prepared.

Hydrogen peroxide

Hydrogen peroxide is a common household disinfectant, used as an antiseptic, and weak solutions are used for cleaning puncture wounds. Benzoyl peroxide is used to treat infections in anaerobic wounds and is also an ingredient in cosmetic preparations for the treatment of acne.

Natural remedies—essential oils

Several essential oils, particularly *tea tree oil*, have gained widespread popularity as antimicrobial agents. A limited number of studies have been done on their effectiveness against various microorganisms. They should be used in accordance with the manufacturer's instructions to ensure they meet the claims for efficacy and to avoid undesirable side effects.

Refrigeration, freezing, drying

These methods are discussed here for completeness, but they are *not* methods of killing microorganisms. They are used mainly for control of microbial growth. Cold temperatures, such as are found in a domestic refrigerator (4°C), slow down the rate of cell division of most microorganisms but do not kill them. The rate of food spoilage can be slowed by refrigeration but, when the food is removed and warms to room temperature, the microorganisms will continue to multiply. Some microorganisms, called psychophiles, can grow at 4°C and thus spoil refrigerated food.

Freezing is a more effective method of food preservation, but it does not kill microorganisms either. The rate of growth is negligible, so the food does not spoil. Some microbial cells may be destroyed by the formation of ice crystals, which disrupt the cell membranes, especially if repeated freezing and thawing occurs, but freezing does *not* cause significant death of microbes. In fact, a standard method for the preservation of bacterial cultures is rapid freezing in liquid nitrogen at –70°C.

Drying preserves food by reducing moisture but does not necessarily kill the organisms present; nor does the process of freeze-drying (lyophilisation) which involves the removal of water under vacuum from a frozen solution. Freeze-drying is also used to preserve microorganisms, which remain viable for long periods (months to years) at room temperature as a lyophilised powder.

Chemical preservatives

Chemicals are used as preservatives in food because they inhibit microbial metabolism or growth. Organic acids such as sorbic acid and benzoic acid inhibit the growth of mould at low pH. Calcium propionate is used as a fungal inhibitor in bread. The benzoic acid derivatives, methylparaben and propylparaben, are used as preservatives in cosmetics and shampoos.

SUMMARY

- Infection control involves the application of procedures, processes and antimicrobial agents that eliminate microorganisms and/or prevent their transmission.
- The main methods for eliminating microorganisms or reducing their numbers on medical equipment and in the healthcare environment are cleaning, disinfection and sterilisation.
- Different methods of control are required for different situations and different types of organisms.

GENERAL PRINCIPLES OF MICROBIAL REMOVAL

- Cleaning is the mechanical removal of material (visible or not) from the surfaces of objects.
- Disinfection is a process that aims to destroy or remove most or all of the pathogenic microorganisms present on an object.
- Sterilisation is a process that completely destroys or removes all microorganisms, including bacterial endospores and viruses, from an object.

SELECTION OF METHOD FOR REMOVAL OF MICROORGANISMS

- Critical items—objects or materials that enter sterile tissue or the vascular system of a patient—must be sterile before use.
- Semi-critical items—equipment that comes into contact with a patient's non-intact skin or mucous membranes—should be free of all microorganisms, but small numbers of bacterial endospores are acceptable.
- A non-critical item—one that only comes into contact with intact skin—should be cleaned, and a low- or intermediate-level disinfectant used if decontamination is required.

CLEANING

- Thorough cleaning of an item to remove organic matter, dirt and grease is essential before a disinfecting or sterilising procedure is applied to it.
- Minimally touched surfaces (e.g. walls, floors) should be cleaned with a detergent solution when they are visibly dusty or soiled.
- Frequently touched surfaces (e.g. bedrails, over-bed tables) should be cleaned with a detergent solution at least daily, when visibly soiled and after known contamination.
- The cleaning of surfaces contaminated with blood or other body substances would usually involve cleaning followed by disinfection.

STERILISATION

- Sterilisation involves the complete destruction or removal of all living organisms from an object.
- The most practical method is moist heat sterilisation, which uses a machine called a steam steriliser or autoclave.
- Dry heat sterilisers are only suitable for sterilising equipment that is not harmed by long exposure to high temperatures.
- Incineration is used for the complete destruction of contaminated articles.
- Ionising radiation is a useful method for heat-sensitive, organic material such as pharmaceutical products for injection, and for disposable equipment.
- Filtration involves the removal of microorganisms from liquids or gases by passage through a filter with pores small enough to retain microorganisms. Ethylene oxide gas is a useful sterilising agent for articles made of rubber or other heat-sensitive components.
- Hydrogen peroxide vapour is used as a low-temperature sterilant suitable for heat- and moisture-sensitive equipment.

DISINFECTION

- Disinfection involves the removal or destruction of microorganisms to an 'acceptable level'.
- Thermal disinfection in healthcare facilities is achieved by the use of automated washer-disinfectors.
- UV radiation is useful for killing bacteria on clean surfaces and for disinfecting small air spaces.
- Microorganisms can be removed from air by the use of high-efficiency particulate air filters (HEPA).
- Phenolic disinfectants are widely used as they retain some activity in the presence of organic material and are inexpensive and stable.
- Chlorine, in the form of sodium hypochlorite (common bleach), is an effective disinfectant that is active against a range of microorganisms.
- Ethyl alcohol and isopropyl alcohol are antibacterial chemicals used in handrubs for the quick and effective disinfection of the hands.
- Glutaraldehyde is used to disinfect delicate heat-sensitive instruments to a high level.
- Antiseptics are chemical disinfectants that are mild enough to use on skin.

STUDY QUESTIONS

1. What is pasteurisation? Why is it used on milk?
2. What is a 'high-risk' (critical) item of equipment? Give some examples.
3. What is a 'semi-critical' item of equipment? Give some examples.
4. Why do instruments need to be cleaned before being treated with disinfectant?
5. What decontamination procedures should be followed for frequently touched surfaces?
6. When is a disinfectant necessary for cleaning of environmental surfaces in a hospital?
7. What is the difference between sterilisation and disinfection?
8. What are the advantages of moist heat sterilisation over dry heat sterilisation?
9. What are the commonly used autoclaving times and temperatures?
10. How do gamma rays have a sterilising effect?
11. What types of materials are sterilised by filtration?
12. What are the disadvantages of sterilisation with ethylene oxide?
13. What are the advantages of hydrogen peroxide plasma as a sterilising agent?
14. What is an antiseptic?
15. What is a HEPA filter, and what is it used for?
16. What are the advantages and disadvantages of phenolic disinfectants?
17. What is the value of bleach as a disinfectant?
18. What antiseptics are the most effective for skin disinfection?
19. What disinfectant is used to decontaminate blood spills?
20. List two chemicals that are used as preservatives.

TEST YOUR UNDERSTANDING

1. Explain what you understand by the term 'killing curve' when applied to sterilising or disinfecting procedures.
2. How do you decide whether equipment needs to be sterilised or disinfected?
3. Why is boiling instruments at 100°C not considered to be a method of sterilisation?
4. Why is sterilisation regarded as an 'absolute' process?
5. How can hazardous substances or infectious microorganisms be safely handled in the laboratory?
6. What factors determine the efficiency of the disinfecting process?
7. Why is chlorine used extensively for disinfection?
8. Why is it important not to reuse disposable equipment?

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CHAPTER

12

Antimicrobial therapy

CHAPTER FOCUS

- Describe the discovery and development of antimicrobial drugs.
- What are the principles underlying the effective use of antimicrobial drugs?
- How do antimicrobials kill, or inhibit the growth of, microbial cells?
- What guidelines are used when prescribing antimicrobial drugs?
- What are the responsibilities of the nurse in the administration of antimicrobial therapy?
- How do microorganisms become resistant to antimicrobial drugs?
- What are the consequences of the development of resistance to antimicrobial drugs?
- What strategies can be used to minimise the emergence and spread of resistant microorganisms?

INTRODUCTION

The discovery of antibiotics and the development of a range of antimicrobial drugs greatly reduced the incidence of morbidity and mortality from infectious diseases in the 20th century. Today most people living in the developed nations assume that their doctor can prescribe a pill that will cure most infections. So, when someone dies from an infectious disease or contracts a serious infection in hospital, it is often seen as due to a failure in the health system.

However, antimicrobial drugs to treat infections have only been available since the middle of the last century and their usefulness is currently being threatened by the emergence of resistant strains of many pathogens. Some of the most serious are the multi-resistant strains of staphylococci and tuberculosis and the carbapenem-resistant strains of Enterobacteriaceae.

The World Health Organization (WHO) has called for a global strategy to overcome the problem of increasing resistance to antimicrobial drugs. In Australia the National Health and Medical Research Council (NHMRC) has set up an expert advisory group to provide continuing advice on antimicrobial resistance and related matters. One of these initiatives is the introduction in hospitals of the concept of 'antimicrobial stewardship' (see page 283).

It is important for health professionals to understand how antibiotics were discovered, used and misused, so that we can protect the future of these valuable therapeutic agents.

In Chapter 1, we described how the work of Robert Koch and other scientists resulted in the isolation and identification of the organisms responsible for most of the known infectious diseases of bacterial origin. At that time there were no methods available to cure patients suffering from these infections. Various physical and chemical methods that killed or inactivated microorganisms had been developed to control the spread of infection (see Chapter 11), but the chemical disinfectants used to kill bacteria on skin, wounds and tissues were relatively non-specific in their action, so the treatment of infectious diseases relied mainly on supportive methods that alleviated the symptoms. Thus, despite all the advances in knowledge of the causes of infection, at the beginning of the 20th century, one-third of all children died from an infectious disease before the age of 5.

THE ORIGINS OF CHEMOTHERAPY

For centuries, extracts from plants and herbs have been used to treat illness. We now know that some of these preparations contain valuable therapeutic drugs. For example: aspirin was first extracted from the bark of the willow tree; morphine is derived from the seeds of the opium poppy; quinine, used against malaria, comes from the bark of the chinchona tree; and digitalis, used in the treatment of heart disease, was first extracted from foxglove leaves. The anti-fever properties of the Qinghao plant (*Artemisia annua*) were first described in China in 340 BC. This plant is also known as the annual or sweet wormwood. The active ingredient of Qinghao was isolated by Chinese scientists in 1971. Known as artemisinin, it is today a very potent and effective antimalarial drug, especially in combination with other medicines.

Ancient Chinese culture has traditionally used combinations of herbs to treat illness. The mouldy soybean preparation that was placed on infected wounds probably contained small amounts of an antibiotic such as penicillin. Plant extracts were used in a variety of ways—as medicinal teas, as inhalants, or as poultices placed on wounds or inflammation. Many of these treatments were successful, but the mechanism by which they effected a 'cure' was, and still is, largely unknown. These alternative remedies are becoming increasingly popular in Australia, but many of them have not been subjected to rigorous clinical trials. It is important for health professionals to be aware of the regulations regarding the licensing and prescribing of therapeutic drugs. Any product for which therapeutic claims are made must be entered in the Australian Register of Therapeutic Goods (ARTG) before it can be supplied in Australia.

The magic bullet

The idea of finding a chemical substance that could specifically kill the microbes responsible for an infection without damaging the host cells (i.e. a 'magic bullet') was proposed by the chemist Paul Ehrlich in 1908. It was known that arsenic was toxic to many bacteria, but it was also toxic to humans. Ehrlich set about trying to synthesise an organic compound containing arsenic that would retain the antimicrobial properties but would be less toxic to humans. The compound he prepared was called *salvarsan* and was found to be effective against spirochaetes—in particular, the bacterium responsible for the sexually transmissible disease syphilis, *Treponema pallidum*. At that time, syphilis was very common. It was incurable and, in later stages had very unpleasant symptoms that were usually fatal. Salvarsan was a great improvement on the use of mercury compounds, which was the only treatment available at that time. It is no longer used, having been replaced by antibiotics.

Ehrlich's work opened up a whole new approach to the treatment of disease. Chemists tried to repeat his success with salvarsan by synthesising various chemical compounds, but most of them were found to be too toxic for human use. However, the new science of chemotherapy had been created.

Chemotherapy involves the introduction of a specific chemical compound, or drug, into the body in order to elicit a particular desired response, preferably without causing harm to the patient. It includes the use of drugs to kill or inhibit microorganisms, as well as drugs used to treat other diseases such as cancer.

Sulfa drugs

Inspired by Ehrlich's synthesis of salvarsan, another chemist, Gerhard Domagk in Germany, tested a dye called prontosil for its ability to kill streptococci. He found that it was active in animal tissues, where it was converted to a compound called **sulfanilamide**, a potent inhibitor of bacterial replication. The discovery of sulfanilamide led to the synthesis of a large number of similar drugs, collectively named **sulfonamides**, or **sulfa drugs**. These sulfa drugs exhibit activity against a variety of pathogens. However, many organisms have been found to develop resistance to them and they frequently cause side effects such as skin rashes.

There are now few specific indications for the clinical use of sulfonamides alone. They are used mainly in combination for the treatment and prophylaxis of *Pneumocystis jiroveci* pneumonia and malaria, and for the topical treatment of severe burns, or when no other suitable drugs are available.

The discovery of antibiotics

A breakthrough in the treatment of infections came with the discovery of antibiotics. In 1928, at about the same time the sulfa drugs were being developed, an English doctor, Alexander Fleming, left a culture of *Staphylococcus aureus* on an agar plate in the laboratory for a few days. He noticed that the growth of the bacteria had been inhibited in the area of the plate that had been contaminated by a common blue/green mould. He correctly deduced that the mould had produced a chemical that diffused through the agar and killed the bacteria. He called the chemical **penicillin**, from the name of the mould *Penicillium*. Surprisingly, he did not realise the therapeutic value of his discovery and it was not until the outbreak of World War II that interest in the compound as a treatment for serious infections was revived.

At that time, an Australian physician, Howard Florey, was working at Oxford University and became interested in penicillin as a chemotherapeutic agent. In collaboration with the chemist Ernst Chain, he was largely responsible for the project that resulted in the isolation and purification of penicillin and the clinical trials that were necessary to establish it as a 'miracle cure'. With the backing of the United States government, they undertook the production of large quantities of penicillin that were used successfully to treat infections in soldiers wounded in the war.

The dramatic results obtained with penicillin encouraged scientists to look for other microorganisms that produced substances that might also have antimicrobial activity.

The term **antibiotic** was coined to describe *a compound produced by a microorganism that in small amounts can kill or inhibit another microorganism*. Antibiotics are natural products of certain microorganisms, including some fungi, actinomycetes and bacteria, many of which are found in soil. More than half the antibiotics in use are produced by different species of filamentous soil bacteria belonging to the genus *Streptomyces* (see Figure 12.1). Although many antimicrobials are derived from natural sources, a number of them have been chemically modified in order to improve their antimicrobial properties and are therefore called 'semi-synthetic'.

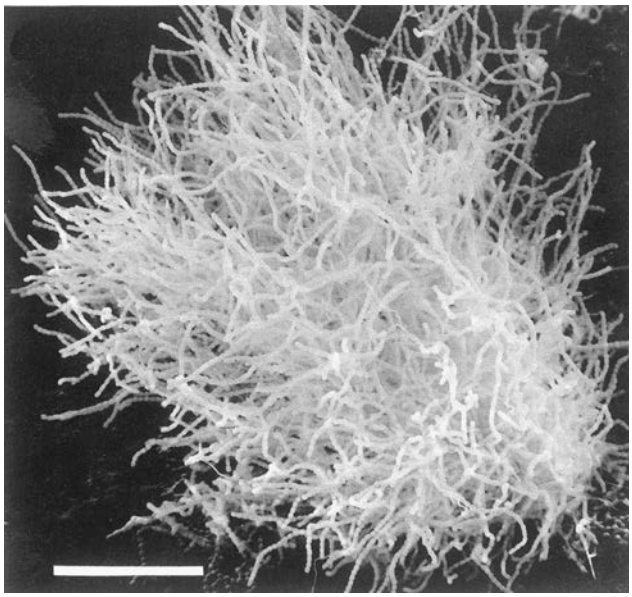


FIGURE 12.1

Scanning electron micrograph of a *Streptomyces* sp. which produces the antibiotic actinomycin, showing aerial mycelium with straight chains of spores

Source: Dr D. Ipek Kurtboke and Prof. Romano Locci, University of Milan, Italy.

THE DEVELOPMENT OF ANTIMICROBIAL DRUGS

Selective toxicity

The principle of **selective toxicity** is fundamental to the successful development, production and use of any chemotherapeutic agent. It requires that the drug is able to kill or inhibit the microorganism responsible for the disease without causing damage to the host cells. It is relatively easy to find chemicals that can kill microorganisms—it is not so easy to find ones that can do so without harming the human host. In order to find a suitable drug it is necessary to identify a reaction or structure that is unique to the target microorganism but which does not occur in, or is not essential to, the host cell. The drug must be able to alter or damage the microorganism in such a way as to either prevent replication, or cause the death of the microbial cell. The principle of selective toxicity, therefore, exploits any differences in structure or metabolism between the microbial cell and the host cell.

Procaryotic (bacterial) cells have a number of characteristics that are unique and provide possible sites of attack

(see Chapter 3). It is more difficult to identify points of difference in eucaryotic microorganisms, because they have more structural and functional similarities to human cells. It is even more difficult to design antiviral drugs because viral replication involves taking over the host (human) cell, so any compound that interferes with viral replication is also likely to damage the host cell. Sometimes, a drug will be considered to be selectively toxic because of the greater affinity of the drug for the microbial enzyme than for the host cell enzyme.

Activity of antimicrobial drugs

So far we have described the discovery of two different kinds of compounds that affect microbial growth. Antibiotics such as penicillin are naturally occurring substances, whereas the sulfa drugs are completely synthetic. Attempts by scientists to find effective antimicrobial agents have resulted in the production of a range of naturally occurring, synthetic and semi-synthetic drugs, with varying activity against the different types of microorganisms.

The term **antimicrobial** is used to describe a compound that is able to kill, or inhibit the growth of, a microorganism. Strictly speaking, antibiotics are a particular type of naturally occurring antimicrobial compound, but in practice the terms 'antibiotic' and 'antimicrobial' are used interchangeably. Thus, an 'antibiotic sensitivity test', performed in a diagnostic microbiology laboratory, should correctly be called an 'antimicrobial sensitivity test' because it tests the susceptibility of a particular microorganism to a range of compounds, some of which are naturally occurring, but some of which may be chemically modified antibiotics, semi-synthetic (e.g. amoxycillin) or completely synthetic (e.g. sulfa drugs and quinolones).

We can further separate antimicrobial drugs into groups depending on the type of organism they are active against. Thus, **antibacterials** are active against bacteria, **antivirals** against viruses, **antifungals** against fungi, and **antiprotozoals** against protozoa. The reason that drugs tend to be active against only one type of organism (e.g. antibacterials do not kill viruses) is related to their mechanism of action. To selectively kill or inhibit the microorganism, the drug must attack or interfere with a structure or a step in a metabolic or replicative process that is unique to the organism and preferably not present in the host cell. Since different types of microorganisms have different cellular components and metabolic processes, the drugs that are most effective against one type of organism may not affect another type.

Compounds that are active against a number of different microorganisms are called **broad spectrum antimicrobial agents**, while those with a limited range of activity are called **narrow spectrum antimicrobial agents**. Some agents are able to kill microorganisms and are identified by the addition of the suffix *-cidal*; for example, penicillin is **bactericidal**. Others inhibit the replication of the organism without killing it and have the ending *-static*; for example, tetracycline is **bacteriostatic**.

These properties are also related to the drug's mechanism of action. In general, drugs that are bactericidal result in irreversible damage to a cell (e.g. the formation of an unstable cell wall) so that the cell is no longer viable. In contrast, agents that are bacteriostatic interfere with metabolism or the synthesis of a compound such as an enzyme protein, which prevents replication but does not necessarily cause the death of the cell. Their effect is thus reversed once they are removed from the cell. Even though they do not kill the cell, bacteriostatic agents are useful because they prevent further replication of the pathogen and allow the host's own immune system time to mount its defences against the infection.

When possible, it is preferable to use a bactericidal agent, especially for immunocompromised patients whose own defences are not able to complete the task of eliminating the pathogens from the body.

One of the major problems associated with the use of antimicrobials is the development of resistance to the antimicrobial drug. This can take many forms and is discussed later in this chapter.

Therapeutic Guidelines—Antibiotic, published by Therapeutic Guidelines Ltd, lists the antimicrobial drugs that are approved for use in Australia and describes in detail recommendations for the management of infections in various body systems. The guidelines are updated regularly, and students are referred to the latest edition for specific details of prescribing practice. A description of the general principles of antimicrobial therapy is given here and is useful for all health professionals. Students should be aware of the nomenclature used for drugs. The **generic name** is the chemical name of the compound. Different drug companies may market the same compound under different names—the **trade name**. For example, the sulfa drug cotrimoxazole consists of a mixture of sulfamethoxazole and trimethoprim (chemical names) and is marketed as Bactrim® or Septrim® by different drug companies.

ANTIBACTERIAL DRUGS

Procaryotic organisms (bacteria) are free-living single-celled organisms with several unique characteristics which distinguish them from their host cells.

There are four main target sites for selective antibacterial action (see Figure 12.2):

- synthesis of the bacterial cell wall
- protein synthesis
- nucleic acid synthesis
- function of the bacterial cell membrane.

Compounds that affect the cell wall

Beta-lactams

The integrity of the cell wall of a bacterium is essential to maintain its shape and provide structural support for the cell. Animal cells are enclosed only by a membrane, so the bacterial cell wall is a very suitable target for antimicrobial action. Peptidoglycan, a major component of the bacterial wall, is unique to bacteria and thus provides an ideal target for selective attack (see Chapter 3). A number of antibacterial drugs have been found that exert their action by interfering with

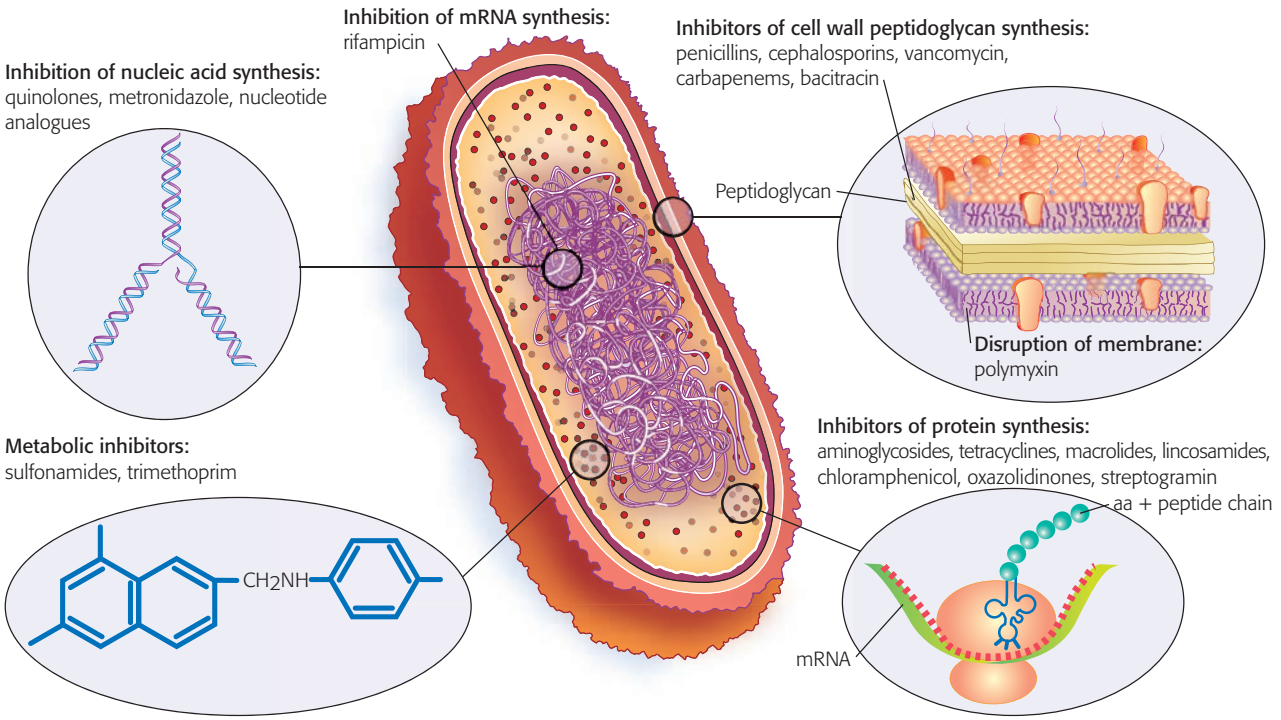


FIGURE 12.2

A summary of the major modes of action of antibacterial drugs

This diagrammatic representation of a bacterial cell shows the mechanism of action of different antimicrobial drugs.

the synthesis of peptidoglycan. One of the most important of these is **penicillin**, which belongs to a group of antibacterials known collectively as **beta-lactams**. Beta-lactam antibacterials derive their name from the four-membered beta-lactam ring which is present in all these compounds (see Figure 12.3).

Resistance to these compounds is frequently associated with the production of an enzyme, **beta-lactamase**, which breaks open this ring and destroys the antibacterial compound. Other members of the beta-lactam group are the cephalosporins, carbapenems and monobactams (see Figure 12.4 and Table 12.2, page 266).

The beta-lactams inhibit cell wall synthesis by combining with the transpeptidase enzyme responsible for cross-linking of the peptidoglycan chains. Thus, these drugs are mainly active against growing cells, preventing the formation of a rigid cell wall structure. This results in a cell without a protective support layer, making it vulnerable to cell lysis (i.e. a bactericidal effect).

Beta-lactam drugs are particularly useful because they affect the synthesis of a structure unique to bacteria and are thus of low toxicity to humans. A small number of people develop an allergy to them; this is thought to be due to the formation of a conjugate of the beta-lactam ring with serum proteins, which elicits an inflammatory immune response, or to impurities in the product. People who are truly allergic to penicillin are often also allergic to most other beta-lactam antibacterials (see page 278 and Case History 12.1: Nursing management of a pneumonia patient, page 279).

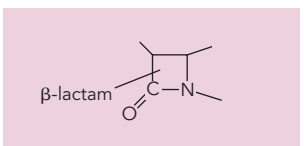


FIGURE 12.3

The beta-lactam ring

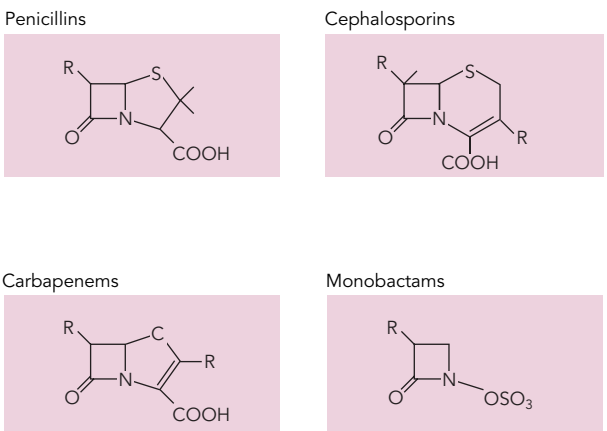


FIGURE 12.4

Comparison of the structures of the beta-lactam inhibitors of cell wall synthesis

Different drugs in each class are synthesised by the substitution of different chemical groups for the R-side chain.

Penicillins

Penicillin is isolated from the mould *Penicillium chrysogenum* and occurs naturally in a number of closely related forms (see Table 12.1). These include penicillin G (benzyl penicillin), which is extremely effective but readily inactivated by the low pH encountered in the stomach. It is usually administered intramuscularly (IM) or intravenously (IV) as the sodium or potassium salt. Another commonly used natural penicillin, phenoxymethylpenicillin (penicillin V), is acid-stable and can be taken orally, although it is not well absorbed and is excreted rapidly.

Research has been directed at developing semi-synthetic penicillins in an effort to improve the efficacy of the naturally occurring compounds and overcome problems such as susceptibility to gastric acid, lack of water solubility, rapid excretion (= short retention) time, narrow spectrum of activity, susceptibility to beta-lactamase enzymes and the development of resistance.

Table 12.1 lists the major penicillins in use. The naturally occurring penicillins are active mainly against Gram-positive organisms, whereas the newer, semi-synthetic compounds have a broader spectrum of activity. For example, ampicillin, amoxycillin, piperacillin and ticarcillin are also active against Gram-negative organisms. Penicillins that are resistant to breakdown by beta-lactamases include methicillin, flucloxacillin and dicloxacillin.

Clavulanic acid (a naturally occurring compound extracted from a streptomycete), sulbactam and tazobactam are compounds that inhibit the beta-lactamase enzymes produced by a number of bacteria. They do not have significant antibacterial activity on their own but, when clavulanate is combined with amoxycillin in Augmentin®, or with ticarcillin in Timentin®, their spectrum of activity is extended.

Cephalosporins

Cephalosporins are produced by a species of the marine fungus *Cephalosporium*. They also inhibit synthesis of the peptidoglycan of the cell wall and have a similar structure to the penicillins, containing a beta-lactam ring (Figure 12.5). They are more active against Gram-negative organisms than the penicillins and chemical modification of the side chains of the cephalosporin nucleus has given rise to a series of compounds with increased antibacterial activity, known as second-, third- and fourth-generation cephalosporins (see Table 12.2). The search for new drugs has provided new cephalosporin derivatives that are made available from time to time. They are now widely used for the treatment of all types of infections in hospitals, the choice of drug being determined by its mode of administration and spectrum of activity, and by its ability to be absorbed and penetrate to the site of infection.

Ceftriaxone is not recommended for use in neonates, and its use should be monitored closely as it can form harmful precipitates if administered in conjunction with calcium-containing solutions.

Cephalosporins have the basic chemical structure illustrated here

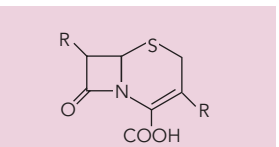


FIGURE 12.5

Cephalosporins

TABLE 12.1 **The penicillins**

PENICILLIN	ROUTE OF ADMINISTRATION	SPECTRUM OF ANTIBACTERIAL ACTIVITY
Natural penicillins		
Benzyl penicillin (penicillin G)	IV, IM	Streptococci, pneumococci, meningococci
Phenoxymethyl penicillin (penicillin V)	Oral	Gram-positives, esp. streptococci
Semi-synthetic penicillins		
Ampicillin	Oral, IM, IV	As for penicillin G plus *E. coli, Haemophilus influenzae*
Amoxycillin		
Amoxycillin + clavulanate (Augmentin®)		Active against β-lactamase producers
Dicloxacillin	Oral, IM, IV	Mostly staphylococci
Flucloxacillin		
Ticarcillin	IM, IV	Pseudomonads
Ticarcillin + clavulanate (Timentin®)		Active against β-lactamase producers
Piperacillin	IM, IV	Pseudomonads
Piperacillin + tazobactam		Gram-negatives Enterococci, Klebsiella

Note: Methicillin is no longer widely used because of nephrotoxicity.

TABLE 12.2 Some cephalosporins and their derivatives

CEPHALOSPORIN	ROUTE OF ADMINISTRATION
First generation—moderate spectrum	
Cephalothin	IM, IV
Cephalexin	Oral
Cephazolin	IM, IV
Second generation—moderate spectrum	
Cefaclor	Oral
Cefuroxime	Oral
Cephamandole	IM, IV
Cefoxitin	IM, IV
Cefotetan	IM, IV
Third generation—broad spectrum	
Cefotaxime	IM, IV
Ceftriaxone	IM, IV
Ceftazidime	IM, IV
Fourth generation—extended spectrum	
Cefepime	IM, IV
Cefpirome	IM, IV

Carbapenems

Carbapenems are synthetic beta-lactam compounds with a wide spectrum of activity and are stable against a wide range of beta-lactamase producers, including enteric Gram-negative bacteria and *Pseudomonas aeruginosa*. They include imipenem, doripenem and meropenem. **Imipenem** is rapidly broken down in the kidney, but high concentrations of the drug can be achieved in the urine and serum if it is administered in combination with the enzyme inhibitor cilastin. Imipenem causes neurological side effects such as convulsions and is being replaced by **meropenem**, which is not metabolised by the renal cells. **Ertapenem** is a newer drug with similar properties to imipenem but with a long half-life so that it need be administered only once daily. However, it has poor activity against *Pseudomonas*, enterococci and *Acenitobacter* species. Carbapenems are available only for parenteral use and so are generally used in hospitals for the specific treatment of pathogenic bacteria that are resistant to other antibiotics. The recent emergence of bacterial strains that produce metallo-beta-lactamase enzymes that inactivate carbapenems is of concern, as the carbapenems represented the last line of defence against some hospital organisms (see Spotlight Box: Global spread of carbapenem resistance, page 282).

Monobactams

Monobactams (e.g. aztreonam) are active against aerobic Gram-negative bacteria (except *Pseudomonas*). They are useful for the treatment of urinary tract infections, septicaemia and gonorrhoea, and are not inactivated by Gram-negative beta-lactamase enzymes. They may be used for the treatment of Gram-negative infections in patients who cannot tolerate aminoglycosides because of impaired renal function, and can be used for patients with penicillin hypersensitivity.

Other cell wall inhibitors

The glycopeptides, **vancomycin** and **teicoplanin**, are large molecules that interfere with cell wall synthesis by binding to the growing peptide chains that are part of the peptidoglycan molecule, thus preventing further synthesis of the cell wall. Because of their size, these compounds cannot penetrate the Gram-negative outer membrane and so they are active mainly against Gram-positive organisms. (Exceptions are the *Flavobacteria* and some *Neisseria* spp.)

Vancomycin is poorly absorbed from the intestine. It *must* be administered by slow intravenous infusion over 1–2 hours in order to prevent histamine release and the 'red man syndrome', which can lead to cardiac arrest (see Case History 12.2: Drug administration, page 280). The drug is important as a last resort treatment for methicillin-resistant staphylococcal infections (MRSA), but there is an increasing number of reports of the isolation of strains of MRSA with reduced susceptibility to vancomycin (called VISA or GISA—vancomycin/glycopeptide intermediate *Staphylococcus aureus*). Although vancomycin is useful as a last resort, it is not as effective as the β-lactam drugs in the treatment of *S. aureus* bacteraemia and increasingly higher concentrations are needed to have an effect. The emergence of vancomycin-resistant enterococci (VRE) is also of concern, especially among seriously ill patients in intensive care units and renal units where vancomycin is also used intermittently (see Chapter 13, page 293). It should be used only in situations where other drugs are not effective.

Inhibitors of protein synthesis

The mechanism of protein synthesis is essentially the same in eucaryotic and procaryotic organisms. However, there are slight differences in the relative sizes and binding properties of the ribosomes in the two types of cell that permit some degree of selective toxicity. Antibacterials that inhibit protein synthesis act by interfering either with the translation of the messenger RNA into protein, or with the binding of the mRNA to the ribosomes. The 70S ribosomes in bacterial cells (made up of a 50S and 30S subunit) are smaller and less dense than the 80S eucaryotic host cell ribosomes. Therefore, drugs that target the 70S ribosomes are able to affect the bacterial cells adversely, while not binding significantly to the host ribosomes. However, eucaryotic mitochondria also contain 70S ribosomes, so drugs that inhibit protein synthesis in bacteria can also affect the mitochondria of the host cells.

A number of different groups of antimicrobials affect protein synthesis in bacterial cells. They include the aminoglycosides, the tetracyclines and the macrolides, as well as lincosamides, chloramphenicol and fusidic acid. Two newer

classes of compounds are the oxazolidones and streptogramins. Compounds that inhibit the synthesis of protein in the cell may be bactericidal or bacteriostatic.

Aminoglycosides

This family of antibacterial agents contains a number of useful drugs, including streptomycin, gentamicin, tobramycin, amikacin and neomycin (see Table 12.3). **Aminoglycosides** are most useful in the treatment of sepsis due to Gram-negative aerobes and *Mycobacterium tuberculosis*. Their action is bactericidal as they cause misreading of the messenger RNA, leading to substitution of the wrong amino acid into the peptide chain, thus forming an inactive protein. They are often used synergistically in combination with another drug to enhance the therapeutic effect. For example, penicillin or ampicillin may be used in combination with gentamicin. The penicillins open up the cell wall, allowing greater penetration by the gentamicin.

Aminoglycosides are not absorbed from the intestine so are administered intramuscularly or intravenously for systemic infections, and must be monitored carefully for toxic side effects. These include **ototoxicity** (vertigo and deafness) and **nephrotoxicity** (kidney damage). They should be used cautiously in patients with impaired renal function.

Gentamicin is the aminoglycoside of choice in hospitals for the short-term treatment of healthcare-associated infections, especially Gram-negative coliforms and *Pseudomonas* infections. Tobramycin is not as active against Gram-negative organisms.

Amikacin is resistant to inactivation by enzymes but is expensive, so it is reserved for use with organisms resistant to other aminoglycosides.

Streptomycin, isolated from *Streptomyces griseus*, was one of the earliest aminoglycosides identified and was used extensively in the treatment of tuberculosis. It is now rarely used due to its toxicity and the fact that resistance to streptomycin develops rapidly.

Neomycin is very toxic but is poorly absorbed, and so is used mainly in topical preparations.

Tetracyclines

Tetracyclines are a group of broad spectrum bacteriostatic agents that were isolated from the members of the genus *Streptomyces*. They are bacteriostatic, inhibiting protein synthesis by preventing the attachment of amino acids to ribosomes. They have a broad spectrum of activity against both Gram-negative and Gram-positive organisms, intracellular chlamydiae and rickettsiae, mycoplasmas and spirochaetes, as well as some non-tuberculous mycobacteria and protozoa. They are useful in the treatment of non-specific urethritis (NSU) since they are active against both *Neisseria gonorrhoea* and *Chlamydia*.

Tetracyclines are used in the treatment of pelvic inflammatory disease, periodontal disease, brucellosis, plague, cholera, Lyme disease and community-acquired pneumonia, and for prophylaxis against all strains of malaria where endemic strains are resistant to other common antimalarial drugs.

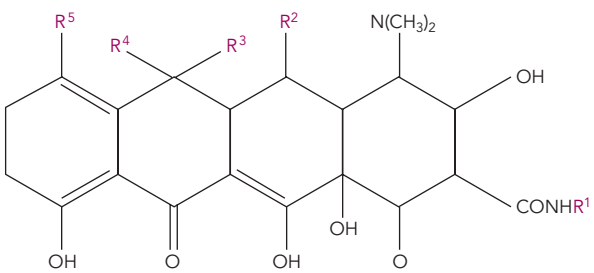
Tetracyclines can be administered orally, and modification of the side chains (see Figure 12.6) has produced a number of useful compounds that are better absorbed and have longer retention times in the body than the parent compound. Side effects of the tetracyclines are due mainly to their broad spectrum of activity, which can cause suppression of the normal flora and lead to a superinfection or overgrowth of undesirable organisms such as *Candida albicans* (thrush).

Tetracyclines should not be given to pregnant women as they may affect liver function, or to young children as the drug is deposited in developing teeth, causing permanent yellow discolouration, and it may interfere with bone formation. Resistance to tetracyclines develops easily, and their widespread use in animal feeds is thought to have contributed to the transfer of resistant strains of bacteria to humans.

TABLE 12.3 Aminoglycosides

AMINOGLYCOSIDE	ROUTE OF ADMINISTRATION	SPECTRUM OF ACTIVITY
Gentamicin, Tobramycin, Amikacin	IM, IV	Gram-negative, may be used with penicillins
Streptomycin, Amikacin	IM, IV	Tuberculosis in combination with other drugs
Neomycin, Kanamycin	Topical or oral for 'gut sterilisation'	Gram-positive, coliforms

TETRACYCLINE STRUCTURE



	R^1	R^2	R^3	R^4	R^5
Tetracycline	H	H	CH_3	OH	H
Chlortetracycline	H	H	CH_3	OH	Cl
Oxytetracycline	H	OH	CH_3	OH	H
Doxycycline	H	OH	CH_3	H	H
Minocycline	H	H	H	H	$H(CH_3)_2$

FIGURE 12.6

Tetracycline structure

Tetracyclines are complex molecules consisting of four rings with five different sites for substitution. This gives rise to a family of molecules that differ more in their pharmacological properties than spectrum of activity.

Doxycycline is the preferred tetracycline in most situations, as it can be administered once daily. It can irritate the oesophagus, so patients should be instructed to wash it down with water and remain upright for 30 minutes after taking it. It can also be used safely for long periods in low doses for the treatment of acne.

Macrolides

The **macrolides** are a group of antibacterials with a complex structure containing a macrocyclic (14-membered) lactone ring (see Figure 12.7).

Macrolides are widely used for community-acquired respiratory infections. They have a broad spectrum of activity against Gram-positive and Gram-negative aerobes, including *Legionella*, *Corynebacteria*, *Mycolplasma* and *Chlamydia*. They are not effective against Gram-negative rods.

The best-known macrolide is **erythromycin**, which is used extensively as an alternative drug for people who are allergic to penicillin. Its action is bacteriostatic, binding to 50S ribosomes and preventing the release of transfer RNA (tRNA) after peptide bond formation. It has a wide spectrum of activity against Gram-positive and Gram-negative cocci, but is not active against Gram-negative rods except *Campylobacter* spp. It is the drug of choice against *Legionella pneumophila* (Legionnaires' disease) and *Bordetella pertussis* (whooping cough). It is also active against *Mycoplasma* and *Chlamydia*. It is not affected by beta-lactamases and has been used extensively for streptococcal infections, especially in children, as it can be administered orally in a pleasant-tasting syrup. However, some resistance has been reported in both streptococci and staphylococci. Clinically, it is used for a variety of community-acquired respiratory infections, tonsillitis, bronchitis and pneumonia, as well as for non-specific urethritis.

Newer macrolides are **azithromycin**, **roxithromycin** and **clarithromycin**. They have better absorption and a longer shelf life than erythromycin. Clarithromycin has a similar spectrum of activity to erythromycin but is also active against *Mycobacterium avium intracellulare*, an opportunistic infection seen in immunocompromised patients. It is used in the treatment of *Helicobacter pylori* infection associated with gastric ulcers. Azithromycin is also used against *Legionella pneumophila* (Legionnaires' disease) and *Bordetella pertussis* (whooping cough) and is recommended for the treatment of community-acquired pneumonia (CAP). It is effective in single doses for chlamydial infections such as trachoma.

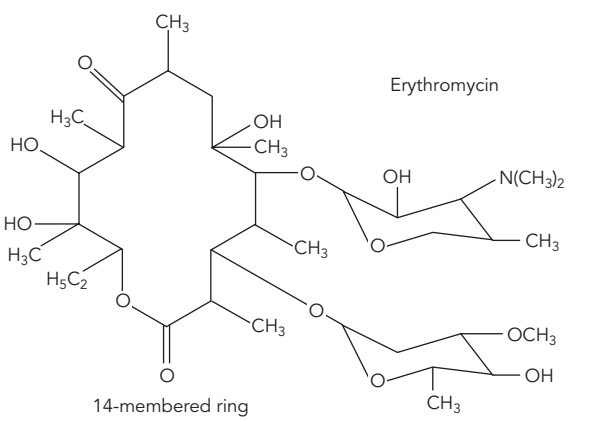


FIGURE 12.7

Structure of the macrolide antibiotic, erythromycin

Oxazolidinones, streptogramins and lincosamides

These new classes of drugs inhibit protein synthesis by binding to 50S ribosomal subunits and interfering with the translation of mRNA to protein.

The **oxazolidinone** drug, **linezolid**, is useful for treating MRSA. In addition, it has activity against streptococci, enterococci (both *Enterococcus faecalis* and *E. faecium*, including VRE), mycobacteria and anaerobes. It can be administered orally or by IV infusion.

Streptogramin (**Q/D**) is a fixed 30/70 combination of two semisynthetic derivatives of pristinamycin: a naturally occurring streptogramin, Quinupristin or streptogramin b, and Dalfopristin or streptogramin a. Q/D is bactericidal for streptococci and staphylococci, including strains resistant to other antibiotic classes. Q/D inhibits *E. faecium*, including vancomycin-resistant strains. It has to be administered via a central venous catheter.

The lincosamides, **lincomycin** and its chlorinated derivative, **clindamycin**, have been widely used in hospitals against Gram-positive aerobes and most anaerobes (*Clostridium* spp. and *Bacteroides*), especially for patients who are allergic to penicillin. They penetrate tissue well and are particularly useful for bone and joint infections. However, a major side effect is the occurrence of antimicrobial-associated diarrhoea—in particular, pseudomembranous colitis caused by *Clostridium difficile*, which is resistant to these drugs. 'Hypervirulent' strains of *C. difficile* have appeared overseas and pose a serious problem in hospitals. The strain has also been reported in Australia (see Case History 13.3: Hypervirulent *Clostridium difficile*, Chapter 13, page 296). Although other antibiotics have also been shown to give rise to this condition, it is particularly frequent following treatment with lincomycin or clindamycin and so their use has been restricted. Clindamycin is used for treatment of streptococcal toxic shock syndrome, as it appears to interfere with superantigen production.

Chloramphenicol

Chloramphenicol has a simple chemical structure (Figure 12.8) and a wide spectrum of activity against

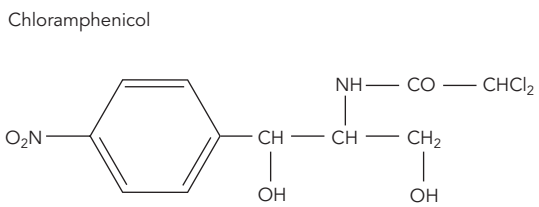


FIGURE 12.8

Structure of chloramphenicol

both Gram-positive and Gram-negative organisms. It was originally isolated from *Streptomyces* but is now synthesised chemically. It is bacteriostatic, inhibiting protein synthesis by binding to the 50S ribosomal subunit and preventing peptide bond formation by the enzyme peptidyl transferase. However, it is also able to bind to human mitochondrial 50S ribosomes, which may explain its toxicity to bone marrow.

Toxicity to chloramphenicol is of two types. The first is a reversible suppression of the bone marrow. The other is a rare type of toxicity (1 in 30 000) involving suppression of red blood cell synthesis in the bone marrow, leading to irreversible aplastic anaemia. The drug is metabolised in the liver, combining with glucuronic acid to be excreted via the kidneys in an inactive form. It is particularly toxic to neonates because they have immature liver enzyme systems. Because of its toxicity, the use of chloramphenicol has been limited to topical eye preparations. It is sometimes used to treat typhoid fever and, since it penetrates readily into the CSF, can be used for the treatment of some forms of bacterial meningitis.

Fusidic acid

Fusidic acid is a bacteriostatic agent that inhibits protein synthesis by complexing with the growing peptide chain. It is active against Gram-positive cocci and is useful in the treatment of staphylococcal infections that are resistant to the beta-lactams, or in patients allergic to penicillin. Resistance to fusidic acid develops easily, so it should always be given in combination with other drugs (e.g. rifampicin).

Inhibitors of nucleic acid synthesis

Antibacterials that interfere with nucleic acid synthesis do so either by inhibiting the synthesis of the nucleotide precursors or by inhibiting DNA or RNA replication.

Sulfonamides

The sulfonamides were among the first synthetic chemical compounds with antibacterial properties. The most commonly used compounds are structural analogues of para-aminobenzoic acid (PABA). They exert their action by competing with PABA in the synthesis of tetrahydrofolic acid (THFA), a compound that is a precursor of the purines and pyrimidines required for nucleic acid synthesis. The basis for the selective toxicity of the sulfonamide drugs is the fact that most bacteria have a pathway of synthesis of THFA, whereas humans are unable to synthesise folic acid and so have a requirement for it in their diet. Drugs that interfere with its synthesis will therefore not affect human cells.

Sulfonamides are broad spectrum bacteriostatic agents active against both Gram-positive and Gram-negative organisms. They can be administered orally and had widespread use in the past for urinary tract infections, but their use has been associated with significant side effects. Sulfonamides, particularly in the elderly, can cause exfoliative dermatitis and may (rarely) be the cause of a fatal bone marrow suppression.

Trimethoprim is an analogue of the pyrimidine bases and competes for the enzyme dihydrofolate reductase, which converts dihydrofolic acid to tetrahydrofolic acid. Although the enzyme is also present in human cells, the selective toxicity of the drug depends on the far greater affinity of trimethoprim for the bacterial enzyme than for the human enzyme. **Cotrimoxazole** (Bactrim®, Septrin®), which combines sulfamethoxazole with trimethoprim, is no longer widely recommended for use. Trimethoprim alone is effective in the treatment of urinary tract infections. The current *Therapeutic Guidelines—Antibiotic* recommends that the combined preparation be restricted to clinical conditions where it is the treatment of choice—for example, *Pneumocystis jiroveci* prophylaxis and pneumonia in AIDS or other immunocompromised patients, *Listeria monocytogenes*, *Salmonella typhi* and *Nocardia* infections. Sulfa drugs are also used for the treatment of melioidosis, and of Q fever in pregnant women and children under 8 years, where doxycycline is contraindicated.

Trimethoprim alone or in combination with sulfamethoxazole may cause nausea.

Quinolones

Quinolones are a group of newer synthetic chemical agents that inhibit the activity of the enzymes, DNA gyrase and topoisomerase that are involved in unwinding the DNA coils during replication. Their action is specific to the bacterial enzymes and so is selectively toxic. The compounds in use are analogues of **nalidixic acid** and have a broad antibacterial activity. They can be administered orally, reach good serum concentrations and are well distributed in the body.

Norfloxacin is used to treat urinary and gastrointestinal infections. **Ciprofloxacin** has the broadest range of activity against Gram-negative bacteria and is the only drug active against *Pseudomona aeruginosas* that can be administered orally. **Moxifloxacin** and **ofloxacin** are extended spectrum fluoroquinolones, effective against Gram-positive bacteria such as those causing pneumonia. Their advantage is that they can be administered orally or parenterally once daily.

Side effects of quinolones include nausea, photosensitivity and neurological disturbances, so it is recommended that they are only used for infections that do not respond to other drugs. Convulsions have been reported, especially in patients with a history of epilepsy or other predisposing factors to seizures. They should be used in lower doses in patients with impaired renal function. They have been shown to damage the joints of immature animals so should not be used in children or pregnant women.

Rifamycins

Rifampicin and rifabutin are members of the family of **rifamycins**. They are bactericidal and affect the cell by binding to the enzyme, RNA polymerase, and blocking the synthesis of mRNA. The basis of their selective toxicity is the greater affinity of the drug for the bacterial enzyme than for the human RNA polymerase. Resistance develops rapidly, so they should always be used in combination with other drugs.

Rifampicin is used against Gram-positive organisms (staphylococci) and mycobacterial infections (e.g. tuberculosis). Rifabutin is used mainly in the treatment of *Mycobacterium avium intracellulare* in HIV patients. Rifamycins can be administered orally and reach high concentrations in serum and saliva. They are metabolised in the liver and excreted in body secretions (tears, urine, saliva), which may be coloured red by the drug. Side effects include thrombocytopenia, renal failure and hepatitis.

Rifamycins interact with a number of other drugs, including anticoagulants, corticosteroids and hypoglycaemic agents. They also interfere with oral contraceptives, and patients should be advised to use other methods of contraception while taking the drug and for 4–8 weeks after.

Nitroimidazoles

Metronidazole (Flagyl®) and **tinidazole** have a wide spectrum of activity against most obligate anaerobic Gram-positive and Gram-negative bacteria, including *Helicobacter pylori*, as well as intestinal protozoa, including *Giardia lamblia*, *Entamoeba histolytica* and *Trichomonas vaginalis*. There are some side effects associated with their use, such as nausea and alcohol intolerance. Patients should be counselled to avoid alcohol during the course of the treatment.

Inhibitors of cell membrane function

The cytoplasmic membrane is a selectively permeable barrier surrounding the cytoplasm of all cells. The **polymyxins** are cyclic polypeptides, which act as cationic detergents and disrupt the phospholipid bilayer of the membrane. They are especially active against Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. They are not readily absorbed if administered orally and are toxic, causing nephrotoxicity and ototoxicity. They are used in topical preparations and occasionally for parenteral treatment of bacteria resistant to all other antimicrobials.

Antimycobacterial drugs

Patients with tuberculosis are managed by specialist doctors and require an extended course of antimicrobial drugs. The most commonly used drugs are combinations of **isoniazid**, **ethambutol**, **rifampicin** and **pyrazinamide** (see Chapter 17). A number of side effects are associated with these drugs. Compliance with the regime is of the utmost importance as drug resistance occurs easily.

ANTIFUNGAL DRUGS

Many common fungal infections are caused by dermatophytes that attack cutaneous or mucocutaneous areas of the body. They include the various forms of tinea (e.g. ringworm, athlete's foot) and, in general, are only mild infections that can be treated by the application of topical antifungal preparations. In recent years, the number of invasive opportunistic fungal infections occurring in immunocompromised patients has increased (see Chapter 6). These infections are potentially life-threatening and require the use of systemic antifungal drugs.

Fungal cells are eucaryotic and it is difficult to identify points of selective toxicity. The main target for attack is the synthesis or functioning of the fungal cell membrane, which, unlike bacteria, contains sterols. **Azoles** and **polyenes** are the main compounds used for this purpose.

Azoles

The **azole** group of antifungal drugs inhibit the enzymes involved in sterol biosynthesis, the basis of selective toxicity being the greater affinity of the drug for the fungal enzyme than for the human enzyme. They include ketoconazole, fluconazole, itraconazole, voriconazole and posaconazole, which are used systemically to treat fungal infections.

Ketoconazole is active against a variety of fungi including yeasts, whereas **itraconazole** has a broader spectrum and is also used to treat *Aspergillus* infections.

Fluconazole penetrates well into tissues, including the central nervous system, and can be used to treat vaginal candidiasis and as prophylaxis (or treatment) against cryptococcal meningitis in HIV-positive patients.

Voriconazole is a newer antifungal agent with a wider spectrum of activity than fluconazole. It can be administered intravenously or orally, and is used to treat invasive *Aspergillus* infections, and serious infections with *Candida*, *Scedosporium* and *Fusarium* spp.

Posaconazole is available only as an oral preparation and is used to treat infections by *Zygomycetes* (e.g. *Mucor*).

The drugs **bifonazole**, **clotrimazole**, **econazole**, **miconazole** and **ketoconazole** are mainly used topically for dermatophyte infections and mucocutaneous candidiasis.

Polyenes

The **polyene** antibiotics **amphotericin B** and **nystatin** act by combining with ergosterol, a major sterol component of the fungal cell membrane, thereby causing leakage of cell contents and cell death.

The **allylamine** drugs, such as **terbinafine**, inhibit ergosterol synthesis. These compounds are quite toxic to humans as they also bind to a lesser extent to the cholesterol in human cell membranes. They are effective in topical preparations.

Caspofungin belongs to a new class of antifungal drugs, echinocandins, that inhibit beta-glucan synthesis in the cell wall. It is effective for the treatment of mucosal and invasive candidiasis and invasive aspergillosis.

Treatment of fungal infections

Treatment of fungal infections is difficult because of the lack of solubility, toxicity and poor absorption of most of the available drugs.

Cutaneous mycoses such as tinea and thrush are treated mainly with topical preparations. Although quite toxic if used systemically, nystatin is poorly absorbed and so is useful for topical application.

Subcutaneous infections can be very persistent and in the past needed to be treated with a systemic drug such as **griseofulvin**. This can be administered orally and appears to act specifically by binding to newly formed keratin present

in cutaneous cells and inhibiting mitotic division. It must be used for a long time for best effect and has a number of drug interactions and toxic side effects, so it is now rarely used in clinical practice.

Chronic mucocutaneous candidiasis (see Figure 12.9) is the most common fungal infection in immunosuppressed patients, followed by infections caused by the yeast *Cryptococcus neoformans*. Severe cryptococcal pneumonia and meningitis are now uncommon in Australian AIDS patients because of the widespread use of HAART (highly active antiretroviral therapy) and prophylactic antifungal agents.

Systemic fungal infections are difficult to treat because of the toxicity of most antifungal drugs. Amphotericin B is probably the main antifungal drug used for serious systemic infections while awaiting cultural diagnosis of the causative agent. Amphotericin B must be administered intravenously and is toxic, especially to the kidneys, but alternative formats involving liposomal preparations are less toxic and allow greater doses to be given.

Azole drugs are useful for systemic infections. Fluconazole and voriconazole can be taken orally, are well absorbed and are widely used as a treatment for sensitive systemic yeast infections (*Candida* and *Cryptococcus*). **Flucytosine** may occasionally be used orally or parenterally in combination with amphotericin against *Cryptococcus neoformans*. High levels of the drug are associated with bone marrow toxicity. **Caspofungin** is useful in the treatment of invasive *Aspergillus*.

Better antifungal drugs are needed, especially as there are increasing numbers of immunocompromised patients susceptible to opportunistic fungal infections.

ANTIPARASITIC DRUGS

A number of parasitic infections, caused by protozoa and helminths (worms), have complicated life cycles and it is difficult to find drugs that are selectively toxic against them.

Infections due to intestinal protozoa such as *Giardia* and *Entamoeba histolytica* can be treated with metronidazole or tinidazole. These drugs are effective against gastrointestinal infections because, although somewhat toxic, they are poorly absorbed from the intestine. Other protozoal diseases such as toxoplasmosis or diarrhoea due to *Cryptosporidium* or *Microsporidium* do not usually require treatment unless the patient is immunocompromised.

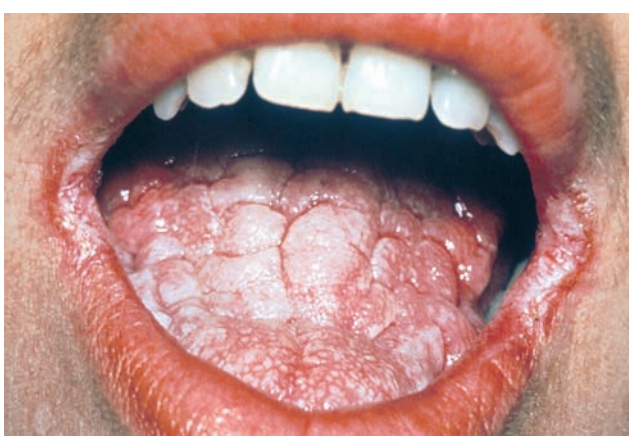


FIGURE 12.9

Chronic mucocutaneous candidiasis

Malaria is one of the most important of the protozoal infections. There are five species of the protozoan *Plasmodium* that are known to infect humans. Derivatives of quinine have been used for years to treat malaria, but quinine itself is now considered too toxic for general use. It is used only if the infection is due to a resistant strain.

Chloroquine is the main drug used to treat clinical attacks of malaria where the malarial strain is still susceptible to chloroquine. Strains of *Plasmodium falciparum* resistant to chloroquine have developed in many areas of the world and some of these are also resistant to **mefloquine**, which was used in some countries as prophylaxis against malaria infection. It is believed that this use has contributed to the rapid emergence of resistance to the drug, so it is not now generally recommended. Once the clinical attack is controlled, **primaquine** is used to eliminate *P. vivax* and *P. ovale* from the liver in order to prevent relapses, which may occur for up to five years after the initial attack.

In recent years, derivatives of the ancient drug artemisinin (artesunate) have been developed. WHO recommends the use of intravenous artesunate to treat patients with severe malaria due to resistant strains—usually in combination with other antimalarial drugs. However, it is ten times as expensive as chloroquine.

Prophylactic chemotherapy for travellers to endemic areas in South-East Asia usually involves the use of **doxycycline**. Recommendations for prevention and treatment of malaria are constantly modified according to location and to accommodate the development of resistance. It is important that travellers to malarial areas consult their local health department or travel advisory clinic or the WHO website <www.who.it/ith> for advice on appropriate antimalarial prophylaxis.

Antihelminthic drugs are discussed in Chapter 6.

ANTIVIRAL DRUGS

A large number of diseases that afflict humans are caused by viruses. However, because viruses replicate inside the host cell, only a few antiviral compounds have been found which can kill or inactivate viruses without being severely toxic to the host. Instead, research has focused with considerable success on the development of vaccines against serious viral diseases. The advent of the human immunodeficiency virus (HIV), which attacks the immune system itself and therefore makes the development of a vaccine particularly difficult, has encouraged the search for antiviral compounds.

For an antiviral drug to be selectively toxic, it must target some point in the cycle of viral replication. This could be, for example, the specific attachment site of the virus to the host cell, or the mechanism of viral uncoating and replication, or a specific viral enzyme synthesised under the direction of the viral genes and required for viral replication. In recent years, a number of specific viral

enzymes have been identified and these have been used as targets for antiviral drugs.

There are a number of difficulties associated with effective antiviral therapy. Many of the drugs that inhibit the viral enzymes also inhibit (but to a lesser extent) the host cell enzymes, so side effects are common. Most available antiviral drugs are **virustatic**—that is, they inhibit viral replication (and relieve the disease symptoms) but they do not eliminate the virus from the body. This is of particular importance in immunosuppressed individuals whose immune system may not be able to attack the virus. Latent (non-replicating) viruses are not affected, so any reservoir of viruses in the body is preserved and can be reactivated later.

Mutations occur frequently during viral replication, giving rise to altered viral proteins with different antigenic properties, so antibodies formed in response to the initial infection may not inactivate the virus. This is important in infections with HIV. Mutation may also produce enzymes with altered substrate specificity so that the antiviral drugs are no longer effective and the virus becomes resistant to the drug.

Nucleoside analogues

The most successful antiviral drugs are analogues of the purine and pyrimidine bases, which are the building blocks of RNA and DNA. These analogues inhibit viral DNA polymerase, preventing further viral replication. They have been found to be effective against the common DNA viruses herpes, cytomegalovirus (CMV) and varicella, and are used as part of the combination therapy for treatment of HIV. Some antiviral drugs are listed in Table 12.4, and new ones are continually being developed.

Aciclovir, an analogue of the nucleoside guanosine, is one of the most effective compounds. It is active against the herpes simplex virus (HSV) types I and II, and varicella zoster virus (VZV—shingles). HSV and VZV contain a virally encoded enzyme, thymidine kinase, which is able to phosphorylate aciclovir to the active monophosphate; this is then converted to aciclovir triphosphate and incorporated instead of thymine into the growing chains of viral DNA, where it prevents further elongation. Thymidine kinase is not present in human cells, which means that aciclovir is incorporated only into the DNA of virally infected cells, and so is relatively non-toxic even in high doses.

Aciclovir is used for primary herpes simplex (HSV) infections (oral, genital and conjunctival) as well as the prevention of recurrent genital HSV. An important use is in suspected cases of herpes encephalitis. It is used in topical applications for HSV or by intravenous infusion, as it is not well absorbed.

Valaciclovir or **famciclovir** replace aciclovir for oral use, as these are better absorbed. They are used for systemic infections in immunocompromised patients or for severe cases of *Varicella zoster* in adults.

Ganciclovir is also a nucleoside analogue of guanosine, but is active against cytomegalovirus as well as HSV and VZV. The mechanism of inhibition is similar to aciclovir, but the drug is more toxic so its use is limited to serious cases of CMV, such as CMV retinitis in AIDS patients. Neutropenia occurs in 25 per cent of patients together with some degree of renal impairment. It is not well absorbed when taken orally.

Valganciclovir is absorbed well when taken orally and is hydrolysed in the body to ganciclovir. **Foscarnet** is a pyrophosphate nucleotide derivative that does not require phosphorylation by host or viral enzymes. It has been used as an alternative to ganciclovir for CMV infections as well as for herpes and varicella zoster but is more toxic and may cause renal failure.

Adefovir, **entecavir**, **lamivudine** and **tenofovir** are used to treat hepatitis B. Tenofovir is a potent analogue of adenosine and behaves in the same way as the guanosine analogues, by inhibiting DNA polymerase and preventing viral replication. Lamivudine is a cytosine analogue and, like tenofovir, is also used in the treatment of HIV.

Ribavarin, a synthetic analogue of guanosine, has a potentiating effect when used with other antivirals. Its main use is in combination with peg-interferon-alfa for the treatment of hepatitis C and for severe cases of respiratory syncytial virus (RSV) infection in hospitalised children. It has a number of side effects and is teratogenic and embryogenic, so is contraindicated in pregnant women and their partners.

TABLE 12.4 Antiviral drugs

VIRUS OR DISEASE	ANTIVIRAL DRUG
Herpes simplex virus (HSV)	Aciclovir, idoxuridine (topical) Fanciclovir, valaciclovir (oral)
Varicella zoster virus (VZV)	Aciclovir (iv, oral)
Cytomegalovirus (CMV)	Ganciclovir Valganciclovir Foscarnet
HIV/AIDS	HAART Combination therapy of three drugs comprising 2 nucleoside analogue reverse transcriptase inhibitors and 1 non-nucleoside reverse transcriptase inhibitor **OR** protease inhibitor **OR** integrase inhibitor see Table 19.6, page 493
Respiratory syncytial virus (RSV)	Ribavarin
Influenza A	Amantadine
Influenza A and B	Zanamivir Oseltamavir
Hairy cell leukaemia	Interferon
Chronic hepatitis B	Interferon, entecavir, adefovir, tenofovir, lamivudine
Chronic hepatitis C	Interferon, ribavirin, zidovudine

Antiretroviral drugs

Retroviruses are a particular group of viruses responsible for a number of serious diseases, including adult T cell leukaemia and AIDS. They have also been linked to the development of cancers in various animal species. In the search for suitable drugs to treat infections caused by these viruses, scientists have been able to make use of the viruses' unique method of replication (see Figure 12.10). Retroviruses contain RNA, and the first step in the replicative process involves the synthesis of DNA from the RNA genome by a specific viral enzyme, an RNA-dependent DNA polymerase called **reverse transcriptase**. The DNA 'provirus' is then inserted into the host cell DNA and subsequently directs the synthesis of new viral RNA and protein.

Nucleoside analogue reverse transcriptase inhibitors (NRTIs)

The reverse transcriptase enzyme, which does not occur in human cells, provides a target for antiviral drugs. Various analogues of the nucleosides that are used to synthesise DNA have been tested for their ability to bind to reverse transcriptase. Of these, zidovudine (AZT), an analogue of thymine, was the first to be licensed for the treatment of HIV infection. However, although the drug has a high affinity for viral reverse transcriptase, it is also incorporated to some extent into host cell DNA by human DNA polymerase. This leads to occasional depression of bone marrow function and may cause inhibition of mitochondrial DNA synthesis, leading to myopathy. Unpleasant side effects of treatment include nausea, myalgia and headaches.

A number of other nucleoside analogues have been developed. All the analogues block the transcription of viral RNA into DNA by combining with and inhibiting the enzyme, reverse transcriptase, leading to termination of the synthesis of the DNA chain. The action of these drugs is virustatic—that is, when the drug is removed, viral replication can resume.

Non-nucleoside inhibitors (NNRTIs)

The newest class of antiretroviral agents are the non-nucleoside reverse transcriptase inhibitors. They stop HIV production by binding directly on to reverse transcriptase,

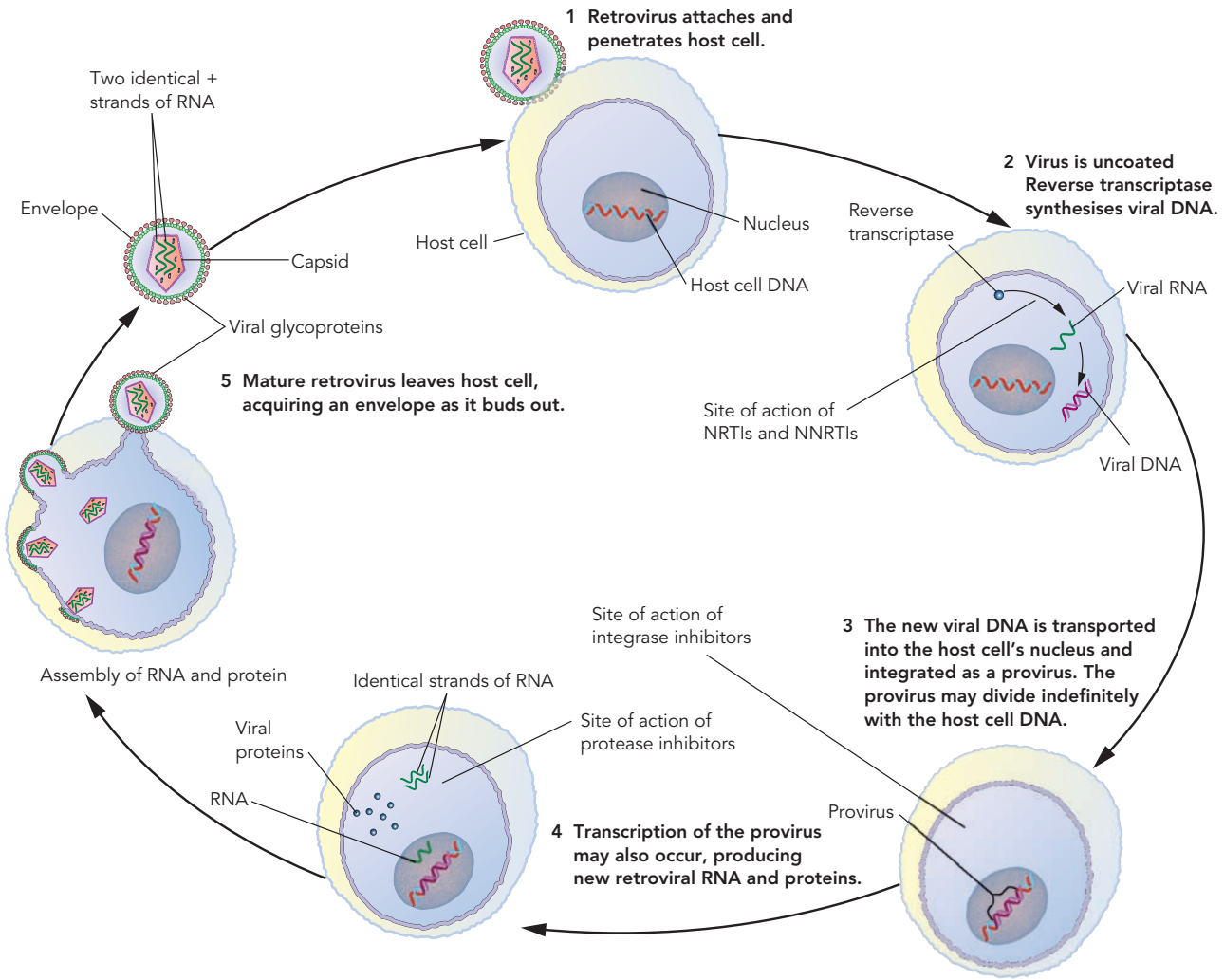


FIGURE 12.10

Sites of action of antiretroviral drugs

preventing the conversion of RNA to DNA. Although they act at the same site as the nucleoside analogues, they exert their effect in a completely different way.

Protease inhibitors (PIs)

Protease inhibitors are a group of compounds that act on HIV protease and interfere with the correct formation of viral proteins, thus reducing viral replication and the spread of the virus to uninfected cells.

Integrase inhibitors

Integrase inhibitors inhibit the integration of HIV DNA into the host cell genome.

Combination therapy (HAART)

Current retroviral treatment for patients who are HIV positive consists of triple-combination therapy or **HAART** (highly active antiretroviral therapy)—two nucleoside reverse transcriptase inhibitors together with a protease inhibitor or an NNRTI, or an integrase inhibitor (see Table 19.6, page 493). Combination therapy reduces the risk of development of resistance to each drug. It has been shown to increase the CD_4 cell count, decrease HIV-RNA, reduce vertical transmission (mother to baby) and improve survival rates. The selection of appropriate combination of drugs is a specialised area and carried out by professionals who are up to date with the current availability and efficacy of HIV drugs. The recommended protocols are continually updated to take into account the development of resistance and licensing of new drugs.

Anti-influenza drugs

Amantadine is a cyclic amine that is active against influenza A but not influenza B. When used to treat influenza it is most effective if given within the first 48 hours after symptoms develop. During influenza epidemics, it can be administered to susceptible people who have not been vaccinated, thus reducing the risk of illness. However, the development of drug resistance is common and it is no longer widely used.

Oseltamavir (Tamiflu®) and **zanamavir** (Relenza®) are newer drugs that are active against both influenza A and B. They inhibit the action of neuraminidase, an enzyme in the spikes of the virus that is essential for the release of new viral particles from infected cells. They have been shown to reduce the duration and intensity of symptoms by one or two days but should be administered within 48 hours of onset of symptoms. Tamiflu®, which is administered orally, has been widely used since its release and there are an increasing number of reports of resistance to the drug. Relenza® has not been widely used as it has to be administered by nasal spray and should be used with caution in people with asthma. These drugs are useful for prophylaxis when there is an epidemic of a novel influenza virus, such as occurred with swine flu in 2009.

Interferons

Interferons are a family of small glycoprotein molecules produced by some mammalian cells, such as lymphocytes, fibroblasts and macrophages, in response to viral infection. The interferons are cell or species specific, not virus specific.

Three classes have been identified—alfa(α), beta(β) and gamma(γ). Gamma(γ)-interferon is a lymphokine produced by activated T cells in response to viral infection; α- and β-interferons are produced by many cell types. Virus-infected cells produce interferons that diffuse to neighbouring uninfected cells, where they bind to specific cell receptors and induce the production of proteins that enhance the cell's immune response.

Interferons have now been produced commercially by genetic engineering. Alpha-interferon (IFN-α) is used in the treatment of chronic hepatitis B and C. Studies of results of interferon treatment of chronic hepatitis C patients showed that, after an initial response rate of 50 per cent, half the patients relapsed, so the overall response to treatment was 25 per cent. Careful selection of patients for therapy—that is, patients without contributing conditions (e.g. cirrhosis)—improves the response rate. Side effects of the therapy include fever, malaise, headache and myalgia; bone marrow depression may also occur. Pegylated interferons are modified interferons with prolonged activity. They have improved the results of treatment of hepatitis C that are obtained with combination therapy of interferon and ribavirin.

THERAPEUTIC USE OF ANTIMICROBIAL DRUGS

Many infections that were once considered life-threatening can now be successfully treated with antimicrobial drugs. This is particularly true of bacterial infections. There is a wide selection of drugs available, and several factors need to be considered before a particular drug is prescribed. The approach taken by the medical practitioner to the treatment of infection may vary depending on whether the patient is in the community or is hospitalised. In the community, the time and cost involved in carrying out a definitive microbiological identification is usually not warranted for most patients so the doctor may prescribe empirically. However, the emergence in the community of antimicrobial resistance in common bacterial infections caused by organisms such as *Streptococcus pneumoniae* and *Staphylococcus aureus* makes some infections with these organisms unresponsive to standard antimicrobial therapies, so laboratory tests may need to be carried out. Most upper respiratory tract infections are due to cold viruses and are not cured by antimicrobial therapy.

In the hospital environment, patients are often sicker and they may have contributing illnesses. There is also a greater likelihood of the infection being due to a resistant organism, especially if the patient has been in hospital for more than three days. In these cases, it is usual to collect a specimen, send it to the laboratory for identification and determination of antimicrobial sensitivities, and start **empirical (best guess) therapy** while waiting for the results (see 'Prescribing principles: Empirical therapy', page 276).

Antimicrobial sensitivity (susceptibility) tests

Specimens taken for microbiological detection and organism identification are sent to the laboratory, where

the predominant organism in the sample (hopefully the one responsible for the infection) is isolated and identified using methods described in Chapters 15. In order to decide which is the best antimicrobial to use in treating a particular infection, it is necessary to determine the susceptibility of the clinical isolate to the available antimicrobial drugs.

The choice of drug is dictated by several factors, including the site of infection, the infecting organism(s), the antimicrobial **susceptibility pattern** of the particular strain of the organism isolated and the available route of administration of the antimicrobial. For example, although penicillin is effective against actively growing cells of many Gram-positive bacteria, a number of strains of these bacteria have developed mechanisms to resist its action (e.g. beta-lactamase enzymes). To select the most effective antimicrobial drug, it is necessary to test the actual strain of the organism that is causing the infection against different concentrations of a range of possible antimicrobial agents.

There are two main methods for carrying out sensitivity tests in the laboratory: disk diffusion tests and the minimum inhibitory concentration (MIC) test. In both methods, an appropriate specimen is obtained from the patient and cultured on a suitable medium to obtain single colonies. Cells from the colonies are suspended in nutrient medium and tested for susceptibility to a range of antimicrobials. The site of the infection, together with the provisional diagnosis, determines which antimicrobials are tested first. The initial choice is also based on the susceptibility pattern of the isolates usually encountered in the particular laboratory, especially for hospital-acquired infections.

Disk diffusion test

The bacteria to be tested are spread uniformly at a known concentration on an agar plate. Disks impregnated with defined amounts of various antimicrobials are then placed on the plate, where the drug diffuses into the agar and inhibits the growth of susceptible organisms in a zone around the disk. The **zone of inhibition**, or clear area (in mm) around the disk, represents the susceptibility of the organism to the drug being tested (see Figure 12.11). The amount of drug in each disk is related to its achievable serum concentration. The diameter of the zone of inhibition is a measure of the susceptibility of the organism to the drug but, as it is influenced by the ease of diffusion (solubility) of the drug in the agar, it is considered a qualitative method. If no clear zone is apparent or there is a reduced zone of growth inhibition compared to a published standard, the organism is considered to be resistant to the drug being tested.

Minimum inhibitory concentration test

The **minimum inhibitory concentration** (**MIC**) test is a semi-quantitative procedure that measures the concentration of an antimicrobial required to inhibit the growth of a standardised inoculum. This can be done by broth dilution in a series of tubes, each containing different concentrations of drug but with a standardised number of organisms. The

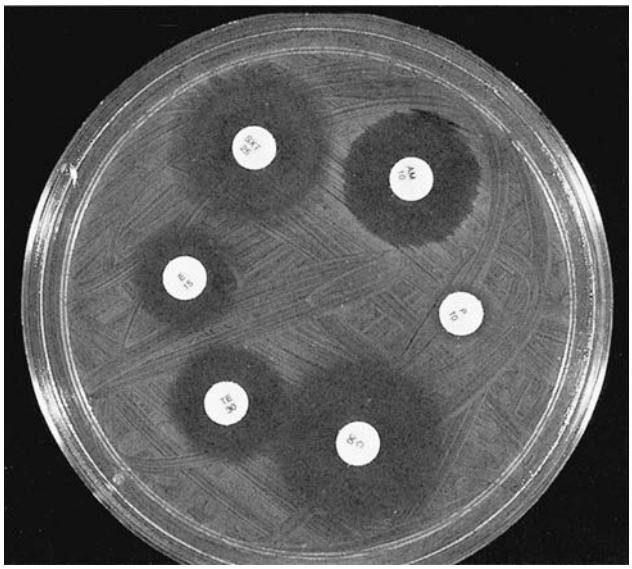


FIGURE 12.11

Disk diffusion test

The diameter of the clear zone around each disk represents the sensitivity of the test organism to the antibacterial drug in the disk. If there is no clearing, the organism is considered resistant to the drugs at that concentration.

method measures the lowest drug concentration (MIC) that will inhibit visible growth. It is a laborious method and has been replaced by the E-test, a commercially available method to determine MICs (see Figure 12.12). Many automated systems use an MIC method but read the growth densities automatically and print out a report of their findings in a few hours. These methods are used in large laboratories where many samples are tested each day.

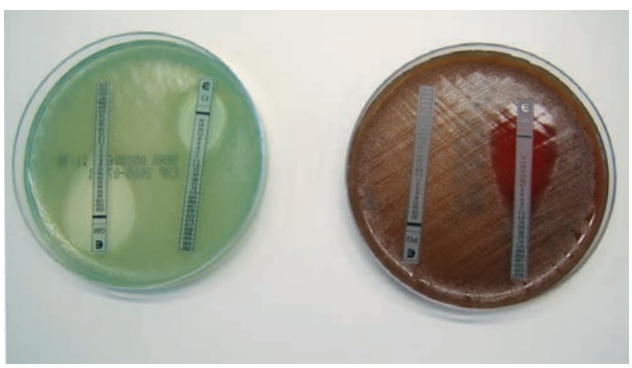


FIGURE 12.12

Measurement of minimum inhibitory concentration (MIC) using the E-test

Calibrated strips with increasing concentrations of antibiotic impregnated into the strip are placed on a plate inoculated with the bacterium to be tested. Visual inspection of the concentration of antibiotic present in the clear zone of inhibition gives the minimum concentration of antibiotic that inhibits growth of the bacterium.

(a) Measurement of growth of Pseudomonas aeruginosa *in the presence of gentamicin (left) and ciprofloxacin (right). The strain is sensitive to both drugs.*

(b) Measurement of growth of Enterococcus *in the presence of penicillin (left) and vancomycin (right). The organism is resistant to penicillin, but sensitive to vancomycin.*

The results of antimicrobial susceptibility testing can vary from one laboratory to another unless standardised control cultures and testing media are used.

Treatment of bacterial infections

In an ideal situation it would be preferable to delay treatment until susceptibility tests had been carried out; however, in practice, by the time the patient shows obvious signs of infection (see Chapter 7) they require treatment as soon as possible. Waiting two or more days for definitive laboratory tests to be carried out before prescribing treatment may well jeopardise the health (or life) of the patient. An infection that has just become established can develop into fulminating sepsis in hours (e.g. meningococcal septicaemia). Balanced against this is the risk of the development of resistant microbial strains or selection of resistant bacteria due to overprescribing of antimicrobials.

Prescribing principles

Empirical therapy

Empirical therapy refers to the prescribing of antimicrobial drugs in a situation where the exact cause of the infection has not yet been identified, but it is important to commence treatment. The antimicrobial is chosen on the basis of an estimation of the most likely causative pathogen(s) and their likely antimicrobial susceptibilities. In general, it is always preferable to prescribe a narrow spectrum antimicrobial, but when the cause is unknown or a mixed infection may be present, the use of a broader spectrum antimicrobial may be indicated

The procedure followed for prescribing antimicrobials to treat bacterial infections will vary somewhat depending on whether the patient is in the community or the hospital.

Community infections

In the community the medical practitioner makes a provisional diagnosis, and usually prescribes medication based on the empirical ('best guess') principle that takes into account the site of the infection, the kind of pathogens circulating in the community at that time and their patterns of antimicrobial susceptibility, together with patient history (including travel). In some cases it is appropriate for a specimen to be sent for analysis and the doctor then reviews the prescription when the results of the pathology tests are available.

Hospital infections

In the hospital, it is more usual to take a specimen and send it to the laboratory for analysis, and start empirical therapy while waiting for the results. It is important that, whenever possible, clinical specimens are collected from the patient *before* any drug therapy is commenced; otherwise, the drug may kill or inhibit the microorganisms present and incorrect or inconclusive results may be obtained. An appropriate clinical sample of blood, sputum, pus, urine, faeces, CSF or tissue is usually collected. It should be adequate in amount, collected in an aseptic manner, correctly labelled and transported to the laboratory as soon as possible. (See Chapter 15 for methods of specimen collection.) A Gram stain or direct antigen detection can often give quick results that allow specific therapy to be commenced.

It is essential to carry out susceptibility testing when:

- the organism is of a type that is frequently resistant to drug therapy (e.g. hospital-acquired Gram-negative bacteria)
- the infection is life-threatening (e.g. meningitis, septicaemia)
- bactericidal drugs are preferred (e.g. for immunocompromised patients or in infective endocarditis).

In deciding which drug to prescribe for a particular patient, the doctor takes into account such factors as:

- ***The site of the body where the infection is located***. This will often indicate the types of organisms most likely to be present and the most effective drug to be used.
- ***Whether the infection was acquired in the hospital or community***. Many hospital organisms are resistant to antimicrobials, and the hospital will have developed a prescribing policy to address this problem. It may involve the use of two or more drugs at the same time.
- ***Whether there is a history of overseas travel***. There are a number of diseases that are not commonly seen in Australia but may be contracted in other countries. The pattern of resistance of the imported bacteria will also often be quite different from local strains.
- ***The age of the patient***. Some drugs are more toxic for children. Some infections are more serious in children and require immediate effective therapy. Some drugs are not suitable for young children, or women during pregnancy and lactation. For example, tetracycline has been shown to bind to calcium and deposit in developing teeth, causing permanent brown staining. It may also interfere with bone development in the foetus. A number of drugs—for example, fluconazole—may cause foetal damage if administered during pregnancy.
- ***Ease of administration***. Oral therapy is generally preferable unless the patient has difficulty swallowing or a gastrointestinal illness. Sometimes urgent treatment is necessary to achieve a rapid high concentration of the drug, so intravenous administration or an intramuscular injection is used. Intravenous infusion has disadvantages, such as the risk of contamination (sepsis) associated with the IV line, the cost of equipment and the need for trained supervision.
- ***Absorption***. The drug must be able to reach the part of the body where the infection is located. Thus, if the drug is to be given orally, it must be unchanged by gastric acid, be absorbed from the intestine and be able to be transported in the bloodstream to the site of infection. Some drugs must be given by intravenous infusion because of their poor absorption from the intestine and so are only suitable for use in a hospital or health facility (see Figure 12.13).

- ***Penetration***. The drug must be able to reach the target tissue. For example, if the infection is in the meninges, a drug must be selected that can cross the blood–brain barrier (although this is sometimes easier when the meninges are inflamed). The drug used to treat a urinary tract infection must be excreted in an active form via the kidneys and must be able to reach a high enough concentration in the urine to kill the microorganisms present in the bladder.

Other medical conditions that may need to be taken into account include a history of hypersensitivity or other adverse reaction to the drug. It is important to avoid inadvertent use of a drug to which the patient is allergic (see Case History 12.1: Nursing management of a pneumonia patient, page 279).

The status of the immune system and the integrity of organ function in the patient is also important. For example, immunocompromised patients should be prescribed bactericidal drugs whenever possible, as their immune system is not able to eliminate the microorganisms left after treatment with a bacteriostatic drug. Infective endocarditis is best treated with a bactericidal drug, as macrophages are unable to penetrate to the site of the infection to destroy residual bacteria.

Metabolism and elimination of drugs depends on hepatic and renal function. Patients with some degree of renal impairment are not able to excrete drugs effectively via the kidneys. This means that high concentrations of the drug may develop in the bloodstream and thus the prescribed dose needs to be lower. Drugs that affect the kidneys (e.g. the aminoglycosides) are not usually prescribed for patients who already have impaired renal function. In particular, elderly patients may have some degree of liver or renal impairment that affects the pharmacokinetic properties of the drug.

Other factors usually taken into account when selecting the most suitable drug are the likelihood of development of resistance, the risk of interactions between the drug and any other medication being taken by the patient and, importantly, the cost of the drug.

Combined therapy

In hospitals it is common for two drugs to be prescribed simultaneously for the treatment of seriously ill patients when the cause of the infection is uncertain, or when delay while awaiting the results of conclusive sensitivity tests may be detrimental to the patient. One advantage of using more than one drug is the efficient treatment of mixed infections. The emergence of resistance can theoretically be retarded by the use of two drugs with different mechanisms of action.

Sometimes, the action of an antimicrobial is potentiated (enhanced) in the presence of another drug or antimicrobial. **Synergism** is the term used to describe the enhancement of one drug in the presence of another. For example, penicillin may be prescribed as well as an aminoglycoside. Penicillin damages the cell wall, allowing better penetration of the aminoglycoside. In systemic fungal infections such as candidiasis or cryptococcosis, amphotericin is used to weaken the cell membrane, allowing uptake of flucytosine.

Some infections respond better to the use of two or more drugs. Enterococcal endocarditis responds to a combination of penicillin or ampicillin, plus gentamicin, vancomycin or streptomycin. Tuberculosis is routinely treated with isoniazid, ethambutol and rifampicin in order to inhibit the development of resistant strains.

Some commercial preparations contain two drugs; for example, cotrimoxazole (Bactrim®, Septrin®) contains sulfamethoxazole and trimethoprim, two drugs which sequentially block the pathway of folate synthesis. Augmentin® contains amoxycillin plus the beta-lactamase inhibitor clavulanic acid, which prevents inactivation of the amoxycillin.

However, there are some disadvantages to the use of combined therapy. There may be **antagonism** when a reaction occurs between the drugs being used, or when one drug interferes with the action of the other. These interactions are usually described in the product information supplied for each drug. If bacteriostatic and bactericidal drugs are administered simultaneously, the action of both

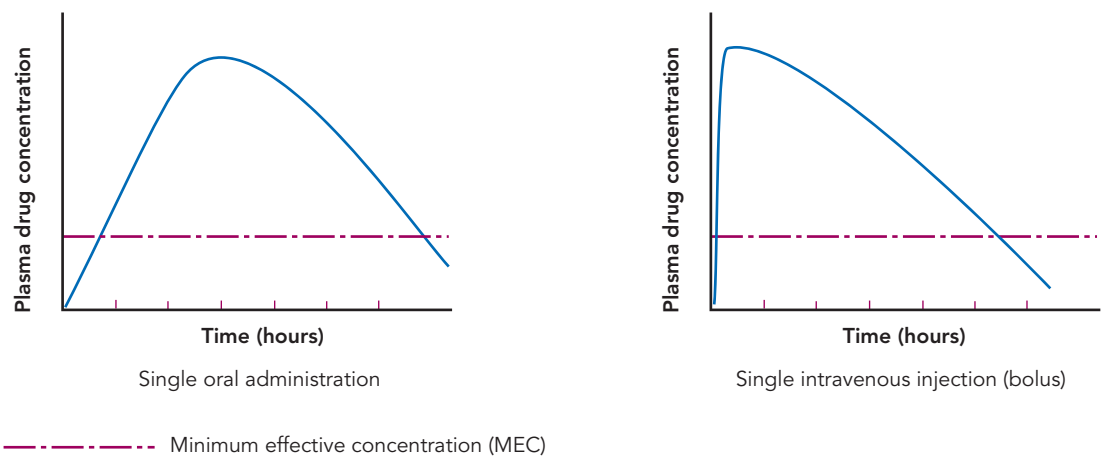


FIGURE 12.13

Comparison of plasma drug concentrations after different methods of administration

may be affected. For example, tetracycline is bacteriostatic, inhibiting protein synthesis and cell replication. Penicillin is bactericidal and is active mainly against dividing cells. If they are used in combination, the tetracycline inhibits cell division and so the bactericidal action of the penicillin is considerably diminished. Some antimicrobials affect the efficacy of other kinds of drugs. Rifampicin stimulates the metabolism (and therefore removal from the body) of oral contraceptives, anticoagulants and corticosteroids.

Duration of therapy

It is important to limit the duration of antibiotic therapy to minimise the development of resistance. Most courses of therapy are five days. Prolonged use of antibiotics can cause suppression of normal flora or selection of opportunistic infections such as candidiasis (thrush) or *Clostridium difficile*-associated diarrhoea.

Special conditions

The management of patients with tuberculosis and HIV/AIDS is usually carried out by specialist doctors in the respective fields. This ensures that patients receive optimum treatment and follow-up with the most appropriate drugs.

Pharmacokinetics

The prescribed dosage must be such that an effective concentration of the drug is achieved at the site of infection. Ideally, the concentration of the antimicrobial agent in the blood or tissues should be maintained above the minimum inhibitory concentration (MIC). The rate of elimination of the drug from the body can be described in terms of **half-life**—that is, the time required for the concentration of the drug in the bloodstream to fall to half its maximum level. The number of times the drug needs to be administered each day will depend on the rate at which the drug is broken down or eliminated from the body.

It is also necessary to take into account the therapeutic index of the drug. The **therapeutic index** is the ratio of the effective concentration of the drug required to kill the pathogen to the concentration that would be a lethal dose for the host. The smaller the number, the more selectively toxic the drug will be and the less risk there will be of toxic side effects.

It is important that blood levels of potentially toxic drugs are monitored regularly, especially in patients with renal or liver impairment.

Prophylaxis

Prophylaxis involves the administration of an antimicrobial drug before there is any evidence of infection, under conditions where the risks of an infection developing are considered to be very high. The risk of a patient acquiring an infection has to be weighed against factors such as cost, the toxicity of the drug and the risk of superinfection (i.e. an overgrowth by opportunistic pathogens).

Prophylaxis may be considered necessary for some surgical procedures, especially where prosthetic devices are involved. A single dose is usually given just before the procedure. It is recommended for patients who are undergoing abdominal surgery or who are susceptible to infection for other reasons. For example, patients who suffer from rheumatic heart disease involving damage to the heart valves are usually prescribed penicillin following certain dental procedures. This is to reduce the risk of infection by oral (viridians) streptococci, which may enter the bloodstream and infect the damaged valves, causing endocarditis.

Pregnant women colonised with group B streptococci are given intra-partem antibiotics to reduce the risk of neonatal infection especially when premature rupture of the membranes occurs or there is prolonged labour.

Limitations of antimicrobial therapy

There are a number of problems associated with the use of antimicrobials.

- The suppression or elimination of normal flora can lead to an overgrowth of opportunists such as *Candida albicans* or *Clostridium difficile.*
- The occurrence of adverse side effects such as hypersensitivity, ototoxicity, nephrotoxicity and bone marrow suppression. Like all drugs, most antimicrobials have some side effects and the nature of the side effects must be weighed against the benefits available from their use. The most important of these adverse effects are listed in Table 12.5. A full description of side effects and contraindications for use are supplied by the manufacturer and should be read thoroughly before the drug is administered. A number of drugs have adverse effects on the foetus and should not be given to pregnant women.
- The emergence of strains of pathogens that are resistant to currently available antimicrobial drugs.

Hypersensitivity reactions and anaphylactic shock

Anaphylaxis due to the parenteral administration of penicillin is a rare but life-threatening occurrence. It has an incidence of about 1 in 5000 of all patients receiving penicillin and a mortality of 1 in 50 000. Prompt administration of adrenaline (epinephrine) by intramuscular injection is essential.

An allergic (hypersensitivity) reaction to penicillin is a genetically determined reaction associated with the individual's immune system. In some people, exposure to the drug elicits an allergic immune response. The penicillin molecule is broken down and reacts with certain tissue proteins to form a complex that stimulates the immune system to produce IgE antibodies. The antibodies attach to the surface of mast cells and basophils, which are distributed throughout the body. These cells are then 'sensitised'. If the patient is exposed to penicillin again, the sensitised cells rapidly release histamine and other active substances that cause anaphylactic reactions ranging from urticaria (rashes), hives and laryngeal oedema, to bronchoconstriction and hypotension. The most severe

TABLE 12.5 Some serious adverse effects of antimicrobial therapy

DRUG	ADVERSE EFFECT
Antibacterials	
■ Aminoglycosides	Ototoxicity (deafness) and nephrotoxicity (renal impairment).
■ Chloramphenicol	Bone marrow depression—aplastic anaemia.
■ Penicillin (and other beta-lactams)	Hypersensitivity occurs in some people. Occasionally fatal.
■ Fluoroquinolones	Nausea, photosensitivity, neurological disturbances.
■ Sulfonamides	Hypersensitivity, skin rashes, agranulocytosis.
■ Tetracyclines	Combine with calcium in developing bones and teeth, cause permanent discolouration of teeth.
■ Vancomycin	Local inflammation—phlebitis. Must be given by slow infusion to avoid histamine release.
Antivirals	
■ Zidovudine	Bone marrow depression, anaemia, nausea, headaches, skin rashes.
Antifungals	
■ Amphotericin	Nephrotoxicity, hypokalaemia.

CASE HISTORY 12.1

Nursing management of a pneumonia patient

A 42-year-old female was admitted to hospital with a temperature of 39.2°C and symptoms of pneumonia. She had difficulty breathing and was coughing up thick sputum. *Staphylococcus aureus* sensitive to penicillin was isolated from her sputum sample. The results were phoned through to the doctor, who authorised a script for parenteral administration of benzyl penicillin.

Questions

1. What is the responsibility of the hospital pharmacist when dispensing the medication?
2. What history should the nurse take before administering the antibiotic?
3. What is meant by 'parenteral administration'?
4. What risks, if any, are associated with the use of penicillin?
5. The patient says she does not approve of taking antibiotics because she doesn't want to become resistant. She does not recall having ever taken penicillin. How should the nurse manage this patient?

form of anaphylaxis is described as anaphylactic shock and can be fatal.

A number of patients report being 'allergic' to penicillin, having reactions that may include symptoms such as a skin rash, but the anaphylactic response is the only major contraindication to its use. It is possible to ascertain whether a patient is hypersensitive to penicillin by the use of a skin test. A positive reaction to the injection of the penicillin complex occurs in 10–15 minutes.

Patients who are hypersensitive to penicillin are likely to exhibit a reaction to the semi-synthetic penicillins as well as to other antimicrobials that have a beta-lactam ring (e.g. the cephalosporins).

It is essential that all patients be asked whether they have a history of allergy to penicillin before the drug is administered. If patients are unclear about their history or have no memory of having had the drug before, then special precautions must be taken. It is essential that adrenaline (epinephrine) is available when penicillin (or any drug) is administered parenterally. The 'use by' date on the ampoule should be checked to ensure the adrenaline is still current.

If penicillin is administered orally, the patient should be observed for any signs of rash, difficulty in breathing, etc.

Note that antihistamines and corticosteroids are *not* effective treatments for penicillin-induced anaphylaxis.

IMPLICATIONS FOR NURSING PRACTICE

Nurses are the health professionals who have most patient contact. Although the microbiologist identifies the organism causing an infection and the doctor prescribes the appropriate antimicrobial, it is usually the nursing staff who are responsible for administering the medication and monitoring the outcome. It is important for the nurses to check medications before administration and to ensure that the drug is administered in the correct manner and at the correct dose. As well, health professionals need to be constantly aware of the importance of handwashing to prevent the spread of infection. This section examines the nurse's role in the treatment of infections.

Specimen collection

The careful collection of specimens for analysis by the microbiology laboratory is essential for correct identification of the pathogen and determination of antimicrobial sensitivities. Whenever possible the specimen should be collected *before* any antimicrobial therapy is commenced. An appropriate specimen (see Chapter 15) is collected in a sterile container, using aseptic technique, labelled and transported to the laboratory as soon as possible. Information such as provisional diagnosis, type of sample, site of infection (or sample site), and date and time of collection

should be included on the request form, together with any other information that might assist the microbiologist, such as travel history, previous illnesses, current medications (especially antimicrobial therapy) and vaccination status.

Interpretation of reports

Nurses need to be familiar with the terms used in microbiology and be able to interpret the reports. Frequently, results are telephoned from the laboratory and the nurse must be able to record the results accurately. The nurse is in a position to check that the treatment and medication the patient is receiving are in accord with the diagnostic results. It is important to be aware of the use of generic and trade names for drugs, and to ensure that the correct drug is used.

Administration of antimicrobial therapy

This is usually carried out by the nursing staff and, as for any drug, it is important that they are aware of the correct procedures. Before administering any drug, the nurse should take a patient history of allergies (especially to penicillin). The manufacturer's directions accompanying the antimicrobial drug should be read carefully to ensure that the administration of the drug is by the correct route, at the correct time and that any special precautions are followed. The nurse should check the package for such information as:

- expiry date
- method of reconstitution
- storage condition
- contraindications for use
- method of administration—oral or parenteral.

Oral administration

For maximum efficacy, the drug should be maintained at a constant level in the blood or tissues throughout the 24-hour period. For this reason, oral doses are generally given at regular intervals throughout the day, depending on the excretion rate of the drug. Sometimes, this needs to be monitored by taking blood samples at regular time intervals to measure the drug level. Other factors may need to be taken into account. The rate of absorption of the drug may be affected by the type of tablet or capsule, the particle size of the drug powder, and the presence or absence of food in the intestine. Some drugs, such as tetracycline, bind to antacids or the calcium in milk and are then poorly absorbed.

Side effects such as nausea can be minimised by the administration of the drug with food. Instructions for administration either before or with food are therefore important. Sometimes, other adverse reactions can occur; for example, metronidazole (Flagyl®) produces alcohol intolerance, so patients should be advised not to drink alcohol when they are taking this medication.

Patients should be educated about the correct way of taking their medication, the need to take it at regular intervals, and the importance of finishing the prescribed course of the antimicrobial so that the drug level is maintained for a sufficient time to kill all the organisms. It is desirable to explain that the relief or disappearance of clinical symptoms does not mean that all the pathogens have been eliminated from the body, and that the full course of the drug should be taken to prevent a recurrence of the disease.

Parenteral administration

The correct procedure for administration should be ascertained (intravenous or intramuscular), and calculations for reconstitution or dilution of the preparation checked by another member of staff. Usually, the drugs are given by slow IV infusion over a period of time. This is important in the case of a number of drugs where a bolus (single injection) can cause histamine release or vasodilation (flushing) and may even lead to cardiac arrest (see Case History 12.2).

Monitoring the outcome

After parenteral administration of antimicrobials (especially penicillin) patients should be closely monitored for any adverse reactions, such as local inflammation or difficulty in breathing. Hypersensitivity reactions causing anaphylaxis can occur in patients who are allergic to penicillin and may require the use of resuscitation equipment and injection of adrenaline.

As with all drugs, the effect of the antimicrobial therapy should be monitored over a period of time and the patient observed for signs of improvement or any side effects

CASE HISTORY 12.2

Drug administration

A patient in a large Sydney hospital suffered a cardiac arrest and died after receiving a 'bolus' injection of vancomycin to treat a *Staphylococcus aureus* infection.

A number of common antimicrobials can cause serious, even fatal, reactions if they are administered by the wrong method. The administration of a bolus injection can cause reactions such as phlebitis (inflammation), histamine release and even cardiac arrest. The drugs that have been shown to have this effect are vancomycin, quinine, lincosamide and macrolides such as erythromycin.

These drugs must be given by slow intravenous infusion, usually over 30 minutes or longer, to avoid local high concentrations. Because phlebitis is also a problem, they are usually given via a centrally placed line.

Questions

1. How could this tragedy have been avoided?
2. Where is information about the correct method of administration to be found?
3. What procedures should be followed to minimise the risks of mistakes in drug administration?
4. What other staff members can be consulted for advice about correct drug administration?

(e.g. rashes, ringing in the ears). Suppression of the normal flora may cause an overgrowth of opportunistic organisms such as *Candida albicans*, causing thrush, or *Clostridium difficile*, causing diarrhoea. Some drugs may build up to toxic levels if renal function is impaired, so blood levels in these patients need to be monitored. Careful taking of patient history is important, as factors such as age, weight, and hepatic and renal functions affect the action of the drug.

DEVELOPMENT OF RESISTANCE TO ANTIMICROBIAL DRUGS

The first antibiotic (penicillin) was introduced in the 1940s. In the 'Golden Age' from 1950 to 1970, most of the drugs described above were developed and used therapeutically. However, strains of *Staphylococcus aureus* resistant to penicillin were isolated in 1944, only a couple of years after its introduction. Methicillin was developed to treat these strains but resistance soon developed, and methicillin-resistant *Staphylococcus aureus* MRSA is now common in most hospitals. Many of these hospital strains are also resistant to a number of other antimicrobials. One of the major challenges confronting medical practice today is the number of microorganisms that have developed resistance to the currently available antimicrobial agents. Initially scientists were able to find new antimicrobials, but there have been no significant discoveries of new classes of antimicrobials for more than two decades—most 'new' antimicrobials are chemical modifications of existing drugs.

Prior to the introduction of penicillin, most hospital infections were caused by Gram-positive staphylococci and streptococci. Very few were due to Gram-negative organisms. However, in the late 1970s and 1980s, due to the selective pressure of antimicrobial use, infections with Gram-negative organisms became more common. Gram-negative bacteria, resistant to many of the antimicrobials in use, have since emerged. These bacteria produce enzymes described as '**extended spectrum beta-lactamases**' (**ESBLs**) because of their ability to destroy beta-lactam antibacterials such as the broad spectrum cephalosporins. More recently, **MBLs** (metallo beta-lactamase enzymes) have been identified that confer resistance to carbapenems (see Spotlight box: Global spread of carbapenem resistance, page 282 and Case History 13.2, page 295).

Some microorganisms (e.g. *Pseudomonas* and *Acinetobacter*) have cellular characteristics that give them a natural resistance to many antimicrobial drugs. But the main problem facing clinicians is that the therapeutic (over-) use of antimicrobial drugs has given rise to an increasing number of microorganisms that have acquired resistance to drugs to which they were previously sensitive.

Worldwide, we have seen the emergence of multiple-drug-resistant strains of organisms such as *Mycobacterium tuberculosis*, *Neisseria gonorrhoeae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Klebsiella*, *Escherichia coli* and *Enterococcus* species.

Bacteria may develop resistance to antimicrobial drugs in any of the following ways:

- By the production of enzymes that are able to destroy or inactivate the drug. For example, the beta-lactamase enzymes produced by many organisms break open the beta-lactam ring in the penicillin or cephalosporin molecule, destroying its structure and preventing the drug from binding to its target, which is the enzyme involved in the synthesis of the structural peptidoglycan of the bacterial cell wall.
- By a change in membrane permeability that results in the drug being unable to penetrate through the membrane into the cell. This may be due to a change in structural protein, a decrease in pore size or an alteration in the transport system.
- The rate of efflux of the drug from the cell may be increased so that the drug is not able to attain a sufficiently high concentration inside the cell to cause inhibition. This is the basis of resistance to drugs such as the tetracyclines, which inhibit protein synthesis on the ribosomes inside the cell.
- By an alteration in the binding sites for the drug in the bacterial cell. Most drugs bind to a specific site in the cell and exert their effect by interfering with enzyme activity or protein synthesis. If the structure of the binding site is altered, the drug is no longer able to bind and so is unable to have any effect on the cell and the cell becomes resistant. Some penicillin-resistant bacteria (e.g. *Streptococcus pneumoniae*) have altered penicillin-binding proteins, so that penicillin is no longer active against them. Alterations to the specific sites on the bacterial ribosome prevent the action of the aminoglycosides and erythromycin which inhibit protein synthesis.
- Some drugs, such as the sulfonamides, exert their effect by inhibiting reactions in essential metabolic pathways. One way in which bacteria develop resistance to these drugs is by the production of other enzymes that have no affinity for the drug and can synthesise the required compounds via an alternate pathway.

The development of these resistance mechanisms involves a change in the genetic properties (DNA) of the cell. This can occur as the result of a spontaneous mutation. Subsequent exposure of the cell population to an antimicrobial drug then exerts a selective pressure, killing sensitive organisms and allowing the resistant mutants to multiply (see Figure 12.14). This may occur during a prolonged course of antimicrobial therapy. It can also occur when a patient does not complete the full course of a prescribed drug. Resistant mutants will also be selected under these conditions, although the level of drug resistance (i.e. the concentration of drug required to kill the cells) may not be quite as high. Without the selective pressure applied by the use of antimicrobial drugs, the population of cells would remain sensitive. The overuse or misuse of antimicrobial drugs can therefore contribute to the development of resistant strains of microorganisms.

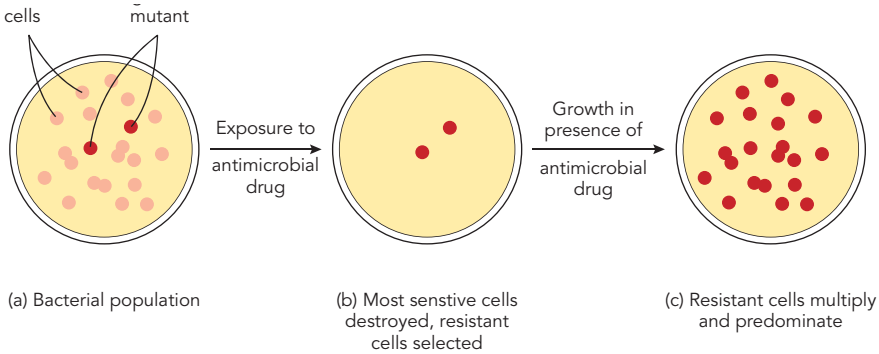


FIGURE 12.14

Effect of exposure to antimicrobial drugs

If the concentration of drug is not sufficient to kill all *cells, the resistant mutant cells are selected and can multiply.*

Transfer of resistance

In the clinical area, an important mechanism of resistance is the transfer of resistance genes from resistant to susceptible strains of closely related genera. This is usually mediated by plasmids during conjugation (see Chapter 4). The initial appearance of resistance in susceptible bacteria is usually due to a mutation in only one gene. However, the level of resistance can be increased if the bacteria acquire additional resistant genes or R-factors from other bacteria.

It is now recognised that an important method of spread of resistance is the conjugative transfer of resistance factors on plasmids, not only within species but also between closely related genera. Thus, if a patient who is carrying a sensitive strain of *Escherichia coli* acquires a multi-resistant strain of, say, *Shigella*, the resistance genes may be transferred in the intestine from the related genus *Shigella* to *E. coli*. The patient then harbours and excretes a strain of multi-resistant *Shigella* as well as multi-resistant *E. coli*, even though the original *E. coli* strain in the patient has not been exposed to any antimicrobial drugs.

Often the genes for resistance to a number of different antimicrobials are located beside each other on a **transposon**, so the transfer of a plasmid will also transfer multiple resistance genes. The increase in resistance in hospital isolates of Gram-negative bacteria is thought to be largely due to the transfer of resistance factors in this manner. In addition, it is thought that the extensive use of antimicrobials in animal feed may contribute to an increase in the numbers of resistant organisms in animal products, which can then be transferred to humans in food or by contact with the animal.

Cross-resistance refers to the situation where an organism that has developed a mechanism of resistance to one drug will also be resistant to related drugs. For example, an organism that produces a beta-lactamase enzyme may be

SPOTLIGHT ON

Global spread of carbapenem resistance

The Gram-negative Enterobacteriaceae are the most abundant group of commensal microorganisms in humans and the most frequent cause of bacterial infections in patients of all ages. They include organisms such as *E. coli, Klebsiella pneumoniae* and *Enterobacter* sp. In recent years, they have acquired various types of resistance mechanisms to evade antibiotics, including the **ESBL**s (extended spectrum-beta- lactamases) enzymes that inactivate beta-lactam antibiotics (penicillins and cephalosporins). Carbapenems that were introduced in 1985 were a new class of antibiotic and were resistant to the ESBLs. However, since then, there have been numerous reports of bacteria producing different types of metallo-beta-lactamase enzymes that inactivate carbapenems, including: **KPC** (*Klebsiella pneumoniae* carbapenemase), **IMP** (active on imipenem), **VIM** (Verona–integron-encoded metallo-beta-lactamase) and **OXA-48** (oxacillinase-type beta-lactamase). These are widespread in Europe, the United States and Asia.

In most cases, the genes encoding for these resistance enzymes are located on transposable elements so are easily transferred between different species of the Enterobacteriaceae.

By 2010 highly resistant **NDM-1** (New Delhi metallo-beta-lactamase-1) producing Enterobacteriaceae appeared in various countries in Europe and have also spread as far as Australia. They are often associated with travel to the Indian subcontinent for medical procedures. Most of the NDM-1 producers are resistant to all antibiotics, so invasive infections have a high mortality rate.

Most carbapenemase-producing strains occur in hospital isolates and pose a serious risk to hospital patients. It is important that there is early detection of these strains to prevent their establishment in the hospital environment.

resistant to both penicillin and cephalosporin, even though it may have been exposed to only one of the drugs. The enzyme inactivates the drug in both cases by destroying the beta-lactam ring.

Drug resistance in hospitals

The high usage of antimicrobial drugs in hospitals, especially in intensive care units, provides an atmosphere in which resistant organisms can flourish. Resistant strains of *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella* are often implicated in ventilator-assisted pneumonia (VAP). Other problem organisms include resistant strains of *Streptococcus pneumoniae*, vancomycin-resistant enterococcus (VRE), MRSA, and strains of *Klebsiella* and *Escherichia coli* with an extended spectrum of β-lactamase resistance and carbapenem resistance. Virulent strains of group A streptococci (GAS) also pose a problem. Attention to infection control procedures, especially handwashing, is essential to prevent the spread of resistant organisms to other patients in the hospital (see Chapter 13).

Antimicrobial stewardship

In Australian hospitals the concept of **antimicrobial stewardship** is being promoted for the responsible use of antimicrobials. It is usually undertaken by a multidisciplinary committee consisting of an infectious diseases specialist, a clinical microbiologist and a hospital pharmacist, and should form part of the quality assurance program. The activities include:

- implementation of policies in line with the latest version of the *Therapeutic Guidelines—Antibiotic*
- establishing prescribing systems that include restriction of broad spectrum and newer antimicrobials to patients where their use is clinically justified
- reviewing antimicrobial prescribing with intervention and direct feedback to the prescriber
- surveillance to determine resistance patterns of hospital organisms
- monitoring performance of antimicrobial prescribing by collecting and reporting unit- or ward-specific usage data, auditing antimicrobial use
- educating prescribers, pharmacists and nurses about good antimicrobial prescribing practice and antimicrobial resistance
- changing from parenteral to oral administration as soon as practicable
- liaising with infection control teams to implement infection control procedures.

Antimicrobial resistance in the community

The availability of drugs to treat even minor infections has led to an overuse of antibacterial drugs, especially in the community. Viruses are responsible for many respiratory tract infections, including the common cold, influenza, RSV, viral pneumonia and viral gastroenteritis. These infections do not respond to treatment with antibacterial drugs, and the prescribing of such drugs for illnesses that are primarily viral in nature is one of the contributing factors to the widespread development of resistance in bacteria.

If the patient's population of normal flora is exposed to an antibacterial drug, a few mutant cells resistant to the drug are likely to arise and they will flourish in an environment where other cells that normally compete for nutrients have been destroyed (see Figure 12.14, page 282). If this occurs, the patient may acquire a population of resistant bacteria, even though the original infection was due to a virus. The use of antibacterials is indicated only when secondary bacterial infections occur following a viral infection of the respiratory tract.

It is important to note that it is not the person who becomes resistant to the antimicrobial—it is the bacteria.

In the past, resistant strains of organisms such as *Staphylococcus aureus* (MRSA) were mainly limited to hospitals where high usage of antibiotics encouraged their selection. However, resistant strains of a number of important pathogens are increasingly being isolated in the community. Research is needed to identify the origin of these strains and to implement policies to limit their spread. The occurrence of these resistant strains may reflect early discharge policies, in that patients may take the hospital strains out into the community when they leave hospital. It may also be due to high levels of prescribing in the community.

A particular problem is the increase in incidence of resistant strains of *Streptococcus pneumoniae*, which is a major cause of community-acquired pneumonia (CAP), upper respiratory tract infections including otitis media (middle ear infections) and meningitis. This problem has been partly addressed with the development of pneumococcal vaccines (see Chapter 14).

Since the 1990s, strains of community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) have been isolated in Australia (CA-MRSA or cMRSA). The first ones were from remote communities in the Kimberley region in Western Australia. They were distinct from other strains of MRSA because they were susceptible to a number of non-β-lactam antimicrobials such as gentamicin. Since then other strains of cMRSA have been identified in the eastern states, some of them carrying a virulence factor called **Panton-Valentine leukocidin** (**PVL**) that is the cause of necrotising pneumonia. Molecular typing studies have linked this strain to the Pacific Islands. Another strain (clone) has been identified in Queensland.

Some MRSA strains have been imported from other countries; for example, EMRSA (epidemic MRSA) from the UK has occurred in some hospitals—probably introduced by healthcare workers. In many Asian countries where antimicrobial drugs are freely available without prescription, there is a high incidence of resistant strains of bacteria, and there is evidence of travellers bringing these strains to Australia.

Worldwide, the occurrence in the community of multiresistant strains of pathogens such as *Neisseria gonorrhoea* and *Mycobacterium tuberculosis* presents a serious public health problem.

The search for new antimicrobials

Although there has been a rapid increase in resistance in recent years, the rate of development of new antimicrobial drugs has decreased. Most antimicrobials are developed by major drug companies who claim that the cost of development, clinical trials, marketing and surveillance of new drugs is prohibitive. In developed countries, there is an insistence on 'zero risk' for any new drug, which means that the clinical trials need to be very extensive (and costly). Unfortunately, drugs soon become obsolete if resistance develops rapidly.

To deal with the dwindling supply of antimicrobials, authorities are advocating more attention to appropriate prescribing practices and increased infection control measures to limit the spread of resistant organisms within the hospital environment. There is renewed interest in phage therapy—a method of treatment that was popular in the former Soviet Union before the discovery of antibiotics (see Spotlight box).

PROBIOTICS

In recent years there has been increasing attention focused on the use of **probiotics**, or 'good bacteria', to maintain health and to treat certain diseases. Probiotics are health-promoting bacteria belonging mainly to the genera *Lactobacillus*, *Bifidobacterium* and *Saccharomyces*. They are often present in commercial yoghurt preparations in addition to the traditional yoghurt-making bacteria, and are also available as live freeze-dried powders and capsules. Oligosaccharides and other carbohydrates that promote the growth of these bacteria are called **prebiotics**. A number of food products contain both pre- and probiotics and are marketed as promoting good health. However, when contained in food the number of viable (live) organisms can vary considerably, depending on the manufacturing process and handling and storage of the product. The organisms must be resistant to acid and bile in order to survive passage through the gastrointestinal tract.

There have been numerous studies on the efficacy of treatment of certain infectious diseases with various combinations of these probiotics. Some of these studies are carried out by food companies to promote their products. Others are conducted by medical practitioners, with the results published in refereed journals. It is important to critically evaluate the results of such studies. Probiotic products that claim specific nutritional or therapeutic properties blur the boundaries between what is a food, a diet supplement or a medicine.

Disturbances to the composition of the normal flora in the gut due to overuse of antibiotics, changes in diet, or stress can lead to an overgrowth of damaging bacteria, resulting in gastrointestinal diseases. The administration of probiotics can help to restore a normal balance.

There is strong supporting evidence that *Saccharomyces boulardii*, *Lactobacillus rhamnosus*, *L. paracasei* (Shirota) and *Bifidobacterium lactis* are effective in the treatment of a number of disorders, including antibiotic-associated diarrhoeas, *Clostridum difficile*-associated diarrhoea, rotavirus diarrhoea, lactose intolerance and irritable bowel syndrome. There is also increasing evidence for their use for bacterial vaginitis. *E. coli Nisse* has been used in the treatment of ulcerative colitis. More research is needed to establish the usefulness of these preparations as an alternative to antibiotic therapy.

ALTERNATIVE MEDICINES

The use of alternative and traditional medicines is widespread in developing countries and is becoming increasingly popular in Western societies. There is an assumption that 'natural' remedies are better and safer. Products containing the Chinese herb *Artemisia annua* are effective against malaria, and therapies such as acupuncture have proven benefits. There is anecdotal evidence for the efficacy of cranberry juice to relieve

SPOTLIGHT ON

Phage therapy

The emergence of antimicrobial resistance in many bacteria has led to renewed interest in bacteriophage therapy—an old method of treatment that was used extensively in the former Soviet Union prior to the discovery of antibiotics. Bacteriophage are viruses that are able to specifically infect and lyse certain bacteria. In recent years they have been used to differentiate between clinical isolates of bacteria (such as MRSA) in diagnostic laboratories (see Chapter 3, page 60).

However, before World War II Soviet doctors used bacteriophage extensively to treat a wide range of bacterial infections, especially in surgery and wound infections such as gangrene. Other uses included treatment of persistent soft tissue infections, the phage preparation being applied as topical liquid. There are a number of reports of oral phage preparations being used to treat severe diarrhoea, and also to treat carriers of typhoid fever.

The advantage of phage therapy is the specific nature of the virus attack. As discussed in Chapter 5, viruses are highly specific for the types of cells they can infect. Theoretically, if a live phage can be delivered to the site of an infection caused by a susceptible bacterial strain, it should be able to destroy the pathogen without damage to the host tissues. It may be that, in the future, scientists will be able to develop this therapy to treat the drug-resistant bacteria that are emerging.

Note: For reviews of phage therapy, see N. Chanishvili and R. Sharp 2008, Bacteriophage therapy: Experience from the Eliava Institute, Georgia. *Microbiology Australia* 29(2), May: 96–101, <www.theasm.com.au> and <www.phageinternational.com>.

symptoms of urinary tract infections. Tea tree oil is used for topical applications and has anti-inflammatory properties as well as some antimicrobial and antifungal effects. Research is being carried out on the antimicrobial effect of other essential oils. They are mostly only suitable for topical application.

However, these alternative medicines are largely unregulated and sold over the counter without prescription, and there may be no medical advice given or follow-up of patients. There have been questions about the quality of products, and reports of people using inappropriate medication for their condition or taking too large a dose. It is important that patients inform their doctor about any 'natural' medication they are taking. For example, *Ginkgo biloba* is a popular herb used to prevent vascular disease, but it can cause excessive bleeding during surgery.

The World Health Organization has issued guidelines to provide information that will facilitate proper use of these medicines. These are available at: <www.who.int/topics/traditional_medicine/en/>.

In Australia, the Therapeutic Goods Administration (TGA) <www.tga.gov.au> regulates all medicines for which there are therapeutic claims. These include prescription medicines, over-the-counter medicines and complementary medicines. Complementary medicines, also known as traditional or alternative medicines, include vitamins, minerals, nutritional supplements, herbal remedies, aromatherapy and homeopathic products.

Any product for which therapeutic claims are made must be entered in the Australian Register of Therapeutic Goods before the product can be supplied in Australia.

SUMMARY

ORIGINS OF CHEMOTHERAPY

- The idea of finding a chemical to kill the specific microbe responsible for an infection without damaging the host cells (i.e. a 'magic bullet') was proposed by the chemist Paul Ehrlich in 1908.
- The first successful antimicrobials were the chemically synthesised sulfonamides, or sulfa drugs.
- An antibiotic is a chemical, produced by a microorganism, that in small amounts can kill or inhibit another microorganism. Some antibiotics are chemically modified to improve their antimicrobial properties and are called 'semi-synthetic'.

THE DEVELOPMENT OF ANTIMICROBIAL DRUGS

- Selective toxicity is the ability of a drug to kill or inhibit the microorganism responsible for the disease without damaging the host cells.
- Antimicrobials are compounds that can kill or inhibit the growth of a microorganism. They include antibacterial, antifungal, antiprotozoal and antiviral drugs.
- Compounds that are active against several different types of microorganisms are broad spectrum drugs; those with a limited range of activity are narrow spectrum drugs.
- Drugs that kill microorganisms have names ending in *-cidal*, while those that inhibit replication end in *-static*.

ANTIBACTERIAL DRUGS

- The main target sites for antibacterial action are the synthesis of the bacterial cell wall, protein synthesis, nucleic acid synthesis and the function of the bacterial cell membrane.
- Peptidoglycan is a compound unique to the bacterial cell wall and therefore provides an ideal target for selective toxicity.
- The beta-lactam antibacterials inhibit cell wall synthesis by combining with the enzyme responsible for cross-linking of the peptidoglycan chains. They include the penicillins, cephalosporins, carbapenems and monobactams.
- The production of the enzyme beta-lactamase, which destroys these antibacterials, is a major mechanism of resistance.
- Other cell wall inhibitors include the glycopeptides, vancomycin and teicoplanin, which interfere with cell wall synthesis by binding to the growing peptide chains of the peptidoglycan molecule.
- Compounds that inhibit the synthesis of protein include the aminoglycosides, the tetracyclines and the macrolides, as well as chloramphenicol, clindamycin and fusidic acid.
- Antimicrobials may inhibit the synthesis of nucleotide precursors, or DNA or RNA replication. They include the sulfonamides, quinolones and rifampicin.
- Metronidazole (Flagyl®) is active against anaerobic bacteria as well as some intestinal protozoa.
- Polymyxins are cyclic polypeptides which act as cationic detergents and disrupt the phospholipid bilayer of the membrane.

ANTIFUNGAL DRUGS

- The main target of antifungal drugs is the synthesis or function of the fungal cell membrane. They include the imidazoles, which inhibit the enzymes involved in sterol biosynthesis, and the polyene antibiotics, amphotericin B and nystatin.
- Cutaneous mycoses such as tinea and thrush are treated mainly with topical preparations containing miconazole, clotrimazole or nystatin.
- Increasing numbers of immunocompromised patients are susceptible to opportunistic fungal infections.

ANTIPARASITIC DRUGS

- Various quinine derivatives are used for the treatment of malaria.
- Prophylactic malaria chemotherapy involves the use of chloroquine or doxycycline.

ANTIVIRAL DRUGS

- For an antiviral drug to be selectively toxic, it must target a unique point in the cycle of viral replication.
- The most successful antiviral drugs are chemically synthesised analogues of the purine and pyrimidine bases.
- Antiretroviral drugs target a specific viral enzyme, called reverse transcriptase.
- The action of these drugs is virustatic (i.e. when the drug is removed, viral replication can resume).
- Interferons are a family of small glycoprotein molecules produced by many kinds of mammalian cells in response to viral infection.

THERAPEUTIC USE OF ANTIMICROBIAL DRUGS

- To select the most effective antimicrobial to use, the actual strain of the organism causing the infection is tested against a range of possible antimicrobial agents. Two methods are disk diffusion tests and the minimum inhibitory concentration (MIC) test.
- The use of antimicrobial drugs can lead to suppression of the normal flora, the occurrence of hypersensitivity and other adverse side effects, and the emergence of resistant strains of pathogens.
- Usually, doctors prescribe on the 'best guess' principle and then review the prescription if and when the results of the pathology tests are available.
- The prescribed dosage aims to achieve an effective concentration of the drug at the site of infection.
- Two drugs may be prescribed at once to treat mixed infections, or to minimise the risk of resistance.
- Prophylaxis is the administration of an antimicrobial drug before there is any evidence of infection, under conditions where the risks of an infection developing are considered very high.

IMPLICATIONS FOR NURSING PRACTICE

- The nurse is responsible for the collection of an appropriate specimen in a sterile, labelled container, using aseptic technique, for arranging transport to the laboratory as soon as possible and for receiving the reports.
- The nurse should be aware of the correct methods of administration of the drugs and any special precautions that apply.
- Patients should be closely monitored for adverse reactions, such as hypersensitivity, which can occur in patients who are allergic to penicillin and may cause anaphylaxis.
- The patient must be observed for signs of improvement and for side effects such as an overgrowth of other microorganisms, causing thrush (*Candida albicans*) or diarrhoea (*Clostridium difficile*).

DEVELOPMENT OF RESISTANCE TO ANTIMICROBIAL DRUGS

- Bacteria may exhibit resistance by the production of enzymes which destroy or inactivate the drug, by a change in membrane permeability, by an alteration in the binding sites for the drug, or by synthesis of the required compounds via an alternate pathway.
- The high usage of antimicrobial drugs in hospitals provides an environment in which resistant organisms can flourish.
- Strategies to control the spread of antimicrobial resistance include hospital policies for prescribing antimicrobials, use of narrow spectrum antimicrobials, improved surveillance to determine prevalence, infection control procedures to prevent hospital-acquired infections, and a continuing search for vaccines against bacterial infections.
- The search for new kinds of therapy includes the use of phage therapy and probiotics.

STUDY QUESTIONS

1. What is meant by the term 'selective toxicity'?
2. Give three examples of ways in which antibacterial drugs can be selectively toxic.
3. Define the terms 'broad spectrum' and 'narrow spectrum' when applied to antimicrobial drugs.
4. Discuss the different ways in which antibacterial drugs can inhibit or damage the bacterial cell.
5. What are some of the major side effects associated with the use of aminoglycosides?
6. Why should tetracyclines not be given to children or pregnant women?
7. Why is chloramphenicol used mainly in topical applications?
8. What is the main point of attack of most antifungal drugs?
9. Why is it difficult to design effective antiviral drugs?
10. What are interferons?
11. How is an antimicrobial sensitivity test carried out?
12. Why do doctors prescribe medication before the laboratory results are available?
13. Should specimens for diagnosis of infection be collected before or after the implementation of antimicrobial therapy?
14. What is meant by the 'half-life' of a drug?
15. List four ways in which bacteria can resist the action of antibacterial drugs.
16. Why are hospital-acquired infections more likely to be resistant to antibacterial drugs than community-acquired infections?
17. In what situations is the prophylactic use of antimicrobials considered necessary?
18. What are the responsibilities of the nurse in administering and monitoring antimicrobial therapy?

TEST YOUR UNDERSTANDING

1. Why is it preferable to prescribe bactericidal drugs under some conditions, rather than bacteriostatic drugs?
2. Why may bacteria that produce beta-lactamase enzymes exhibit resistance to both penicillins and cephalosporins?
3. Name two major disadvantages of the indiscriminate use of antimicrobial drugs.
4. Explain why it is more difficult to treat systemic fungal infections than cutaneous infections.
5. Why should travellers to malarious areas seek advice from the Health Department?
6. Explain why analogues of nucleic acid precursors are effective as antiviral agents.
7. Why is it necessary to perform antimicrobial sensitivity tests?
8. What precautions should be taken with patients who are allergic to penicillin?
9. What are the advantages/disadvantages of prescribing two drugs simultaneously?
10. Explain how bacteria can become resistant to antimicrobial drugs.

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CHAPTER

13

Infection control in healthcare facilities

CHAPTER FOCUS

- Why are healthcare-associated infections (HAIs) important?
- What factors contribute to the incidence of infections in healthcare facilities?
- How are infections transmitted in hospitals?
- What is the relevance of community-associated organisms and infections to healthcare facilities?
- What are 'Standard Precautions' and 'Transmission-based Precautions'?
- What is the importance of and methods used for hand hygiene in healthcare facilities?
- Which groups of patients are most susceptible to infection by hospital pathogens?

The very first requirement in a hospital is that it should do the sick no harm.

—Florence Nightingale

INTRODUCTION

The World Health Organization (WHO) states that 'patient safety is a fundamental principle of health care'. However, it also acknowledges that care-giving is a complex process that has a certain degree of inherent unsafety, and that unexpected and unwanted events can take place in any setting where healthcare is delivered. In the last decade the importance of improving patient safety has been increasingly recognised throughout the world, and in 2002, the WHO World Health Assembly agreed on a resolution on patient safety, based on the need to reduce the harm and suffering of patients and their families, as well as the compelling economic benefits. As a result, in October 2004 the WHO launched a Patient Safety program that urged countries to pay close attention to patient safety in healthcare.

Infection is the most common adverse event affecting patient safety in hospitals, and hence, one of the key aims of the WHO Patient Safety program is to reduce the incidence of healthcare-associated infections. Each year, hundreds of millions of patients throughout the world are affected by an infection acquired in a healthcare setting, and these infections are a major cause of death and disability worldwide. Surgical patients, patients who have an invasive procedure in hospital, and patients in intensive care units (ICUs) are particularly prone to acquiring an infection; however, infection is also a problem in other types of healthcare settings.

Up to 12 per cent of hospitalised patients in developed countries acquire an infection (see Figure 13.1). In some developing countries, the rate of healthcare-acquired infection can be a high as 25 per cent. In Australia it is estimated that around 200 000 patients develop a healthcare-associated infection each year. Of these, around 4500 develop *Staphylococcus aureus* bloodstream infections, with one-third of these patients dying.

The Australian Commission on Safety and Quality in Health Care (ACSQHC) was established by state and territory governments and commenced on 1 January 2006. The focus of the work of the Commission is on areas of the health system where current and complex problems or community concerns could benefit from urgent national consideration and action to achieve a measurable reduction in healthcare-associated infection. The Healthcare-Associated Infection

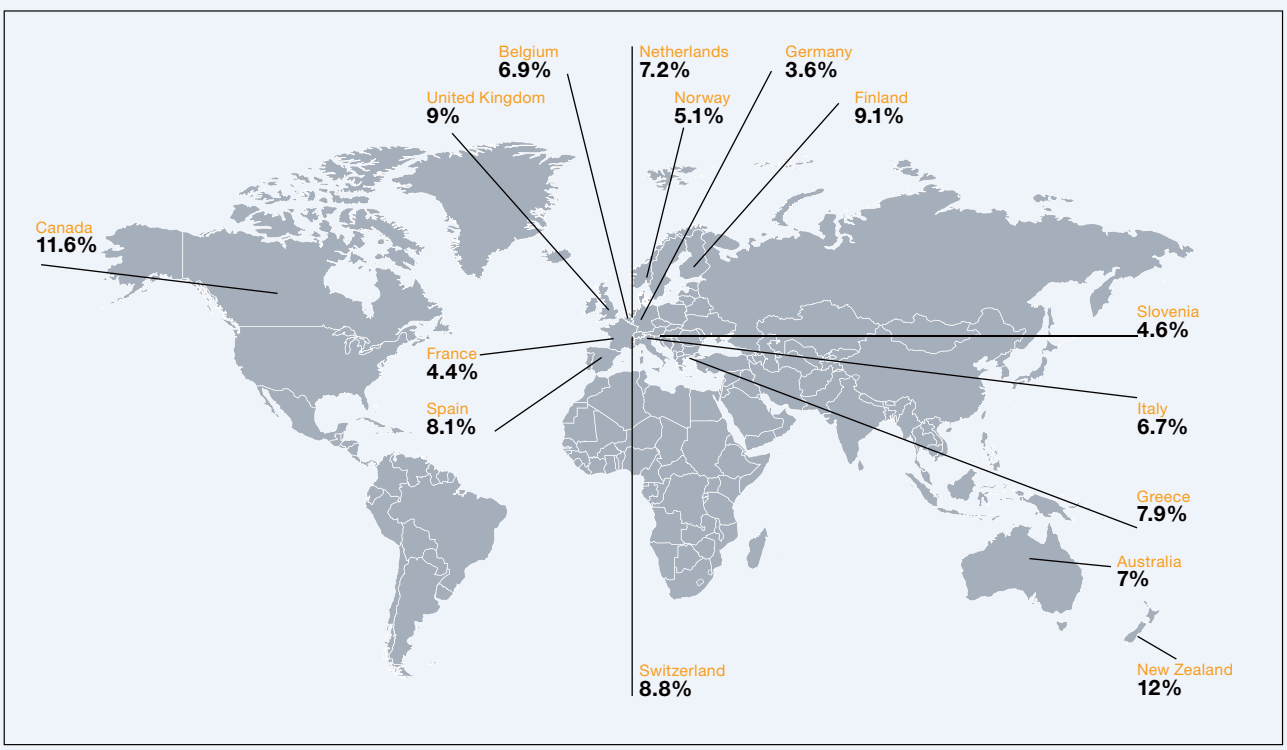

FIGURE 13.1

The prevalence of healthcare-associated infection in high-income countries, 1995–2010*

* For countries with more than one study, the most recent figures are included.

Source: Adapted from World Health Organization, *Report on the Burden of Endemic Health-Care Associated Infection Worldwide* (2011), Figure 3.3, p. 13.

program was nominated as one of the priority areas for 2007–10, and it aimed to develop a national approach to reducing healthcare-associated infection by identifying and addressing systemic problems and gaps, and ensuring comprehensive actions are undertaken in a nationally coordinated way.

In October 2010 the ACSQHC released its *Australian Guidelines for the Prevention and Control of Infection in Healthcare*. The guidelines, which were developed by the National Health and Medical Research Council (NHMRC), detail the core principles for the prevention and control of infection in all healthcare settings. Their focus is on hospital infection control, but they are designed to be readily adapted to other healthcare settings, including primary and community care.

A **healthcare-associated infection** (**HAI**) is defined as an infection occurring in a patient in a hospital or other healthcare facility in whom the infection was not present or incubating at the time of admission. This includes infections acquired in the healthcare facility but appearing after discharge, and occupational infections among healthcare staff. While the focus tends to be on hospitals, it is important to recognise that infection may occur in other types of healthcare facilities, including doctors' consulting rooms, day-surgery centres, residential aged care, home nursing services and ancillary health services such as dental and physiotherapy practices. It is also important to recognise that HAI may also afflict healthcare workers, visitors or any other people who enter these settings. Infected patients are a major reservoir of infection, but contaminated equipment and instruments, as well as the hospital environment, are potential sources of pathogenic microorganisms. HAIs are sometimes called **nosocomial infections**, from the Ancient Greek words *nosos*, meaning 'disease', and *komeion*, meaning 'to take care of'. The term **hospital-acquired infection** refers to an infection specifically acquired in a hospital.

A BRIEF HISTORY OF HOSPITAL INFECTION

Specialised buildings for the care of the sick have existed for over 2000 years. In early times these buildings were designed to allow for plenty of fresh air, and bathing and hygiene were considered very important. During and after the fall of the Roman Empire the emphasis on good hygiene seemed to be eroded. Ignorance and superstition blamed evil spirits and miasmas ('toxic emanations from the earth') for the occurrence of infectious diseases. Hospitals in the Middle Ages were overcrowded, unsanitary buildings with poor ventilation and several patients to a bed (Figure 13.2). More than 50 per cent of hospitalised patients died.

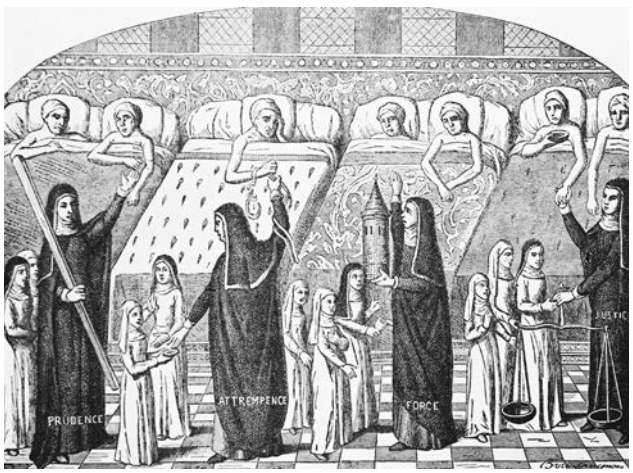

FIGURE 13.2

A ward in the Hotel Dieu, Paris, from a 15th century engraving

Note the overcrowding and sharing of beds. The nun–nurse tutor is holding a large incense dispenser to camouflage the foul odours and neutralise the 'miasmas'.

Source: © Bettmann/Corbis.

During the early 19th century, some physicians tried to draw attention to the appalling rate of infection and to devise ways of reducing mortality. However, there were many who did not believe that diseases were contagious and blamed high mortality rates on 'intrinsic defects' in the patients, such as poverty or ignorance.

A major problem during the first half of the 19th century was puerperal fever (childbed fever), a septicaemia that occurred in women following childbirth and that had a high mortality rate. Oliver Wendell Holmes published an essay in 1843 claiming that puerperal fever was contagious. This was at a time when most people believed that bad air was responsible for such diseases. It was almost 20 years before his tenet was accepted.

At about the same time, the Hungarian physician, Ignaz Philipp Semmelweis, who was head of the maternity service in a Vienna teaching hospital, noticed the smell of cadavers on the hands of doctors and medical students who were in the habit of going directly from a post-mortem examination to a delivery room without washing their hands. He suggested that the cause of puerperal fever was being spread from the corpses in the autopsy room to the women in the labour rooms. He demonstrated that, when the doctors washed their hands in a chlorinated solution before entering the obstetric ward, there was a dramatic decrease in the incidence of puerperal fever and mortality (see Figure 13.3). We now know that these infections are mainly caused by streptococcal bacteria. Unfortunately, Semmelweis's classic paper, published in 1860, was not accepted at the time by his profession. He was later confined to a mental institution where, ironically, he is alleged to have died from a streptococcal infection in 1865.

The Scottish surgeon James Simpson made a study of the epidemiology and prevention of 'surgical fever', which he believed was similar to puerperal fever. In 1869 he reported

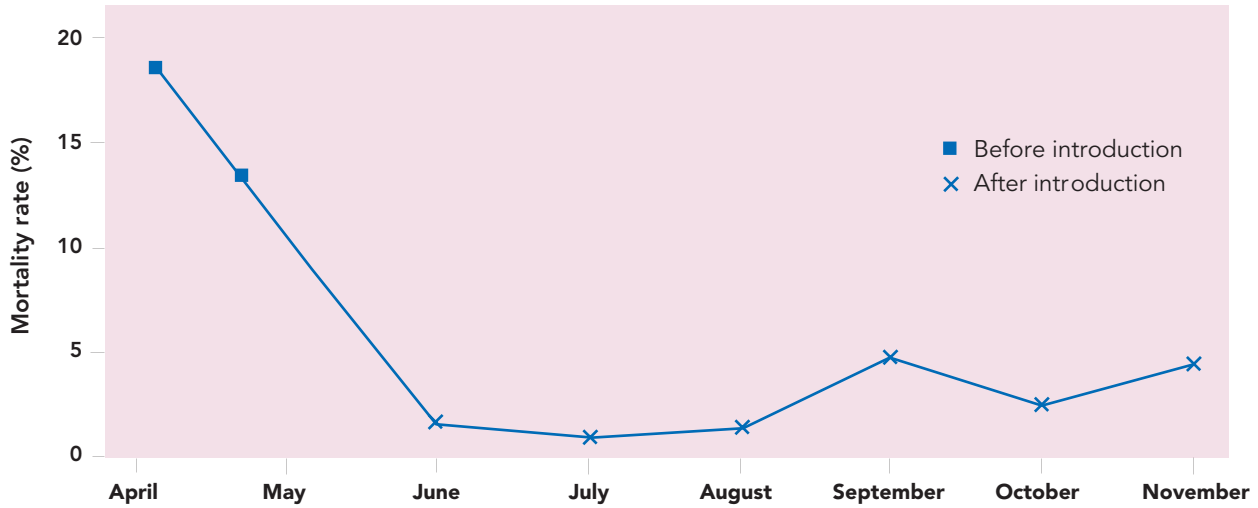

FIGURE 13.3

Effect of hygienic hand disinfection as introduced by Semmelweis in May 1847 on maternal mortality at the Vienna Lying-in Hospital

Source: Data from I. Semmelweis; K. Codell Carter (translator and extensive foreword) (1861). *Etiology, Concept and Prophylaxis of Childbed Fever*, Data for Jan 1841 to May 1847 from table 3, p. 72; for Jun 1847 to Dec 1847 from table 6, p. 90.

that over 40 per cent of amputees in large metropolitan hospitals died after surgery, a rate that was four times higher than for amputations performed by country physicians. He noted that death rates increased with the size of the hospital and reported that in large hospitals pyaemia (septicaemia) accounted for 60 per cent of all deaths. He concluded that the bringing together of so many sick people in a confined area was 'perilous', and made a number of recommendations to reduce the incidence of infection, one of which was that dressings should not be reused!

The British surgeon Joseph Lister is considered to be the first person to introduce the use of antiseptics in surgery. In the 1860s he began to use a fine spray of carbolic acid to disinfect operating rooms and as an antiseptic in wound dressings, and reported a decrease in mortality after amputation from 46 per cent to 15 per cent.

Florence Nightingale was also aware of the high mortality rates in hospital wards. She is noted for many achievements in nursing, not the least for her efforts in English field hospitals in Turkey during the Crimean War. She was sent there in 1854 as Superintendent of Nursing because of a public outcry over the death rate of British soldiers in these hospitals. The conditions in these 'medical facilities' were appalling, and the death rate of over 50 per cent was due mainly to infection. Within six months of her arrival the mortality rate had dropped to under 3 per cent, thought to be at least partly due to the much-improved sanitary measures that she introduced. Ten years later, still concerned with infection rates in hospitals, Florence Nightingale wrote that 'in all probability a poor sufferer would have much better chance of recovery if treated at home'.

Towards the end of the 19th century, Robert Koch and Louis Pasteur (see Chapter 1) identified microorganisms as the causes of many diseases. The early 20th century saw moves for hospital reform and the development of methods of infection control. To reduce the spread of infection, specialised disease hospitals, such as smallpox hospitals, fever hospitals and tuberculosis sanatoriums, were established.

In the 1940s the introduction of antimicrobial drugs led to a significant reduction in mortality from infectious diseases (see Chapter 12). However, this was not matched by a reduction in the number of infections. Despite the many advances in hospital infection control, it is estimated that in developed countries in the 21st century 5–10 per cent of patients in hospitals are at risk of acquiring an infection that will, in some cases, directly or indirectly contribute to their death.

This chapter examines the causes of healthcare-associated infections, the risks associated with various medical and hospital procedures, and the measures being taken to reduce the incidence of infection in healthcare facilities. It is the responsibility of each healthcare worker to be able to identify the factors that put patients or themselves at risk, understand the principles of infection control, be aware of national and local guidelines and be able to apply this knowledge in the clinical setting. In this chapter we focus on hospitals, but the core principles described are applicable to all health settings.

THE IMPORTANCE OF HEALTHCARE-ASSOCIATED INFECTIONS

Given the continuing high incidence of healthcare-associated infections throughout the world (see below), it is clear that, to date, most efforts to control them have been largely unsuccessful. HAI create additional suffering for patients and their families. Also, for the patient there may be long-term effects on their quality of life, loss of personal income and sometimes death. HAIs also prolong the length of hospital stay

(or 'hospital bed-days'), resulting in an additional financial burden on the health system. Other impacts of HAIs include the costs of antimicrobial therapy, of replacing infected workers, and of reduced productivity. HAIs also add substantially to the problem of resistance to antimicrobial drugs (see Chapter 12). Another important consequence is that, once a patient is infected, they become an additional reservoir of infection in the hospital or community.

The WHO estimates the prevalence (proportion of patients who have an infection at a given time—see Chapter 8) of HAI in hospitalised patients in developed countries to range from 3.5 per cent to 12 per cent (average 7 per cent). The European Centre for Disease Prevention and Control has reported an average prevalence of HAI of around 7 per cent, affecting over 4 million patients. In the United States, it is estimated that 1.7 million hospitalised patients acquire an infection each year. In an Australian national survey of hospital infection rates, McLaws and co-workers (1988) found an overall prevalence of 6.3 per cent, which rose to 8.6 per cent in hospitals with more than 500 beds. More recent Australia-wide data is not available because of the lack of a coordinated approach to data collection, but the prevalence is considered to be still around 7 per cent, affecting around 200 000 patients per year.

In Australia, estimates suggest that HAIs may take up as many as 2 million bed-days per annum. This significant increase in patient bed-days not only increases costs, but also limits the availability of health resources for other patients. The annual cost of healthcare-associated infections in Europe is estimated to be €7 billion, and in the United States around US$6.5 billion. The cost of HAIs to the Australian health system is thought to be over $1 billion per annum.

Data on mortalities due solely to healthcare-associated infections is difficult to obtain, but in the United States HAIs are believed to account for around 99 000 deaths a year, and in Europe the figure is around 37 000. It is estimated that, in Australia, HAIs contribute to around 7000 deaths annually.

TYPES OF HEALTHCARE-ASSOCIATED INFECTIONS

The most common sites of HAIs are the urinary tract, surgical sites, the lower respiratory tract, the skin and blood. The relative frequency and seriousness of each type of infection is influenced by a number of factors, including the age of the patient, the type of surgery, the patient's degree of immunosuppression, and the length of time a catheter or cannula is in place.

Urinary tract infections are the most common HAI, and are most often associated with indwelling urinary catheters. Although inconvenient, urinary tract infections do not usually lead to a prolonged stay in hospital, but occasionally can lead to a more severe infection—septicaemia. A detailed description of these infections is provided in Chapter 21.

Surgical site infections are less common than urinary tract infections, although up to 15 per cent of surgical wounds become infected. The incidence varies according to the type of surgery and the underlying health of the patient (see Chapter 16). Surgical site infections are associated with substantial morbidity and mortality.

Bloodstream infections represent a relatively small proportion of HAI (around 5 per cent), but cause significant morbidity with a case-fatality rate of up to 30 per cent. An intravascular catheter is the most common factor predisposing to a bloodstream infection. The risk increases the longer the catheter is in place. Bloodstream infections are described in detail in Chapter 19.

A number of factors can predispose patients in healthcare facilities to pneumonia. Ventilator-associated pneumonia is a significant risk for patients requiring mechanical ventilation in ICUs. Other risk groups are patients with a decreased level of consciousness, young children in paediatric units, the elderly and the immunocompromised. The types, causes and risk factors of pneumonia are discussed in detail in Chapter 17.

There are many other types of HAI, including skin infections, skin ulcers (open sores) and burns wounds. Outbreaks of gastroenteritis, mainly caused by viruses, may occur in paediatric wards. *Clostridium difficile* is also an important gastrointestinal pathogen in hospitalised adults (see later in this chapter and Chapter 18). The risk of occupational exposure of healthcare workers to pathogenic microorganisms should also be recognised, particularly in relation to blood-borne viral infections, such as hepatitis B or C, and respiratory infections.

ORGANISMS THAT CAUSE HEALTHCARE-ASSOCIATED INFECTIONS

Many different microorganisms can cause HAIs. Bacteria are responsible for the great majority of HAIs, and can come from the normal flora (see Chapter 7) of the patient, or they may be pathogenic strains, originating in the healthcare facility. The latter are more problematical because they tend to be much more resistant to antimicrobial drugs, and this is a major threat to the successful treatment of these infections. Microorganisms that are unaffected by multiple types of antibiotics are said to be **multi-drug resistant organisms**, and are sometimes called **superbugs**. Although these organisms cause infections that can be very hard to treat, by virtue of their resistance to a variety of antibiotics, they are not necessarily more virulent. That is, they are not necessarily better able to cause infection, nor do they necessarily cause more severe infections. They are common hospital pathogens because they are able to survive better than other organisms in an environment where antibiotics are heavily used. The sources of HAI-causing microorganisms are discussed in a following section.

Staphylococci

Staphylococci cause a wide variety of infections, affecting the bloodstream, wounds, soft tissue, the lungs and a variety of other sites. The most important species is *Staphylococcus aureus* (called 'golden staph' because of its yellow pigment), which is a dominant cause of hospital infections (see

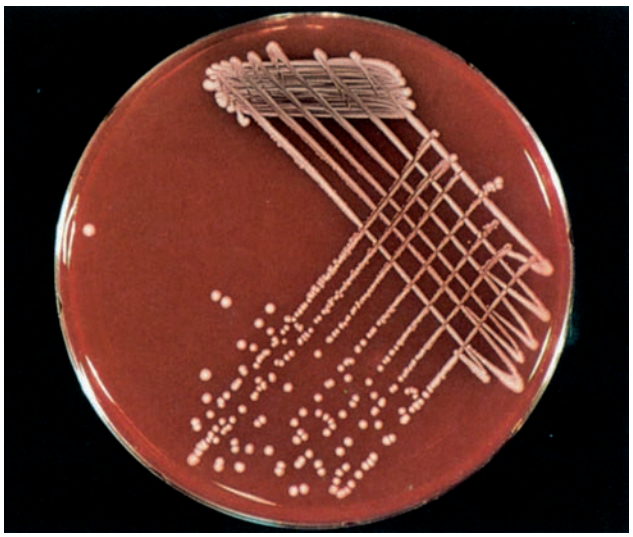

FIGURE 13.4

Culture of *Staphylococcus aureus* (golden staph)

Note the golden colour of the colonies.

Source: Courtesy of G. Jayachandran, Sydney Medical School, University of Sydney.

Figure 13.4). Penicillin was initially very effective against staphylococci when introduced in the 1940s, but by the 1950s, strains of *S. aureus* had appeared that were resistant to it. Methicillin (a derivative of penicillin) was introduced in the early 1960s, but strains of **methicillin-resistant *Staphylococcus aureus*** (**MRSA**) appeared by 1961 in Europe and by 1966 in Australia. Subsequently, strains of *S. aureus* emerged that were resistant to methicillin and a number of other drugs, and now MRSA are usually multi-drug resistant. MRSA has been responsible for numerous outbreaks in hospitals and is now considered to be endemic in many hospitals throughout the world, including most hospitals in Australia. At one time the only drug available to treat MRSA was vancomycin. In the last decade, strains of MRSA with decreased sensitivity to vancomycin have been isolated. The latter are known as VISA (vancomycin intermediate *Staphylococcus aureus*) if they have low-level resistance to vancomycin, or VRSA (vancomycin-resistant *Staphylococcus aureus*) if they have high-level resistance to vancomycin. Antimicrobial resistance has also appeared in strains of *S. aureus* of community origin (see Chapter 14).

MRSA has classically been associated with hospitals and hospital infections, and is sometimes referred to as **hospital-associated MRSA** (**HA-MRSA**). However, since the 1990s MRSA strains that appear to have originated in the community have been identified—called **community-associated MRSA** (**CA-MRSA**). CA-MRSA was initially found in remote regions of Western Australia (the Kimberleys), but strains have now been reported throughout Australia and in most other countries. CA-MRSA strains tend to have a different antibiotic resistance pattern to HA-MRSA—they are not as broadly resistant as HA-MRSA and usually not resistant to the non-beta-lactam antimicrobials (e.g. gentamicin). Many CA-MRSA strains possess a virulence factor called Panton Valentine leukocidin (PVL), which also distinguishes them from HA-MRSA. Strains of CA-MRSA have been identified in outpatient clinics and have been found to cause infections in healthcare facilities.

Patients and healthcare workers in hospitals are frequently colonised by MRSA without showing any evidence of infection. HA-MRSA does not seem to be particularly invasive in healthy individuals. The patients who are most at risk of infection by HA-MRSA are those who are debilitated or have diminished resistance to infection: infants in high-dependency units, cancer patients, patients who are immunodeficient, patients in whom foreign bodies have been implanted (such as heart valves, joint prostheses, IV lines or catheters), and patients who have undergone extensive surgery (see Case History 13.1). In contrast, CA-MRSA tends to cause infections in non-hospitalised, healthy people, although hospital acquisition of these strains can occur.

MRSA has been shown to be readily transferred from person to person and from person to fomites via hands. However, it is readily removed from hands by appropriate hand hygiene. Healthcare workers should be aware of the risks they pose if they have been working in the environment of an MRSA patient and then move to an area where patients are particularly susceptible to infection. This applies especially to agency staff, who may move from one ward to another or one hospital to another.

Staphylococcus epidermidis and other coagulase-negative staphylococci are commonly found on human skin as part of the normal flora. Formerly considered non-pathogenic, they are now recognised as responsible for many cases of HAI, especially associated with indwelling intravenous lines, cerebrospinal fluid shunts, prosthetic heart valves and other prostheses. They are naturally resistant to a number of antibiotics.

Streptococci

The pattern of microorganisms responsible for wound infections has changed over the last 80 years. In the 1920s streptococci, especially *Streptococcus pyogenes*, were the most feared microbes in hospitals. However, with the introduction of sulfonamide drugs in the 1930s, their importance waned. Within ten years, *S. aureus* had virtually replaced streptococci as the dominant cause of hospital infections. In recent years there has been a resurgence of *Streptococcus pyogenes* (group A streptococcus) as a cause of HAIs (see Figure 13.5). These bacteria are often carried in the nose or pharynx of healthy people. However, they are a common cause of wound infections and cellulitis, and some strains carry virulence factors that cause invasive infections such as necrotising fasciitis.

Vancomycin-resistant enterococci (VRE)

Vancomycin is an antibiotic which was, at one time, the drug of last resort for the treatment of serious hospital infections caused by MRSA. And as mentioned above, strains of MRSA with reduced sensitivity to vancomycin have been isolated. The emergence around the world of strains of enterococci resistant to vancomycin has also been a cause for alarm.

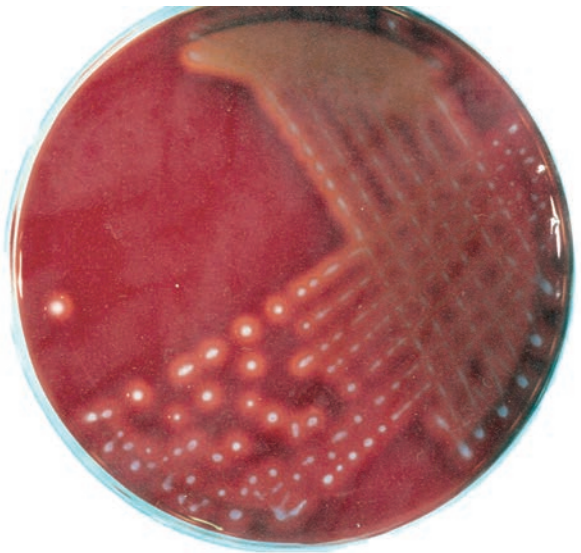

FIGURE 13.5

Culture of *Streptococcus pyogenes*

Note the clearing of the blood, called β-haemolysis, around the colonies.

Source: Courtesy of G. Jayachandran, Faculty of Health Science, University of Sydney.

Enterococcus faecium and *E. faecalis* are normal inhabitants of the human intestine and can cause hospital-acquired infections such as surgical wound infections, urinary tract infections, septicaemia and endocarditis. They are naturally resistant to a number of antimicrobial drugs including the cephalosporins and sometimes to the aminoglycosides, tetracyclines and erythromycin. In recent years, strains resistant to penicillin have appeared. The first vancomycin-resistant enterococci (VRE) appeared in Australia in 1994. Patients in ICUs are most at risk of infection with VRE, and are more likely to suffer fatal outcomes. Vulnerability to infection is largely determined by the presence of an indwelling device such as a peripheral vascular line, central line, urinary catheter or surgical drainage. Exposure to broad spectrum antibiotics predisposes a patient to colonisation and possible infection with VRE.

Because VRE is shed in the faeces, transmission of VRE in hospitals is usually due to dissemination by health personnel or contact with fomites. Enterococci can survive for long periods on surfaces, so strict attention to infection control practices and hand hygiene are essential. Patients need to be isolated and Transmission-based (contact) Precautions (see later section) implemented.

CASE HISTORY 13.1

MRSA outbreak in an ICU

Following the detection of *Staphylococcus aureus* with reduced susceptibility to vancomycin in the ICU of a hospital in Dandenong, Victoria, routine screening of patients was instituted in January 2002. Swabs of the nares and groin were taken from patients on admission, and weekly to detect MRSA colonisation.

Rifampicin was not used for surgical prophylaxis at the hospital. However, central venous catheters impregnated with rifampicin and minocycline were introduced into the ICU in June 2002 for patients predicted to require a catheter for more than 72 hours. Their use was discontinued in May 2004, when they were replaced by silver-impregnated catheters.

Between July and September 2004, seven patients with rifampicin-resistant MRSA were identified in the ICU. Six of these seven patients screened negative for MRSA colonisation on admission to the ICU, indicating nosocomial acquisition. Five of the seven patients overlapped during their ICU stay. One affected patient was admitted to the ICU on the same day that another patient with rifampicin-resistant MRSA was discharged. One patient with rifampicin-resistant MRSA did not overlap with any other affected patients. The length of stay of these patients in ICU prior to acquisition of the rifampicin-resistant MRSA ranged between 6 and 16 days.

Isolates from six of the seven patients were typed by pulsed-field gel electrophoresis (PFGE), and were identical.

All MRSA isolates from the ICU over a period of 18 months after this cluster displayed susceptibility to rifampicin.

It was considered that this outbreak of rifampicin-resistant MRSA was most probably due to horizontal transmission of the organism within the ICU. In fact, infection control staff noted that contact isolation precautions were poorly adhered to in the ICU at that time.

Source: Adapted from N.D. Friedman et al. 2006, A clonal outbreak of rifampicin-resistant methicillin-resistant *Staphylococcus aureus* (MRSA) in an intensive care unit. *Australian Infection Control* 11(2): 53. <www.publish.csiro.au/nid/241/paper/HI06053.htm>.

Questions

1. What was the significance of the vancomycin resistance in the MRSA strains in the ICU?
2. What was the significance of the rifampicin resistance in the MRSA strains in the ICU?
3. Could any other healthcare workers, apart from the unit nurses, have been involved in the transmission of MRSA in this outbreak? Explain.
4. What specific precautions should be followed when caring for a patient infected with MRSA?

Gram-negative bacteria

Enteric Gram-negative bacteria, such as *Escherichia coli*, *Klebsiella*, *Proteus* and *Enterobacter*, have always played a prominent role in HAIs. Some strains now exhibit transmissible resistance to two or more antibiotics (see Chapters 4 and 12). Some of these bacteria, in particular *Klebsiella* and *E. coli*, produce ESBLs (extended spectrum β-lactamases)—enzymes that inactivate β-lactam antibiotics such as the cephalosporins. Some are resistant to most, and sometimes all, available antibiotics.

Other Gram-negative bacteria that are significant causes of HAIs include *Acinetobacter* spp., *Pseudomonas aeruginosa* and *Burkholderia cepacia*. These organisms mainly cause opportunistic infections in severely compromised patients. *Acinetobacter* spp. occur widely in nature, and hospital infections due to *Acinetobacter baumannii* occur in patients compromised by intravascular lines, ventilator equipment, urinary catheters or surgery. *Pseudomonas* is an important cause of infection in patients with burns or suffering from cystic fibrosis. *Pseudomonas* and *Acinetobacter* tend to have intrinsic resistance to many antimicrobial drugs.

Infections with multi-resistant Gram-negative organisms are associated with significant patient morbidity and mortality.

CASE HISTORY 13.2

A new superbug: NDM-1

A 59-year-old male patient was originally from India but had lived in Sweden for many years. He had type 2 diabetes mellitus and had had multiple strokes. In November 2007, he travelled to India and was hospitalised in Ludhiana, Punjab, for surgery on a large gluteal abscess. In December 2007, he was admitted to a hospital in New Delhi, where he was again operated on and where he developed a decubital ulcer. During his stay in New Delhi he received amoxicillin-clavulanic acid, metronidazole, amikacin and gatifloxacin. On 8 January 2008, he was transferred to a hospital in Sweden.

In Sweden he was found to be colonised in various body sites (urinary tract, wound site, intestine) by a strain of *Klebsiella pneumoniae* and one of *Escherichia coli*, both of which possessed the type of enzyme designated NDM-1 (New Delhi metallo-beta-lactamase-1). This enzyme degrades β-lactam antibiotics, making these organisms resistant to a wide variety of drugs. The organism, or at least the gene that codes for the β-lactamase enzyme, was considered to have originated in New Delhi, India.

Comment

The NDM-1 gene has been found to be on plasmids, which are easily transferred between bacteria. Furthermore, the plasmid carrying *the NDM-1 gene* often contains additional genes that confer resistance to other antibiotics. Enterobacteriaceae (e.g. *Escherichia coli* and *Klebsiella pneumoniae*) are among the most serious causes of nosocomial and community-associated infections, and resistance of these bacteria to antimicrobial drugs is a serious concern. Carbapenems (β-lactam drugs) are often the last line of effective treatment against these bacteria. There is evidence that NDM-producing Enterobacteriaceae are widespread in India and Pakistan. Since 2008, NDM-producing enterobacteria have been isolated from patients in other countries, including the United Kingdom, the United States, the Netherlands and Australia. Many cases are clearly associated with the receipt of medical care in India, although there is evidence that in some cases local circulation of the organisms has occurred. Many people are choosing to undergo surgical procedures in countries such as India and China, to avoid long waiting times and to save money. This so-called medical tourism has some risks, not the least of which is inadequate infection control procedures.

Source: Adapted from D. Yong, M.A. Toleman, C.G. Giske, H.S. Cho, K. Sundman, K. Lee and T.R. Walsh 2009, Characterization of a new metallo-β-lactamase gene, *bla*$_{NDM-1}$, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrobial Agents and Chemotherapy* 53(12): 5046–54.

Questions

1. Where are Gram-negative enterobacteria most commonly found in hospitals?
2. What infection control precautions should be followed when caring for a patient with a wound infection caused by *E. coli*?
3. Explain how a hospital physiotherapist might be involved in the transmission of *Klebsiella* to patients in the hospital.
4. Which families of antibiotics are affected by β-lactamases?

Clostridium difficile

Clostridium difficile is a common cause of HAI. It usually occurs as an opportunistic infection in patients receiving broad spectrum antimicrobial therapy. Infected patients are the major reservoir, and it can readily be transmitted to other susceptible patients since it produces spores that can survive for long periods in the hospital environment and are difficult to remove.

Clostridium difficile infection (CDI) is one of the most frequently occurring HAIs, and has a high fatality rate. It is usually associated with broad spectrum antibiotic usage, because these antibiotics (e.g. clindamycin, cephalosporins and fluoroquinolones) can severely disrupt or diminish the intestinal microflora. This provides an opportunity for *C. difficile* to establish in the intestine and cause damage. Symptoms of CDI include watery diarrhoea, fever, nausea and abdominal pain, and in severe cases the patient may develop a pseudomembranous colitis (see Chapter 18, Figure 18.4). Age is a risk factor, with most cases occurring in patients over 60. The major reservoir of the organism is infected patients in hospitals and long-term care facilities. The bacterium is transmitted by the faecal/oral route—that is, via hands or fomites contaminated with faeces from an infected person.

The impact of CDI on the health system is significant, as infected patients require additional infection control precautions, supportive treatment and extra hygiene measures, and take up additional bed-days (often 1–3 weeks). Recently, a highly virulent strain (PCR ribotype 027) that causes higher mortality rates has emerged in North America and Europe, and first appeared in Australia in 2010 (see Case History 13.3).

CASE HISTORY 13.3

Hypervirulent *Clostridium difficile*

An 83-year-old man underwent an aortic valve replacement in late January 2010 at a hospital in Melbourne. In the four months prior to the surgery, he had not received any antibiotics except for a single preoperative dose of cephalothin. He was admitted to the hospital the day before surgery. Two days after the surgery, he developed severe sepsis, for which he received ticarcillin–clavulanate and a noradrenaline infusion. A coagulase-negative *Staphylococcus* was isolated from blood cultures, and he was given vancomycin.

Five days after the surgery, he developed watery diarrhoea. *Clostridium difficile* was isolated from stool samples. Therapy with metronidazole (400 mg orally, 8-hourly) was commenced for presumed *C. difficile* infection (CDI), and the patient was placed under contact precautions. Alcohol-based handrub was replaced with traditional soap-and-water handwashing. Therapy with ticarcillin–clavulanate was subsequently ceased. After nine days of metronidazole therapy, the diarrhoea became more frequent and vancomycin (250 mg orally, 6-hourly) was substituted. Repeat stool specimens were tested and *C. difficile* was isolated again. The stool sample was positive by real-time polymerase chain reaction when tested for the presence of *C. difficile* organisms carrying genes characteristic of the PCR ribotype 027 strain.

Nineteen days after surgery, the patient's condition deteriorated further. His temperature was 39.2°C and an abdominal X-ray showed a distended right colon. The oral vancomycin dose was increased to 500 mg, 6-hourly, and therapy with intravenous metronidazole was commenced along with vancomycin enemas (500 mg in 500 mL normal saline, 6-hourly). Ticarcillin–clavulanate therapy was recommenced. After five days, the fever and diarrhoea improved. The enemas were ceased after eight days and metronidazole therapy stopped after 14 days. The patient subsequently recovered, and the diarrhoea had not recurred at three-month follow-up.

Comment

This was the first case of hypervirulent CDI diagnosed in Australia with apparent local acquisition. There were at least two other subsequently confirmed cases of infection with *C. difficile* PCR ribotype 027 in the hospital at the time the patient developed symptoms of infection, but it was not known where these cases were acquired.

Source: Adapted from M. Richards et al. 2011, Severe infection with *Clostridium difficile* PCR ribotype 027 acquired in Melbourne, Australia. *Medical Journal of Australia* 194(7): 369. © Copyright 2011. Reproduced with permission.

Questions

1. Why did this patient develop *Clostridium difficile* infection?
2. Why is *C. difficile* PCR ribotype 027 regarded as being hypervirulent?
3. How is *C. difficile* transmitted from patient to patient in hospitals?
4. Why was the alcohol handrub replaced with soap-and-water handwashing?
5. What is the risk of this man's infection being transmitted to hospital staff in contact with him?

Other pathogens

The number of systemic infections caused by fungi is increasing in hospitalised people. These opportunistic infections occur because of the increasing use of procedures and medications that lower the resistance of the patient. Not only are immunocompromised patients more susceptible to these previously rare causes of infection, but once infected, they then serve as reservoirs of these pathogens in hospitals. The main fungi involved in HAI are *Candida albicans*, *Aspergillus fumigatus* and *Cryptococcus neoformans*. Other moulds such as *Scedosporium* spp. are also being seen with increasing frequency.

Viruses may also be transmitted in healthcare facilities. The blood-borne hepatitis B and C viruses can be transmitted to a patient via contaminated equipment (e.g. dialysis machines or endoscopes) or to a healthcare worker, usually via a needlestick injury. Respiratory viruses, such as influenza viruses or respiratory syncytial virus, can be transmitted in healthcare, as can gastrointestinal viruses, such as rotavirus, noroviruses and enteroviruses, usually via the faecal–oral route. Respiratory syncytial virus and rotavirus are particularly important in paediatric units. Latent viruses such as cytomegalovirus and herpes simplex, which may be reactivated in patients whose immune system is compromised, can infect other patients.

Bacterial and viral diseases that normally occur in the community can also be transmitted in the hospital environment. Patients admitted to hospital with an infectious disease or incubating a disease can be a source of infection for other patients and for healthcare workers. Colds, chickenpox and measles are examples of community diseases that can be spread in hospitals. The 2003 epidemic of SARS (severe acute respiratory syndrome) occurred because it was a new viral disease and, in many instances, was spread in hospitals to patients and health workers before it could be diagnosed.

The common causes of and risk factors for hospital-acquired infections are summarised in Table 13.1.

SOURCES OF HOSPITAL INFECTIONS

Infections may be caused by microorganisms that are derived from either inside or outside the hospital. Those that originate outside and are brought into the hospital by patients, staff and visitors are generally regarded as **community strains**. They include the normal flora of these people,

TABLE 13.1 Common causes and risk factors of hospital-acquired infections

INFECTION	COMMON CAUSES	RISK FACTORS
Urinary tract	*Escherichia coli*, *Klebsiella* spp., *Enterococcus* spp., *Pseudomonas aeruginosa*, *Proteus* spp.	Urinary catheter; anatomical or functional abnormality of the urinary tract
Gastrointestinal tract	*Clostridium perfringens*, *Salmonella*, *Staphylococcus aureus*, *Bacillus cereus*, noroviruses, *C. difficile*	Poor food handling practices, enteral feeding, age, antibiotic usage
Surgical wound (SSI)	*S. aureus*, *Klebsiella* spp., *Streptococcus* spp., *E. coli*, *P. aeruginosa*, *Enterococcus* spp., *Enterobacter* spp.	Debility, implant of prosthesis, prolonged surgery, surgery on heavily colonised sites
Pneumonia	*S. aureus*, *Klebsiella* spp., *P. aeruginosa*, *Candida* spp., *Enterobacter* spp., *Streptococcus pneumoniae*, viruses	Underlying disorder, surgery, mechanical ventilation
Bloodstream infections (Septicaemia)	*S. aureus*, *Klebsiella* spp., *E. coli*, *Staphylococcus epidermidis*, *Enterococcus* spp.	Indwelling IV catheter, infection at a primary site
Skin	*S. aureus*, *Candida* spp.	Newborn
Immunocompromised patient	Viruses, *Aspergillus* spp., *Candida* spp.	Immunosuppression, immunodeficiency, AIDS

as well as any pathogens they may be infected with or are carrying asymptomatically. If these pathogens are shed from the body, they can be transmitted to other patients or staff. The patient's own normal flora may also be the source of an infection—for example, bacteria in the gastrointestinal flora that gain access to the urinary tract, or *Staphylococcus epidermidis* from the skin that infects an IV cannula insertion site. In general, community strains are likely to be sensitive to antimicrobial drugs, although some organisms, such as *S. epidermidis*, are naturally resistant to a number of antibiotics.

Most HAIs are caused by **hospital strains**. As the name implies, these are strains of microorganisms that are found mainly in hospitals or associated with people (patients and staff) who are, or have been, in hospitals. Hospital strains differ from community strains in that they usually have a greater resistance to antimicrobial drugs and may be resistant to some disinfectants. Multi-resistant strains of bacteria such as *Staphylococcus aureus* (MRSA), *Pseudomonas*, *E. coli*, *Klebsiella*, *Acinetobacter*, *Enterobacter* and *Enterococcus* cause infections that are difficult to treat and are therefore associated with significant morbidity and mortality. The major sources of hospital strains are the hospital environment, patients with infections, and colonised patients and staff.

Colonisation

Colonisation refers to the presence and growth of a microorganism on the skin or mucous membrane of a person without any evidence of infection. The organisms that make up a person's own normal flora are the usual colonisers. However, hospital-derived microorganisms can colonise patients within days of their admission to hospital. The major sites of colonisation of patients by hospital organisms are the nose, the skin, the oropharynx, the lower intestine and the urinary tract. Colonisers do not usually pose a threat to a healthy person but are often the cause of infection in the individual if their resistance becomes lowered. Colonising organisms tend to prefer certain sites, as shown in Table 13.2.

TABLE 13.2 Sites of colonisation with hospital strains in patients and staff

ORGANISM	COLONISATION SITES
MRSA	Nose Skin: hands, perineum, groin, axillae
Escherichia coli	Bowel, urinary tract
Klebsiella, Enterobacter	Oropharynx, urinary tract
Pseudomonas	Bowel, oropharynx, urinary tract, skin
Staphylococcus epidermidis	Skin, urinary tract
Enterococci	Bowel
Candida	Bowel, urinary tract, oropharynx

Colonisation may result in the short-term presence of unusual organisms in a particular site—for example, Gram-negative bacteria on the skin or in the oropharynx. Having taken up residence with (or displaced) the normal flora, hospital strains may then remain there for weeks to months, even after discharge from hospital. Colonisation with hospital microorganisms becomes increasingly common the longer a person is in hospital, and is favoured if the patient receives antimicrobial drugs. The presence of an antibiotic in the tissues of a patient means that, if colonisation occurs, it will probably be by a drug-resistant strain.

Healthcare staff are also commonly colonised by hospital organisms, and thus become carriers of these potential pathogens. For example, approximately 30 per cent of the general community carry *Staphylococcus aureus* in the nose, while as many as 70 per cent of hospital staff may carry this organism, with a significant proportion carrying multi-resistant strains (MRSA).

Burns wounds and surgical wounds are ideal sites for microbial growth and are frequently colonised by hospital organisms that can ultimately cause serious infections. These colonised or infected wounds act as potential sources of pathogens in the spread of hospital-acquired infections.

Exogenous versus endogenous infection

Identification of the source of a causative agent of an infection is important in the epidemiological investigation of outbreaks, and for the selection of appropriate methods of infection control to prevent further transmission. HAIs can be divided into two general categories based on the source of the causative organism—*exogenous* or *endogenous* (see Figure 13.6). However, the distinction between exogenous and endogenous sources may be difficult to make in practice.

Exogenous infections are infections caused by microorganisms from a source external to the patient—mainly healthcare workers, other patients, or visitors. That is, humans are the major exogenous sources of pathogens. The hospital environment is a less common, but nevertheless important, source of exogenous pathogens. Wet areas that support the growth and multiplication of microbes are the major environmental reservoirs.

Other patients and healthcare workers are particularly important sources of pathogens in healthcare infections. Patients can be sources in three ways:

1. *Patients with infections*. For example, in a surgical ward, the patient with an infected wound is a potential source of pathogens for any other patient who has just had surgery.
2. *Patients who are colonised with hospital strains*. Typically, a patient who has been in hospital for a while may be colonised by MRSA on various skin sites. If the MRSA are then transferred to another, susceptible patient, the latter may be infected by the organism.
3. *The normal flora of a patient*. The normal flora of one person may infect another person, particularly if the latter is immunocompromised in some way—for example, has severe burns, or is a transplant recipient on immunosuppressive therapy.

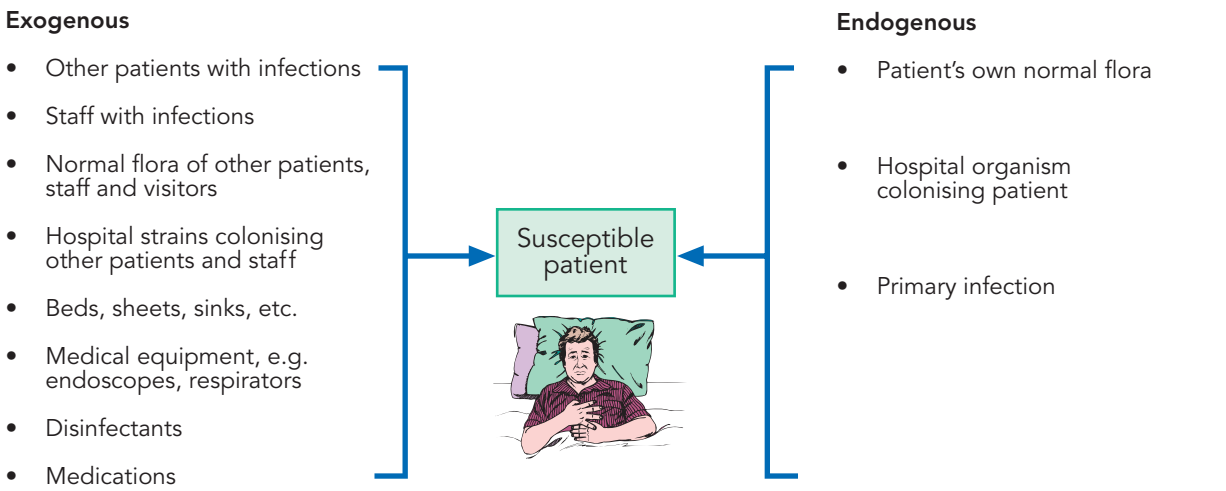

FIGURE 13.6

Sources of infections in healthcare facilities

Healthcare workers may also act as exogenous sources of infection in the same three ways. Members of staff can be infected, and if they are, can serve as sources of infection for the patients in their care. Healthcare workers are frequently colonised with hospital pathogens. And the normal flora of healthcare workers are potential pathogens for immunocompromised patients. Workers who have close patient contact are particularly important potential sources.

Inanimate objects (called *fomites*) in the hospital environment may be contaminated with microorganisms capable of causing infection but they are rarely the primary source of pathogenic microorganisms. Mostly they are contaminated with organisms from human sources. While there is plenty of opportunity for the hospital environment to become contaminated, many microorganisms are unable to multiply or even survive for long on clean, dry surfaces such as floors, walls and furniture. For example, Gram-negative rods such as *E. coli*, *Klebsiella* and *Pseudomonas* are susceptible to desiccation and survive for only a few hours on dry surfaces. However, the endospores of some bacteria (e.g. *Clostridium* spp.) and some other bacteria, such as *Staphylococcus aureus* and *Streptococcus pyogenes*, are somewhat resistant to desiccation and can survive on surfaces for days. Noroviruses, which cause gastrointestinal disease and are transmitted by the faecal/oral route, are also able to survive on surfaces for long periods and can be responsible for outbreaks in hospitals. Standardised cleaning and appropriate disinfection procedures are important in minimising the risk of infection from these sources (see Chapter 11).

Environmental organisms, such as *Pseudomonas*, *Burkholderia* and *Aspergillus*, may cause HAIs. *Pseudomonas* is regularly found in moist areas of the hospital environment and is a common cause of infection in burns patients and patients with cystic fibrosis. Burkholderia is a less common cause of HAI, but is noted for its ability to infect the lungs of people with cystic fibrosis. The spores of the fungus *Aspergillus* are carried on air currents and cause lung infections, especially in immunocompromised patients. Outbreaks of atypical pneumonia caused by *Legionella* are usually associated with water-cooling towers of air-conditioning units.

Many bacteria multiply readily in warm moist environments, and items such as sinks, mops, sponges, respiratory equipment, suction apparatus and humidifiers have all been implicated as potential sources of infection (see Figure 13.7). IV fluids, especially TPN (total parenteral nutrition), can also be a source of pathogens if not handled correctly. Even disinfectant and antiseptic solutions can sometimes become contaminated, especially if not freshly prepared or if contaminated with organic material (e.g. blood). *Pseudomonas aeruginosa*, for example, can grow in povidone-iodine solutions.

In summary, the most important exogenous sources are places where microbial survival and multiplication can take place. These include infected patients, human carriers, infected wounds and moist areas of the hospital environment. Dry objects and surfaces in the hospital environment are less

FIGURE 13.7

Reservoirs of infection in hospitals

Source: © Christine Bishop.

important, but should not be ignored as potential sources of infection, particularly if they have been recently contaminated.

Endogenous infections are caused by microorganisms that are part of the patient's own normal flora, or are organisms (including hospital strains) that have colonised the patient after admission to hospital. Sometimes, in patients that have an infection, the pathogen can travel to another site in the body—that is, the primary infection acts as the source of microorganisms for a second site. The transmission may occur by mechanical transfer on the hands of the patient or staff, or may occur directly if the organism is carried in the blood or lymphatic system from one part of the body to another. Latent infections which are reactivated when the patient's immune system is compromised are also considered endogenous.

ROUTES OF TRANSMISSION OF MICROORGANISMS IN HEALTHCARE

There are three main modes of transmission of infectious organisms in healthcare facilities: contact transmission, droplet transmission and airborne transmission. Each potential pathogen is typically transmitted in one of these ways, although some are transmitted by multiple modes. Occasionally, an infection can be transmitted to multiple individuals in a healthcare facility via contaminated medications or equipment—this mode of transmission is called **common vehicle transmission**.

Contact transmission

Contact transmission refers to transmission of infectious microorganisms to a susceptible host by touch or via contact with blood or other body substances. Two types of contact transmission are recognised. In some cases, infectious organisms are transferred directly from one person to another—termed **direct contact transmission**. For example, direct contact transmission occurs when blood from an infectious person comes into contact with a mucous membrane or breaks in the skin of another person, or when a healthcare worker with infected lesions on his or her hand infects the wound of a patient while changing the patient's dressings. Alternatively, organisms can be transferred via an intermediate object (fomite) or person—termed **indirect contact transmission**. This is the more common type of contact transmission. A typical scenario is an instrument becomes contaminated when used on a patient with an infection, and then the instrument is used, without adequate decontamination, on another patient. Other situations when indirect contact transmission can occur are when staff clothing becomes contaminated after care of a patient colonised or infected with an infectious agent, or when environmental surfaces become contaminated. Contaminated hands of healthcare workers have been shown to be very important contributors to indirect contact transmission. Organisms that cause skin and wound infections (e.g. *Streptococcus pyogenes*, *Staphylococcus aureus*) and viruses that cause outbreaks of gastroenteritis in hospitals (e.g. rotavirus, noroviruses) are typically spread via contact transmission.

Direct or indirect contact transmission of microorganisms during patient care is responsible for the majority of healthcare-associated infections in patients and healthcare staff.

Droplet transmission

Droplet transmission occurs when a person with a respiratory infection coughs, sneezes or talks (sometimes) and thereby expels large infectious droplets that directly infect the mucosal surfaces (throat, nose, eyes) of another person. The two people need to be physically close to each other, because the droplets are large and travel only short distances. Droplet transmission can also occur indirectly when hands or fomites are contaminated with droplets. Influenza viruses are examples of microbes that are typically transmitted by droplet transmission.

Airborne transmission

Airborne transmission occurs via small particles (aerosols) expelled during coughing, sneezing or talking, or when large droplets (above) partially evaporate to form smaller, lighter particles. Small particles remain suspended in air and can be dispersed over long distances by air currents, and, if infectious, can transmit the microbes into the airways of a susceptible host. The measles virus is an example of a microbe that is transmitted in this way.

The modes by which infections are transmitted, in general (including in the community), are described in Chapter 8.

FACTORS CONTRIBUTING TO THE INCIDENCE OF HOSPITAL-ACQUIRED INFECTIONS

Microbial factors

The capacity of a microorganism to cause disease (i.e. its virulence) is not directly related to its sensitivity or resistance to antimicrobial drugs. A particular strain of an organism can be resistant to a number of drugs but is not necessarily more virulent than a sensitive strain of the same organism. However, the use of large amounts of antimicrobial drugs in hospitals favours the selection and persistence of resistant strains (see Chapter 12). A resistant strain is therefore more likely to be responsible for an infection in the hospital environment, and the infection will often be serious because of the limited availability of drugs to treat it. Certain bacteria have also developed resistance to some disinfectants and antiseptic solutions normally used to control their spread (see Chapter 11).

As explained in Chapters 9 and 10, the outcome of exposure of a human to a microorganism depends on the balance between the virulence of the microbe and the resistance of the host. Generally, the virulence of a microorganism is a key factor in determining whether infection occurs, but an equally important factor is the patient's overall state of health and resistance to infection. While some hospital pathogens have exceptional virulence, such as the 80/81 phage type of *Staphylococcus aureus* ('golden staph') which caused hospital outbreaks in the early 1950s, this is not a prerequisite for infection. Some hospital infections are caused by

organisms of low virulence (e.g. *S. epidermidis*) in patients with compromised body defences.

Patient susceptibility

A large proportion of hospitalised patients have lowered immune defences and are thus at increased risk of infection (see Chapter 9). Age is an important factor. The newborn are highly susceptible to infection because of the immaturity of their immune system. Elderly people also have a greater risk of infection because of the deterioration of their immune system, or because of impairment to other body functions such as the blood supply to tissues. Immobility in the aged may lead to stasis of body fluids and hence to increased susceptibility to infection in organs such as the lungs or bladder.

Some underlying diseases lower the resistance of an individual. For example, patients with leukaemia are more susceptible to infection because they have inadequate numbers of functional white cells. People with diabetes may have lowered resistance because of vascular changes leading to poor blood circulation, causing tissue hypoxia and necrosis. Prior infection may lower a person's resistance. For example, elderly patients with an influenza infection are more susceptible to bacterial pneumonia. A more extreme example is that of HIV infection, which greatly increases patient susceptibility to opportunistic infections because of the severe damage the virus causes to the immune system.

Some medical treatments lower the patient's resistance to infection. Cytotoxic drugs and radiotherapy used for the treatment of cancer impair the immune system by reducing white cell production. The drugs given to transplant patients to prevent rejection are designed to suppress the immune system, as are some anti-inflammatory drugs, especially the corticosteroids.

Conditions in which the protective skin barrier is damaged, such as surgical wounds, skin lesions or pressure sores, provide a portal of entry for microorganisms and increased risk of infection. Patients with severe burns are highly susceptible because of the loss of substantial areas of skin (see Chapter 16). Accident victims or trauma patients with extensive skin damage or crushing injuries to tissues often suffer serious infections because microorganisms may be carried deep into the tissues.

Medical procedures

While the skin and mucous membranes remain intact they provide very effective barriers against infection (see Chapter 9). However, many diagnostic and therapeutic procedures performed in hospitals involve the penetration of these barriers by the introduction of a device into the body, thus providing microorganisms with easy access to susceptible tissues. Surgery usually involves the breaching of a patient's skin, and surgical site infections (SSIs) are the second most common type of infection in hospitals. Infections resulting from medical procedures or treatment are termed **iatrogenic infections**.

Other procedures that carry a risk of infection include the insertion of intravascular cannulae and urinary catheters, and endotracheal and drainage tubes. All of these allow microorganisms to circumvent the body's external barriers.

Urinary tract infections are the most common type of HAI, the major risk factor for which is an indwelling urinary catheter. Urinary catheters allow microorganisms to enter the bladder by travelling up either the outside or through the inside of the catheter tube. Bacteria are able to colonise the urethra (outside the tube) and move upwards towards the bladder because the normal flushing action of urine flow over the surface of the urethra is lost. This flushing action is a major defence mechanism against bladder infection. Additionally, if the urine drainage bag becomes contaminated, organisms can gain entry to the inside of the tube and then spread to the bladder. The use of closed drainage systems reduces the risk of spread of organisms to the bladder via the inside of a catheter tube. The longer a urinary catheter is in place, the greater the risk of infection.

Indwelling intravascular catheters are frequently used to administer fluids, medications or nutrients to patients. Insertion of an intravascular catheter is one of the more commonly performed invasive procedures in hospitals. These devices provide access for microorganisms into tissues by bypassing the skin barrier. The catheter tip and the skin around the insertion site can become colonised and subsequently infected, leading to bacteraemia and then sometimes septicaemia. *Staphylococcus epidermidis*, a normal skin inhabitant, is the most frequent cause of these infections, but other organisms, particularly *Staphylococcus aureus* and enterococci, are also common causes. Very occasionally, the fluid being administered or parts of the equipment may become contaminated, resulting in microorganisms being passed directly into the bloodstream. Again, the longer the device is in place, the greater the risk of infection.

Lower respiratory tract (lung) infections may result from tracheostomy (an opening made into the trachea through the neck) and intubation (insertion of a tube into the trachea). Intubation increases the risk of infection because it gives microorganisms direct access to lower airways, and because the procedure often causes injury and irritation to the tracheal lining. The use of respiratory equipment to administer oxygen or medication deep into the lungs can also predispose a patient to infection. Furthermore, nebulisers, humidifiers and other respiratory equipment provide a moist environment for bacteria to persist and multiply, and are difficult to sterilise.

The use of endoscopes to examine mucosal passageways increases the risk of infection. They can irritate or break the delicate mucosal linings of the passageways through which they are passed and thereby increase the risk of infection, and they are difficult to sterilise.

Implantation of prostheses, such as heart valves or artificial joints, has a high risk of infection, since the surgical site is usually exposed to the air for an extended time and organisms may be introduced during the procedure. The prosthesis may act as a site of colonisation for microorganisms that later enter the bloodstream. Under normal circumstances, small numbers of organisms entering the bloodstream are

quickly removed by the body's defences, but inert implants (which do not have a blood supply) may provide a site for colonisation that is inaccessible to these defences.

Even the common practice of giving injections using a hypodermic needle brings with it the chance of infection. For such a simple device and common procedure, there are several potential hazards—the needle or syringe may be contaminated, the solution to be injected may be contaminated, or the skin at the injection site may not have been properly disinfected.

The more severely ill a patient is, the more likely they are to acquire an infection, either as a direct result of their clinical condition or because of the medical or surgical procedures to which they are subjected. The combination of invasive procedures and extensive use of immunosuppressive or antimicrobial therapy in some patients leaves them very susceptible to infection. Intensive care units, with severely ill patients, often have the highest incidences of infection. As medical practice develops, more complex and invasive procedures are used, with an ever-increasing risk of infection for those subjected to them.

The host factors and medical procedures that predispose patients to hospital infection are summarised in Table 13.3.

Movement of patients

Patients are frequently transferred from ward to ward or from one healthcare facility to another. This has the potential to spread pathogens into different locations. For example, a patient colonised with MRSA and transferred from an intensive care unit to a renal unit would represent a new reservoir of the organism in the renal ward. Colonised patients transferred from a hospital to a nursing home, or vice versa, might spread a pathogen from one facility to the other. Some healthcare facilities require patients to have negative swabs for resistant organisms, such as MRSA, before they can be moved. However, in practice, when there is a shortage of beds in a hospital, patients may have to be transferred. It is important to have proper communication between the transferring facility and the receiving institution about the infection status or colonisation status (if known) of the patient in such cases.

Trauma patients or patients evacuated from other countries may also introduce new strains of pathogens (see Spotlight box: Imported infections).

TABLE 13.3 Factors that predispose patients to hospital infection

FACTOR	SPECIFIC EXAMPLES
Age	The newborn have an immature immune system; the elderly have an immune system that is deteriorating.
Underlying disease	Leukaemia, diabetes, cancer, neutropenia.
Existing infection	Viral influenza can lead to bacterial pneumonia; HIV infection; herpes virus lesions can be infected by bacteria (e.g. *Staphylococcus aureus*).
Prescription drugs and treatments	Cancer therapy (cytotoxic drugs, radiotherapy); immunosuppression for transplant patients; anti-inflammatory drugs; antibiotics that alter the normal flora; chloramphenicol (suppresses the bone marrow).
Medical procedures	Surgery; indwelling IV catheters; indwelling urinary catheters; intubation of the trachea; tracheostomy; use of respiratory equipment (which is difficult to sterilise); endoscopes; injections; implantation of prosthesis (e.g. heart valves); dialysis.
Broken skin	Burns, accident, pressure sores.

Type of healthcare facility

In every healthcare facility there is a risk of infection for patients. The number of HAIs that occur varies from one facility to another, influenced by such factors as:

- the type of hospital or facility
- the size (number of beds) of the hospital
- the location of the hospital (e.g. inner-city or country).

An Australian survey carried out by McLaws and coworkers in the 1980s showed a higher prevalence of HAIs in large teaching hospitals. To some extent, the figures are a reflection of the hospital facilities as well as the type of patient. Large hospitals usually have high-dependency units, specialised burns units and facilities for transplant operations. Patients in these units are particularly susceptible to infection, because of severe illness and the weakened status of their immune system. Patients admitted to accident and emergency units are likely to be severely traumatised and therefore at greater risk of infection. In contrast, aged-care facilities do not usually have a population of severely ill patients or patients who are actually infected. There is a smaller reservoir of microorganisms present, and thus fewer infections.

A common feature of hospitals is the high frequency of physical contact that occurs between staff and patients. Depending on the size and nature of the unit, a nurse might be required to perform routine procedures such as temperature, pulse and blood pressure readings, change dressings, or administer medication for each patient on several occasions during the day. Other health workers, such as doctors and physiotherapists, may attend to these patients as well as to patients in other units throughout the hospital. Thus, not only will a staff member have physical contact with many patients, but the patient will usually have contact with a number of different staff. The contact between patients and staff is thus frequent and varied, allowing easy transmission of microorganisms from person to person. Isolation procedures are sometimes instituted to prevent this type of transmission, especially for patients who are severely immunocompromised or who have a highly communicable infection.

Chapter 8 gives a general description of the ways in which diseases can be spread. Figure 13.8 and Table 13.4 outline how microorganisms can be spread in the specialised hospital environment.

Imported infections

SPOTLIGHT ON

There were many casualties from the bombing of the Sari nightclub in Bali in 2002. Patients were first treated in Bali and some of the more serious cases were then flown to Australian hospitals for specialised medical treatment. The patients carried microorganisms derived from Indonesia, the environment and some of the hospital strains circulating in Bali, as well as their own normal flora.

A strain of *Acinetobacter baumannii*, resistant to multiple antibiotics, including gentamicin, was among the bacteria introduced into one Adelaide hospital on one of these patients. The organism has since been implicated in several outbreaks in the intensive care unit and other wards of the hospital and has necessitated a surveillance program to monitor its spread. A collaborative infection control effort, including extra cleaning and hand hygiene, had to be instituted to contain the spread of this pathogen.

Comment

Although *Acinetobacter baumannii* is common in the environment, in this case it was introduced by a patient and then persisted in the hospital. Multi-drug-resistant strains pose a serious risk to patients who are immunocompromised or critically ill. The ability of the organism to persist in a range of environments and to resist desiccation means that it is particularly difficult to eradicate. This case highlights the potential for drug-resistant pathogens to be introduced into a hospital and to spread to susceptible patients.

Antibiotics are freely available without a prescription in many Asian countries. As a result, there are many multi-resistant strains of bacteria circulating in these communities and they can be carried into Australia by tourists or, in this case, by patients brought for specialist treatment.

Source: Based on H. Brettig et al. 2004, *Proceedings of AICA Conference*. © 2011 Australian Infection Control Association.

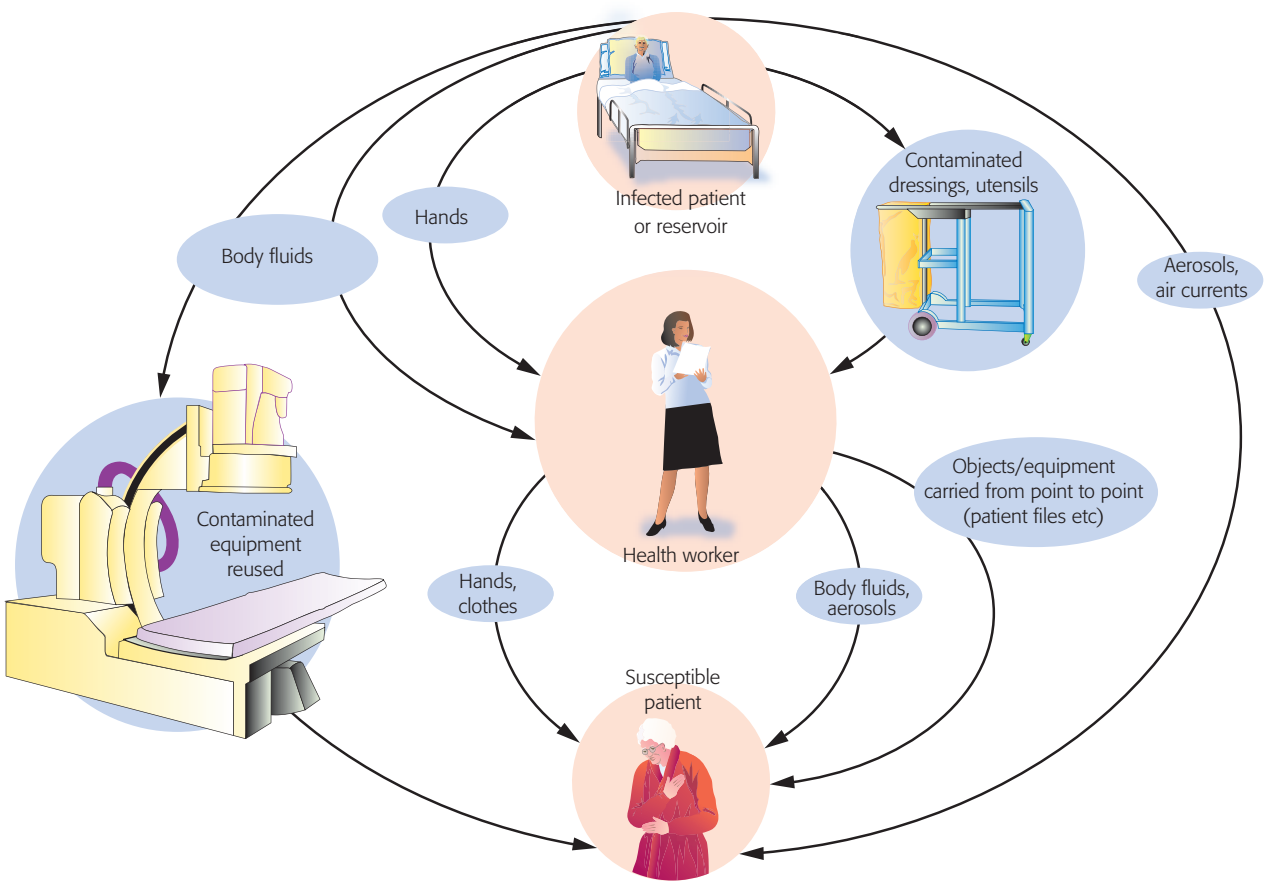

FIGURE 13.8

Spread of microorganisms in hospital

TABLE 13.4 Transmission in the healthcare environment

SOURCE	METHOD OF TRANSMISSION	PORTAL OF ENTRY
Endogenous (patient)	Patient (hands or systemic)	Wound, IV or other body site
Environment		
Contaminated fomites (e.g. patient records, keys, computer terminals)	Hands of healthcare workers: doctors, nurses, other personnel	Wounds, IV or injection site, or other break in skin
Air currents and aerosols, e.g. bacteria (*Legionella*, TB), viruses, (influenza, RSV), fungal spores	Air conditioning, inhalation	Upper respiratory tract
Contaminated (non-sterile) equipment or materials, e.g. endoscopes, IV solutions	Iatrogenic	Various, depending on procedure
Contaminated food	Hands Food and food utensils	Oral ingestion
Contaminated material (dressings, used equipment)	Hands Incorrect disposal	Break in skin, IV line, wound
Body substances, faeces	Hands, poor hygiene	Oral ingestion, break in skin, wound, IV line
Body substances, blood (HBV, HCV, HIV), urine (CMV)	Sharps injury	Needlestick (sharps) injury, mucocutaneous splashes

CONTROL OF INFECTION IN HEALTHCARE FACILITIES

As part of its Healthcare Associated Infections priority program, the Australian Commission on Safety and Quality in Health Care released its *Australian Guidelines for the Prevention and Control of Infection in Healthcare* in October 2010. As we saw in the introduction to this chapter, the guidelines were prepared by the NHMRC at the request of the ACSQHC, and supersede the previous national guidelines published by the Communicable Diseases Network of Australia in 2004. The exact procedures specified to prevent the occurrence of infection may vary from one healthcare facility to another, but should be based on sound microbiological principles and the ACSQHC guidelines. The guidelines are a comprehensive document which provides a coordinated, national approach for the prevention and management of HAI. The implementation of the guidelines, through development of detailed protocols and processes, is the responsibility of individual healthcare establishments. Some of the key points of the guidelines are included in this chapter, but the reader is referred to the document for a complete description: <www.nhmrc.gov.au/_files_nhmrc/file/publications/synopses/CD33_InfectionControlGuidelines2010.pdf>.

The guidelines are organised into three major sections.

- Part A provides background information on the basics of infection prevention and control 'that should be read by everyone working in health care'.
- Part B describes the effective work practices that minimise the risk of transmission of infectious agents.
- Part C describes the responsibilities of management in healthcare facilities in relation to implementing, monitoring and reporting of infection control practices.

Similar to previous infection control approaches, these guidelines recommend a two-tiered approach to provide protection to patients, healthcare workers and other people in healthcare settings:

- *Standard Precautions*—routinely applied strategies (all of the time) to minimise infection risk to patients and healthcare workers.
- *Transmission-based Precautions*—specific interventions where Standard Precautions may not be sufficient, designed to interrupt the mode of transmission of specific microorganisms.

Although infection risks differ in different types of healthcare facilities, these precautions are generally applicable to all settings. The following sections outline the major elements of Standard and Transmission-based Precautions.

Standard Precautions

Standard Precautions are the work practices that should be applied to all people, regardless of their infection status. The precautions are based on the principle that all people potentially harbour pathogenic microorganisms. As a first-line approach, they are designed to minimise the risk of transmission of infectious microbes from one person to another, and from one place to another. These precautions should be followed in all situations, because a person's infection status is not always known. For example, an infected person (patient or healthcare worker) may be in a phase of the disease where signs and symptoms are very mild or non-existent, such as the incubation phase or the convalescent

phase (see Chapter 7). In some cases, a person can be infected but be asymptomatic (or very mildly symptomatic) through the whole course of the disease. Furthermore, as described earlier in this chapter, both patients and healthcare workers have a normal microbial flora that can cause infection in certain circumstances and they can also be colonised with potential pathogens, including resistant hospital strains.

Standard Precautions should be used for all patients, whether symptomatic or asymptomatic and regardless of their infective status. Standard Precautions are based on the following eight work practices:

- personal hygiene practices, especially hand hygiene
- appropriate use of personal protective equipment
- safe handling and disposal of sharps
- environmental controls, including cleaning and spills management
- appropriate reprocessing of reusable equipment and instruments, including appropriate use of disinfectants
- respiratory hygiene and cough etiquette
- aseptic technique
- appropriate handling of waste and linen.

Hand hygiene

Healthcare workers' hands are the most common vehicle for the transmission of potential pathogens from patient to patient and within the healthcare environment. The oldest, simplest and most effective method of preventing cross-infection is good hand hygiene. A healthcare worker's hands harbour microorganisms that are present most of the time (resident flora), and may also be contaminated with microorganisms that are acquired during activities involved with healthcare (transient flora). Hands can become contaminated with infectious agents through contact with a patient, a patient's surroundings, other parts of the hospital environment or other healthcare workers, or through contact with respiratory secretions from coughing or sneezing. Once hands are contaminated, cross-contamination can occur from one site to another site of the patient, from healthcare worker to another patient, from healthcare worker to the environment, or between healthcare workers. Although the resident flora is potentially infectious, especially for the immunocompromised, it is the transient flora that contaminate the healthcare worker's hands that are the biggest danger. They are more likely to be hospital strains acquired from a patient or the hospital environment. Good hand hygiene practice in healthcare has been shown to significantly decrease the risk of HAI, and is regarded as the single most effective measure for prevention of HAI.

The Australian infection control guidelines recommend hand hygiene practices based on the 'Five Moments for Hand Hygiene' developed by the World Health Organization (see Figure 13.9). Essentially, this involves practising hand hygiene *before* every episode of patient contact (including between caring for different patients and between different care activities for the same patient) and *after* any activity or contact that potentially results in hands becoming contaminated. That is, the five moments for hand hygiene are:

- before touching a patient
- before a procedure
- after a procedure or body substance exposure risk
- after touching a patient
- after touching a patient's surroundings.

It is now generally agreed that hand hygiene is best achieved using an alcohol-based handrub. Alcohol handrubs are considered more effective in removing infectious microorganisms from hands than plain or antiseptic soap and water. The handrubs that have been shown to be most effective contain at least 60 per cent alcohol (isopropanol) plus 0.5 per cent chlohexidine (a disinfectant with residual activity).

It has been recognised for many years that very low compliance rates for hand hygiene (often around 40 per cent, but as low as 20 per cent) are common in healthcare facilities in both developed and developing countries. The reasons for poor compliance are complex, and numerous attempts to rectify this problem have had limited and mostly temporary success. It is expected that hand hygiene based on the use of an alcohol-based handrub will result in improved hand hygiene compliance, since alcohol handrubs have the added advantages of being able to be made available at point of care, requiring less time than soap and water, and causing less irritation to the skin. These three factors, in conjunction with educational and heightened awareness campaigns, will hopefully improve the conviction and capability of healthcare workers to undertake appropriate hand hygiene. Handwashing with soap and water is still recommended when hands are visibly soiled or when the healthcare worker has been in contact with a patient with *Clostridium difficile* infection, since the spores of this organism are resistant to alcohol.

Cuts and abrasions on hands are possible sources of infectious microorganisms which may not be removed by hand hygiene practices. Skin lesions should therefore be covered with waterproof dressings to reduce the risk of transmission of microorganisms via damaged hands.

Effective hand hygiene also relies on the correct technique being followed. The techniques for using an alcohol handrub and for soap handwash are outlined in Table 13.5. A detailed step-by-step set of instructions for an alcohol handrub is shown in Figure 13.10.

Gloves are an important means of reducing cross-infection, but their use *does not remove the need for hand hygiene*. Hand hygiene should still be performed, preferably before and after the use of gloves.

Patients should also be instructed about the importance of hand hygiene. Many hospital-acquired infections are caused by the patient's own normal flora and may actually be transmitted to a wound site by the patient's own hands. Visitors should also be encouraged to perform appropriate hand hygiene (see Figure 13.11).

TABLE 13.5 **Techniques for hand hygiene**

USE OF ALCOHOL-BASED HANDRUB

1. Apply the amount of alcohol-based handrub recommended by the manufacturer on to dry hands.
2. Rub hands together so that the solution comes into contact with all surfaces of the hand, paying particular attention to the tips of the fingers, the thumbs and the areas between the fingers.
3. Continue rubbing until the solution has evaporated and the hands are dry.

USE OF SOAP (INCLUDING ANTIMICROBIAL SOAP) AND WATER

1. Wet hands under tepid running water and apply the recommended amount of liquid soap.
2. Rub hands together for a minimum of 15 seconds so that the solution comes into contact with all surfaces of the hand, paying particular attention to the tips of the fingers, the thumbs and the areas between the fingers.
3. Rinse hands thoroughly under running water, then pat dry with single-use towels.

Source: NHMRC 2010, *Australian Guidelines for the Prevention and Control of Infection in Healthcare*. Commonwealth of Australia.

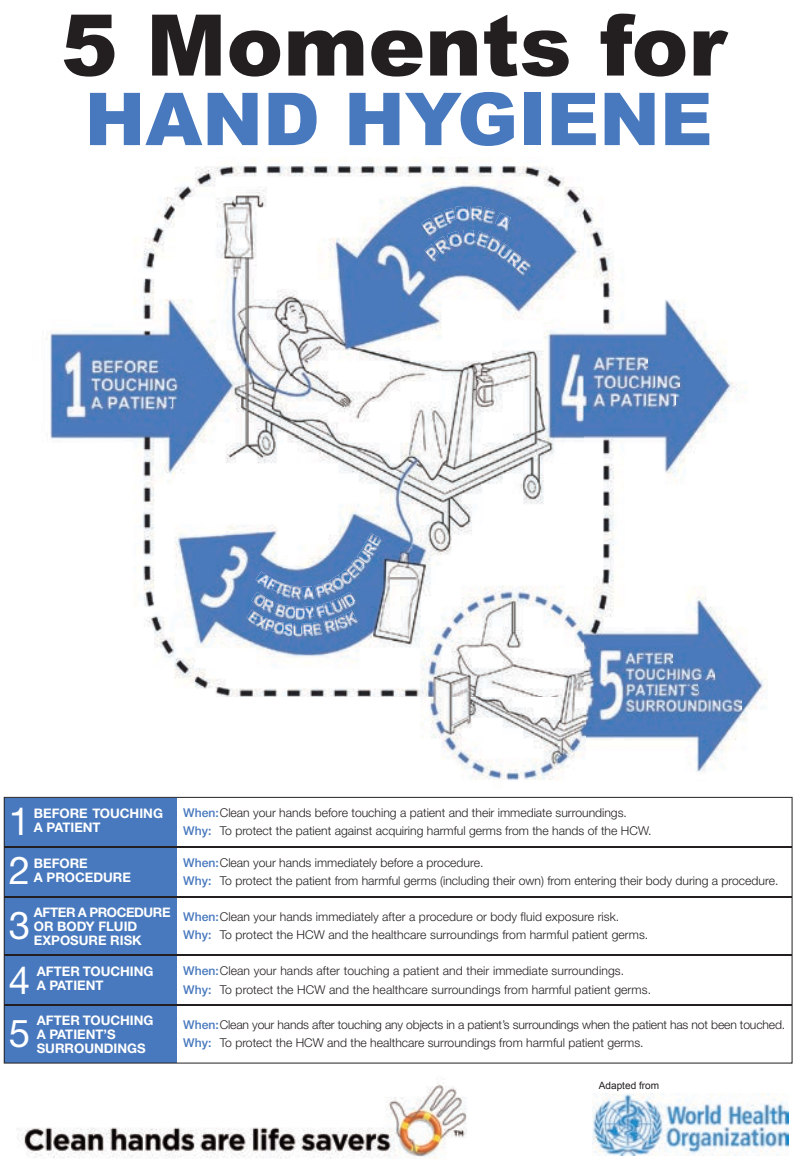

FIGURE 13.9

Five moments for hand hygiene

Source: World Health Organization/Queensland Health. <www.who.int/gpsc/5may/background/5moments/en/index.html>. © World Health Organization 2009. All rights reserved.

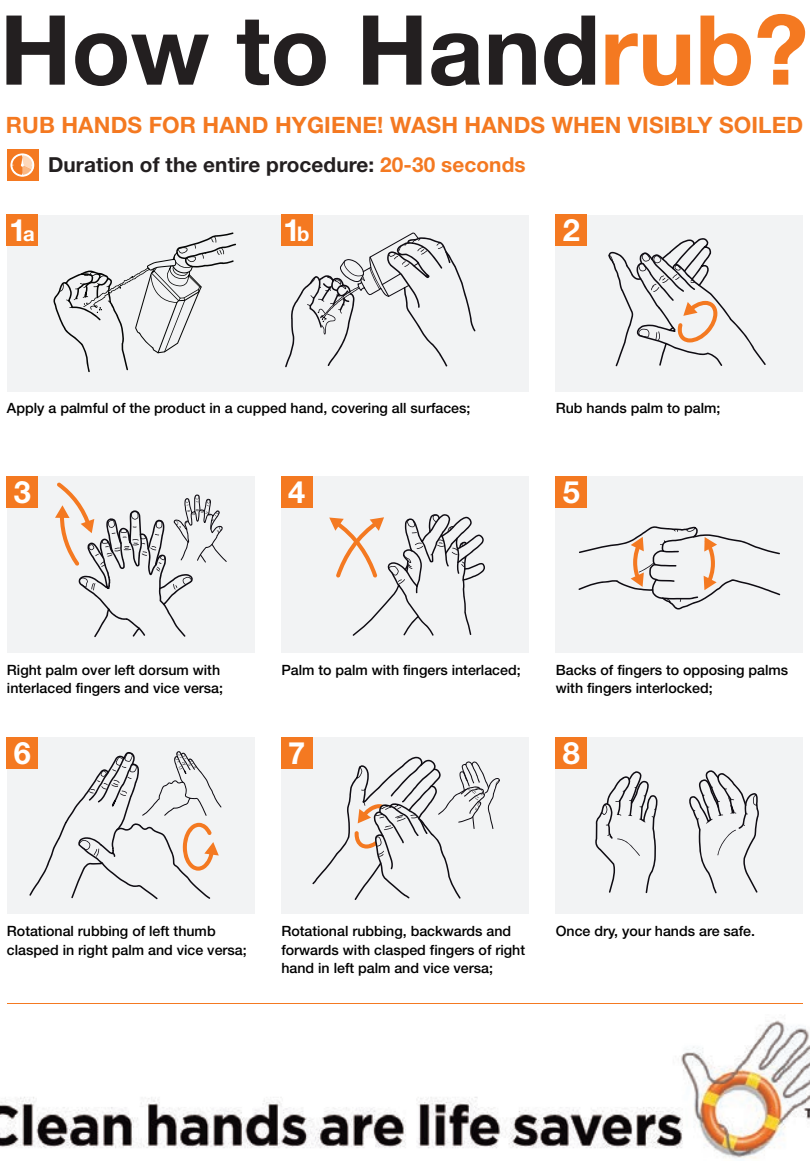

FIGURE 13.10

How to handrub with alcohol

Source: World Health Organization/Queensland Health. © World Health Organisation.

Personal protective equipment

Pathogens that are normally spread by contact or droplet transmission can contaminate the hands, other parts of skin, or the clothing of a healthcare worker. Cross-contamination may then occur between the healthcare worker and patients or other healthcare workers, or between the healthcare worker and the environment.

Personal protective equipment (**PPE**) consists of the barriers used to protect the mucous membranes, airways, skin and clothing of the healthcare worker and/or patient from contamination with infectious microorganisms. Use of PPE is a key component of Standard Precautions, and mainly involves the use of aprons, gowns, gloves, surgical masks, protective eyewear or face shields. Which protective item(s) should be used by a healthcare worker in a given situation is based on an assessment of the risk of transmission of infectious microbes to the patient or healthcare worker, and the risk of contamination of the clothing or skin of the healthcare worker with the patient's blood or other body substances.

A protective apron or gown should be worn when close contact with a patient or equipment could lead to contamination of the healthcare worker's skin or clothing, or when there is a risk of contamination with blood or other body substances. A clean, non-sterile apron or gown is generally adequate to protect skin and prevent soiling of clothing. A fluid-resistant apron or gown should be worn when there is a risk that clothing may become contaminated with blood

FIGURE 13.11

Poster for visitors about hand hygiene

Source: Victoria Quality Council.

or other body substances. Gowns and aprons should be considered as single-procedure items—that is, they should be changed between patients.

The mucous membranes of the respiratory tract and eyes are portals of entry for a number of infectious microorganisms. Face and eye protection reduces the risk of exposure to splashes or sprays of blood or body substances and is an integral part of Standard Precautions. Surgical masks cover the nose and mouth and are used to protect them from splashes of body fluids. They are also worn when caring for patients for whom droplet precautions have been specified. Eyes are protected with safety glasses or goggles, depending on the situation. Personal eyeglasses and contact lenses are not considered adequate eye protection. Face shields are an alternative to protective eyewear. A face shield provides protection to other parts of the face as well as the eyes.

Gloves protect both patients and healthcare workers from infectious microorganisms carried on hands. Gloves should be used when hand contact with blood or other body substances, mucous membranes, damaged skin, and other potentially infectious material is anticipated, or when handling potentially contaminated equipment and surfaces. As with all PPE, the need for gloves is based on careful assessment of the task to be performed and the risk of transmission of microorganisms to the patient or healthcare worker. Gloves should be considered single-use items—that is, they should be put on immediately before a procedure and removed on completion of the procedure, and changed between procedures on the same patient. They must be changed between patients. Sterile gloves are used for aseptic procedures or contact with sterile sites. Hand hygiene should be performed before putting on gloves and after their removal.

Table 13.6 outlines the general indications for the use of PPE, but it is stressed that the healthcare worker should a make a decision about PPE based on an assessment of the specific situation and its inherent risks. It is also important that items of PPE are correctly put on, and correctly removed and disposed of to prevent contamination of the wearer and the environment. The correct sequence for removing PPE is shown in Figure 13.12, a poster developed by NSW Health. A similar poster for donning PPE can be obtained from the NSW Health website. Note that hand hygiene must be performed before putting on and after removing PPE.

The use of protective clothing by staff is sometimes seen as creating a psychological problem for patients, who are already feeling isolated in a strange environment. For this reason, health workers should ensure they use only the necessary clothing and explain its use to the patient.

Handling and disposal of sharps

Sharps injuries can occur in any healthcare setting during the use of a sharp device on a patient, or prior to or during its disposal. A recent survey by the Australian Safety and Compensation Council (ASCC, 2008) found that 11 per cent of nurses had sustained at least one needlestick or other sharps injury. Injuries may occur during procedures, when cleaning used instruments, during disposal of used needles, and when handling sharp instruments after procedures. Sharps must not be passed directly from person to person, and handling of sharps should be kept to a minimum. The user of a single-use sharp is responsible for its immediate safe disposal in an approved container. The containers should be located as close as possible to the point of use.

Environmental controls

Potentially pathogenic microorganisms may be found throughout the environment of healthcare facilities. Transfer of these microbes from the environment to patients may occur via direct contact, or indirectly via healthcare worker hands. Most hard surfaces can be adequately cleaned with warm water and detergent. Allowing the cleaned surface to dry is an important aspect of cleaning. Frequently touched surfaces in patient-care areas should be cleaned using a detergent solution at least daily, as well as when visibly soiled and known to have been contaminated. Examples include bedrails, over-bed tables, light switches and tabletops. Touched surfaces of clinical equipment should be cleaned between use on different patients. All healthcare facilities should have a documented cleaning schedule that details

TABLE 13.6 Examples of use of PPE for specific procedures

PROCEDURE	HAND HYGIENE	GLOVES	STERILE GLOVES	SURGICAL MASK	EYE PROTECTION	GOWN
Activities of daily living (washing, toilet, etc.)	✓					
Routine observations (e.g. blood pressure measurement)	✓					
General medical examination	✓	✓ For contact with broken skin/ rash/mucous membrane		✓ If splash risk likely	✓ If splash risk likely	✓ If splash risk likely
Wound examination/ dressing	✓	✓ For contact with body substances	✓ For direct contact with wound	✓ For wound irrigation if splash likely	✓ For wound irrigation if splash likely	✓ For grossly infected wounds
Intravascular access device insertion	✓		✓	✓	✓	✓ (Where maximum barrier precautions are used)
Insertion of urinary catheter	✓		✓	✓ If exposure risk likely	✓ If exposure risk likely	✓ If exposure risk likely
Urinary catheter care	✓	✓			✓ When emptying drainage bag	✓ If exposure risk likely

Source: Adapted from NHMRC 2010, *Australian Guidelines for the Prevention and Control of Infection in Healthcare*. Commonwealth of Australia.

responsibilities of staff, a roster of duties, the frequency of cleaning, and the products that should be used.

Procedures for decontaminating spills of blood and other body substances (e.g. vomit, urine) differ based on the setting in which they occur and the volume of the spill. Appropriate PPE should be worn. The spill should be confined and visible matter removed with absorbent material (e.g. disposable paper towel), which is then discarded in an appropriate waste container. The spill area can then be cleaned with a detergent solution. Chemical disinfectants, such as sodium hypochlorite, may be used, based on risk assessment. Alcohol solutions should not be used for spillages.

Appropriate reprocessing of reusable equipment

The type of reprocessing required for reusable instruments and equipment depends on the individual situation—that is, the type of instrument, the body site and the way in which the instrument will be used. Consult the Australian guidelines for specific details.

Respiratory hygiene and cough etiquette

Respiratory hygiene and cough etiquette should be undertaken as a standard precaution by staff and patients with signs and symptoms of respiratory infection. The recommended procedures are as follows:

- Cover the nose/mouth with disposable tissues when coughing, sneezing, wiping and blowing noses.
- Use tissues to contain respiratory secretions.
- Dispose of tissues in the nearest waste receptacle after use.
- If no tissues are available, cough or sneeze into the inner elbow rather than the hand.
- Perform hand hygiene after contact with respiratory secretions or contaminated objects/materials.

Healthcare workers should instruct patients with a respiratory infection on these procedures.

Aseptic technique

An **aseptic technique** aims to prevent pathogenic organisms from being introduced into susceptible sites by hands or equipment. If performed correctly, aseptic technique protects patients during invasive clinical procedures.

Aseptic no touch technique (**ANTT**) is a special form of aseptic practice, in which key parts (of equipment) and sites (of the patient's body) are not touched by hands. ANTT should be undertaken when performing procedures such as cannulation, wound care, urinary catheterisation, and central and peripheral line management. Asepsis is ensured by identifying and then protecting key

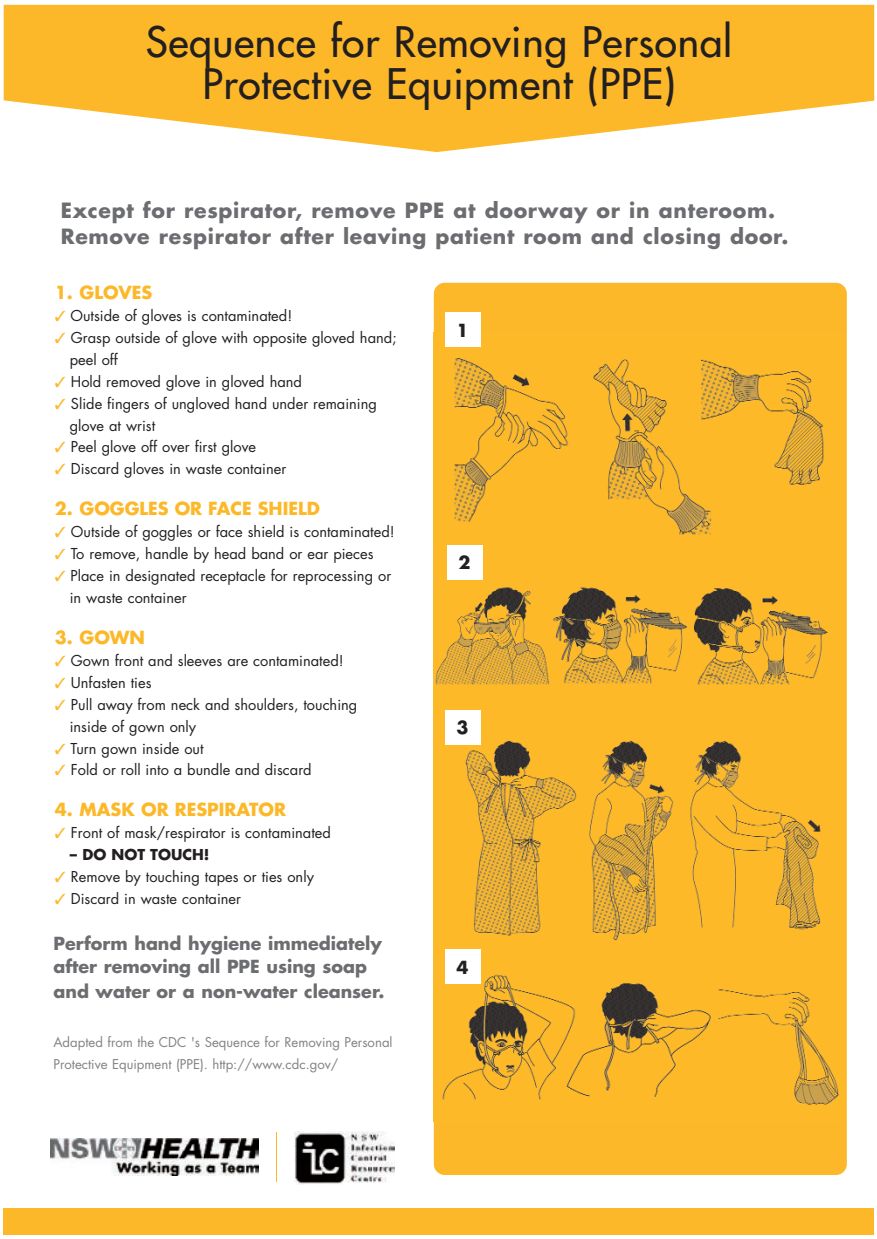

FIGURE 13.12

Poster showing the sequence for removal of personal protective equipment

Source: NSW Health. <www.health.nsw.gov.au/resources/quality/hai/pdf/tool_protective.pdf>.

parts and key sites. The essential components of ANTT are:

- key part and key site identification and protection—not touched by hands
- hand hygiene
- glove use—if it is necessary to touch a key part or key site directly, sterile gloves must be used, otherwise non-sterile gloves should be used.

Sterile technique is defined as the maintenance of objects and areas free from *all* microorganisms. In practice, it is not usually possible to achieve a sterile technique in healthcare settings because of the presence of microorganisms everywhere on people and in the facility environment. Near-sterile technique can only be achieved in highly controlled environments such as in a specially equipped operating theatre.

Handling of waste and linen

Healthcare facilities should have documented policies and procedures for the management of clinical waste and linen. These policies and procedures should conform to relevant state or territory legislation and regulations.

Transmission-based precautions

Transmission-based Precautions are applied in addition to Standard Precautions to patients infected (suspected or confirmed) with agents transmitted by the contact, droplet or

airborne routes. The aim is to prevent transmission by these specific routes. The measures used depend on the route(s) of transmission of the infectious agent involved, and involve a combination of:

- continued implementation of Standard Precautions
- appropriate use of PPE
- patient-dedicated equipment
- allocation of single rooms or cohorting of patients
- appropriate air-handling requirements
- enhanced cleaning and disinfecting of the patient environment
- restricted transfer of patients within and between facilities.

Note that Transmission-based Precautions are applied where necessary, in addition to Standard Precautions.

Contact precautions are used when there is a risk of direct or indirect contact transmission of infectious agents that are not effectively contained by Standard Precautions. Microorganisms typically transmitted in this way are MRSA, *Clostridium difficile* and *Streptococcus pyogenes*.

The key components of contact precautions are:

- maintenance of Standard Precautions
- use of appropriate PPE
- special handling of equipment—preferably use patient-dedicated or single-use equipment
- patient placement—a single-patient room is recommended
- minimising patient transfer or transport—limiting transfer of a patient to reduce the risk of environmental contamination, or cover infected or colonised areas of the patient's body if transfer is necessary.

Droplet precautions are intended to prevent transmission of infectious agents spread through close respiratory or mucous membrane contact with respiratory secretions. Droplet precautions are indicated for such organisms as respiratory syncytial virus (RSV) and meningococcus.

The key components of droplet precautions are:

- maintenance of Standard Precautions
- use of appropriate PPE—put on a surgical mask when entering the patient-care environment, with hand hygiene before putting it on and after taking it off
- special handling of equipment
- patient placement—place patient in a single-patient room if possible
- minimising patient transfer or transport—and patient to wear a mask and to observe respiratory hygiene and cough etiquette while being transferred.

Airborne precautions prevent transmission of infectious agents disseminated through airborne droplet nuclei or small particles that remain infective over time and distance when suspended in the air. Microbes for which airborne precautions are indicated include rubeola virus (measles), varicella virus (chickenpox) and *Mycobacterium tuberculosis*.

The key components of airborne precautions are:

- maintenance of Standard Precautions, including respiratory hygiene and cough etiquette
- use of appropriate PPE—especially a correctly fitted P2 respirator when entering the patient-care area
- minimising exposure of other patients and staff—patients on airborne precautions should be placed in a negative-pressure room or in a room from which the air does not circulate to other areas.

The recent outbreak of SARS illustrates the importance of airborne precautions for certain infections. Before the causative agent or method of transmission was identified, approximately 20 per cent of all SARS cases occurred in healthcare workers exposed to respiratory secretions in the hospital environment . Respiratory infections such as SARS and tuberculosis are difficult to contain unless the patient is placed in an appropriate room (e.g. single occupancy).

Figure 13.13 summarises the key aspects of Standard and Transmission-based Precautions

OTHER INFECTION CONTROL CONSIDERATIONS

Risk assessment

Although infection control guidelines provide a framework for the management of infections in the healthcare environment, it is important that the healthcare worker is able to assess the risk of transmission and susceptibility to infection for each individual patient and situation. Health workers need to be able to identify a potential hazard and implement strategies to prevent transmission.

To evaluate the infection risks involved in a given situation, health workers should consider the following questions:

1. *Is there a source (reservoir) of pathogenic microorganisms?*
   Potential sources should be identified and the nature of the pathogen and its method of transmission assessed.
2. *What is the risk of the patient acquiring an infection?*
   It is necessary to assess factors relating to patient susceptibility, such as the patient's immune status, underlying or contributing illnesses, the presence of wounds or pressure sores, or the presence of a urinary catheter, IV line or other relevant medical device.
3. *What are the risks of transmitting the infection to or from the patient?*
   The actual procedures to be carried out when caring for a patient should be assessed for their potential to spread microorganisms into the environment, to another patient or to a healthcare worker.
4. *What protocols and work practices exist to prevent transmission?*
   The protocols developed for the prevention of transmission are similar in all hospitals. They should be ascertained, monitored and modified according to experience.

Effective implementation of infection control is based on a sound understanding of the nature and properties of the

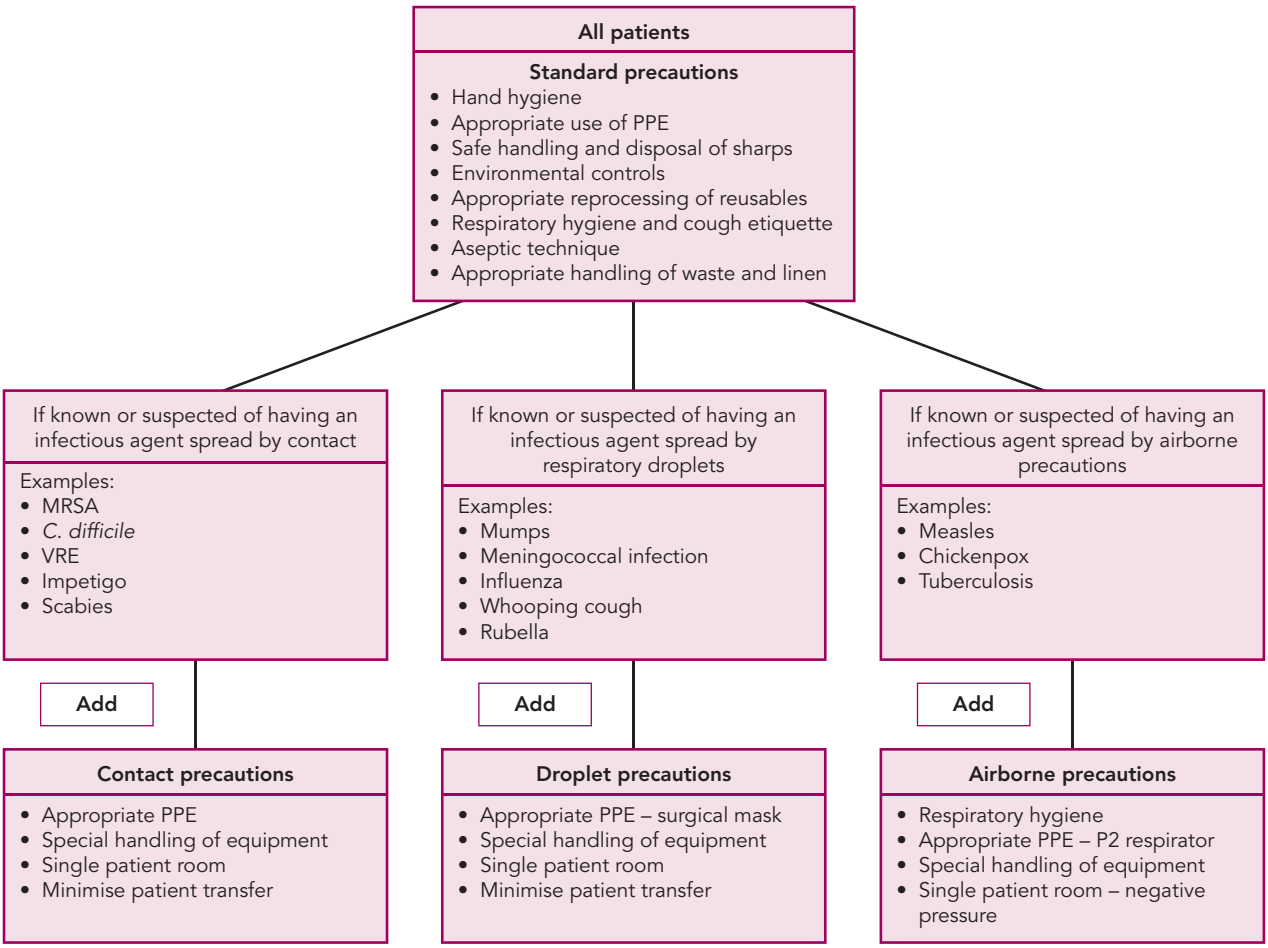

FIGURE 13.13

Standard and Transmission-based Precautions

infectious agent(s) involved, their method of transmission and the strategies that can be used to 'break the chain of transmission'. There are several routes by which a pathogen can reach a susceptible host. The reservoir may be an infected patient or healthcare worker, or the pathogen may be present in the environment. The source may be the air-conditioning, fomites, or the clothing or hands of a healthcare worker. Ways of 'breaking the chain' are indicated in Figure 13.14.

Sterilisation and disinfection of equipment

The risk of transmission of infection on instruments or equipment is determined by the type and number of micro-organisms present on the instrument, the area of the body where it is to be used and the procedure to be undertaken. Instruments used in areas of the body classified as critical must be sterile. Those used in semi-critical areas must be sterile or subjected to high-level disinfection. Those in non-critical areas must be clean (see Table 13.7).

The use of disinfectants and antiseptics, and of different methods of sterilisation, are all important in the prevention of HAI. Bacteria do not multiply on clean, dry surfaces. They need a warm, moist environment in which to reproduce. If surfaces and equipment are kept clean, dry and well ventilated, their contribution as a source of infection is greatly reduced. Most hospitals now use disposable presterilised instruments, needles, syringes, dressing packs and other items.

Procedures for disinfection and sterilisation of instruments are described fully in Chapter 11.

Isolation

Patients are placed in single rooms (isolation) to prevent the transmission of infectious agents either to or from the patient or health worker. Standard and Transmission-based Precautions are used to prevent transmission from infectious patients to other patients or healthcare personnel. Patients with an infection transmissible by the airborne route (e.g. tuberculosis) are preferably placed in a single room with negative-pressure ventilation. Negative-pressure ventilation maintains a flow of air into the room, thus preventing pathogens from being disseminated from inside to outside the room. This type of room is particularly important if the patient's infection is caused by a multi-drug-resistant organism.

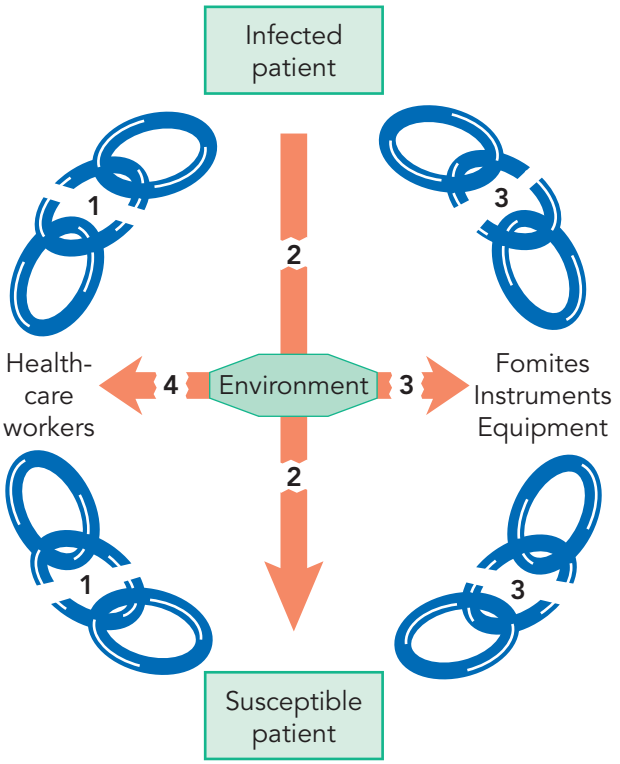

FIGURE 13.14

Breaking the chain of transmission

Ways of 'breaking the chain' are indicated by the corresponding numbers.

1. *HAND HYGIENE, Standard and Transmission-based Precautions, aseptic technique, protective clothing.*
2. *Transmission-based Precautions (contact, droplet, airborne), use of negative pressure, masks.*
3. *Cleaning, disinfection, use of sterile equipment.*
4. *HAND HYGIENE (personal hygiene, gloves and protective clothing).*

Patients whose immune system is compromised, either from illness or medical treatment, pose a particular problem in the hospital setting. They may be extremely susceptible to infection by many microorganisms, including opportunistic pathogens (i.e. microorganisms that only infect immunodeficient hosts). These patients should be accommodated in a room with positive pressure to prevent the entry of pathogens. Positive-pressure ventilation maintains a flow of air out of the room, thereby restricting movement of microorganisms into the room.

Sometimes immunocompromised patients may already have an infection (e.g. TB) that would be hazardous if transmitted to hospital personnel and/or other patients. This would require measures to prevent the spread of the infection by containing it within the patient's environment. The isolation procedures required would therefore need to prevent the passage of microorganisms *in both directions* across a 'barrier'.

INFECTION CONTROL TEAMS

With increasing awareness of the importance of infection control in Australian hospitals has come recognition of the need for staff with a specialised infection control role. The first infection control nurse was appointed in 1965. Subsequently, the Australian Infection Control Association (AICA) was established, with a network of members in every state.

In most large hospitals the policies and procedures for infection control are the responsibility of the Infection Control Committee. This committee usually comprises representatives of the Medical Board, Nursing Administration, Microbiology, Pharmacy, Theatre Staff, Clinical Nurse Consultants and other hospital departments. In practice, the responsibilities and composition of this body will vary considerably depending on the size of the hospital, the resources available and financial considerations. In large city hospitals an infection control team, consisting of one or two Clinical Nurse Consultants and the Microbiologist and Infectious Diseases specialist, is responsible for the daily implementation of infection control policies. In smaller country hospitals the responsibility may rest with one part-time Clinical Nurse Consultant.

Infection control personnel are usually responsible for the production of a local infection control manual containing details of procedures for infection control, including cleaning, disinfection and sterilisation. Procedures should comply with Australian and New Zealand Standard AS/NZS 4187. Increasingly, infection control programs are included in the quality assurance audits for accreditation of healthcare facilities. An ongoing responsibility of the infection control team is to conduct information sessions for all hospital staff, to make them aware of the importance of preventing transmission and of their responsibility to observe correct practices.

TABLE 13.7 **Spaulding classification system for possible contact sites of instruments**

APPLICATION	CLASSIFICATION	DESCRIPTION	EXAMPLES
Entry into or penetration of sterile tissue cavity or bloodstream	Critical	Sterile	Surgical procedure with entry into sterile tissue; intravascular cannulation
Contact with intact sterile mucosa (or non-intact skin)	Semi-critical	Sterile or high-level disinfection	Respiratory therapy; non-gastrointestinal endoscopy
Contact with intact skin	Non-critical	Clean	Non-invasive procedures (e.g. palpation, abdominal ultrasound)

Source: CDNA 2004, *Infection Control Guidelines for the Prevention of Transmission of Infectious Diseases in the Health Care Setting*, Table 4.2. Commonwealth of Australia.

Surveillance

In order to devise appropriate strategies to control the spread of infection in hospitals, reliable information is needed on the incidence of infections, their causes, and the effectiveness of any prevention strategies implemented. **Surveillance** systems are essential in providing this information. An effective surveillance system provides timely information for hospital managers and clinicians to act on.

Surveillance of HAI in Australia has mostly been performed at a state or regional level, with no systematic Australia-wide system in place. The Australian Commission on Safety and Quality in Health Care has stressed the importance of surveillance in its 2008 report titled *Reducing Harm to Patients from Health Care Associated Infection: The Role of Surveillance* and has recommended a national coordinated, standardised approach to surveillance of healthcare-associated infections, and the incidence and prevalence of resistant microorganisms. It recommends that each healthcare facility should collect and report surveillance data, since good-quality surveillance systems have been shown to result in reduction of infection rates, and of associated morbidity and mortality.

Staff should understand that prevention of HAI is the responsibility of all those who care for patients. Infection should no longer be considered an unpredictable 'complication', but rather as a potentially preventable 'adverse event'. Collection, analysis and reporting of surveillance data on HAIs has been shown to be associated with a reduction in infection rates, morbidity and mortality.

An important function of the infection control team is to monitor the incidence of infections within the hospital. Infection control staff are in a position to collect epidemiological data on the type of hospital infections that occur, and on patterns of antibiotic resistance of isolated organisms. This constant surveillance helps to detect an increase in infection rates or an outbreak or cluster of similar infections in the hospital, so that measures can be taken to determine the source and means of transmission and prevent further cross-infection. Surveillance is essential for quality assurance and to monitor the effect of interventions designed to reduce infection.

Two major types of surveillance are employed. *Process surveillance* involves auditing practice against standard guidelines or policies—for example, compliance with hand hygiene, intervention strategies, or guidelines for antimicrobial prescribing. *Outcome surveillance* measures adverse events, such as the incidence of infections.

SPECIFIC PROBLEMS IN INFECTION CONTROL

Although the general principles of infection prevention and control apply to all areas of the hospital, there are certain situations where the risk of infection is very high. Standard and Transmission-based Precautions should be applied, but patients and staff may still be susceptible to infection because of the nature of the treatment, the state of their immune system or the type of microorganisms to which they are exposed. Some units of the hospital are more likely than others to harbour a population of resistant organisms. These tend to be units where there are seriously ill or immunocompromised patients, and thus an associated high level of antibiotic usage (e.g. intensive care units). Healthcare workers in these units are much more likely to be nasal carriers of resistant organisms and have the potential to spread them in the hospital.

Operating theatres and surgical patients

Since Joseph Lister pioneered the use of antiseptics in surgery in the 1860s, many advances have been made in the prevention and control of surgical site infections (SSI). However, SSI remain a leading type of HAI, resulting in significant morbidity and mortality. Surgical procedures inherently carry a risk of infection because the patient's external defences are breached during surgery. This risk has increased, especially over the last 50 years, with the introduction of more complex and invasive procedures, increased insertion of implants, and performance of surgery on an ageing population with increasing comorbidities. Around 5 per cent of surgical patients acquire an SSI, which adds an average of around a week to their hospital stay.

The risk of SSI varies according to the type and site of the surgery. Surgical procedures may be classified according to the level of microbial contamination at the site—for example, classifications often include: clean, clean-contaminated, contaminated, and dirty. This enables a prediction to be made about the risk of the patient developing an SSI. Other factors that significantly influence the risk of SSI include the duration of the operation (i.e. the length of time that tissues are exposed), host susceptibility and underlying health factors, and existence of infection at any other body sites. Patients may develop post-surgical infections at the incisional site, or in the organs or spaces accessed during the operation.

The greatest risk of infection occurs during the period that the surgical wound is open. The most common source of organisms causing SSI is endogenous—that is, the patient's own normal flora at or near the surgical site, including colonisers possibly acquired while in hospital. Thus, skin flora such as staphylococci, enterococci and Gram-negative enteric bacteria (e.g. *Pseudomonas* and *E. coli*) are the most common causes. It is clear that current methods of surgical antisepsis (see Figure 13.15) can reduce but not eliminate the risk of SSI by endogenous skin flora. Seeding of the operative site from a distant focus of infection can occur, particularly in patients having a prosthesis implanted.

Exogenous microorganisms are occasionally the cause of SSI, and are most often derived from colonised or infected surgical personnel, the operating theatre environment, or contaminated equipment or instruments. As many as 50 per cent of hospital personnel carry *Staphylococcus aureus* (including MRSA) in the nose or on broken skin. SSI caused by Group A streptococci have been traced to colonised operating room personnel. Unusual environmental organisms from sources in the operating theatre are rarely

FIGURE 13.15

Staff working in operating theatres 'scrub' and put on special protective clothing in order to minimise the risk of transmission of infection

associated with SSI, including *Legionella*, *Mycobacteria* and *Pseudomonas*.

SSIs are potentially more serious, even life-threatening, when the infection is due to a resistant hospital organism. One way in which this problem has been addressed is by minimising the patient's stay in hospital, especially pre-operatively. Previously, patients undergoing major operations (e.g. open heart surgery) were admitted two to three days before the operation in order to carry out tests and stabilise the patient. However, it was found that this allowed time for patients to become colonised with resistant hospital organisms. If wound infection subsequently occurred, it was often caused by a hospital strain. Patients for major surgery are now admitted as close as possible to the time of surgery, to minimise exposure to hospital strains. Many operations are now done in day-surgery facilities, which reduces not only the risk of infection, but also costs.

Table 13.8 Outlines procedures for reducing the risk of SSI. The types, causes, incidence and risk reduction of SSI are discussed more fully in Chapter 16.

Burns units

The survival rate of patients with extensive burns has improved in recent years, partly because of improved

TABLE 13.8 **Major risk factors for SSI and approaches for reducing the risk**

RISK	RISK REDUCTION
Age	No realistic approach
Remote site of infection	Treat infection before surgery
Pre-operative colonisation with hospital organisms	Reduce pre-operative stay to as short as possible
Hair removal at surgical site	Do not remove hair unless it interferes with the procedure
Patient skin flora	Wash and clean skin around surgical site, using appropriate antiseptic
Staff skin flora	Perform pre-operative surgical scrub; use appropriate PPE
Antibiotic prophylaxis	Use only when indicated, use appropriate antibiotic, and within one hour of incision
Incision time	Minimise incision time
Environmental sources	Follow recommended sterilisation and disinfection procedures

infection control practices, as well as new treatments. Burns vary in their degree of severity, depending on the cause of the burn and the area of the body affected. A large percentage of children admitted to accident and emergency departments suffer from burns caused by boiling water, which can cause extensive damage to their skin. Inhalation burns from smoke or fire damage the epithelial lining of the lungs and may predispose to pneumonia.

Patients suffering from burns to a large part of their body are at increased risk of infection, since one of the primary body defences (intact skin) has been destroyed (see Chapter 9). Furthermore, other components of the immune system may be impaired following severe burns (see Chapter 16). The nature of the tissue damage in a severe burn means that phagocytic cells and antibodies have difficulty in reaching the area and preventing bacterial multiplication from taking place. The burn site itself provides a moist area, full of nutrients, ideal for the growth of organisms.

When burns become infected, treatment is difficult because the damage to the vascular tissue around the burn site prevents antibiotics from reaching the infection. Similarly, topical application of antibiotics is not always successful because of the large quantities of dead tissue. The two most common organisms found in infected burns are *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Both pathogens have strains that are resistant to many antibiotics. It is therefore very important to maintain a high level of asepsis in a burns unit (see Case History 13.4).

As the burns unit can be a significant source of antibiotic-resistant microorganisms, staff should exercise appropriate infection control precautions when moving from burns units to areas of the hospital that have particularly susceptible patients (e.g. transplant units, oncology and intensive care units).

Intensive care units

Patients in an intensive care unit are usually seriously ill and so their body defences are highly compromised. The invasive nature of their treatment, which may involve procedures such as vascular catheterisation, urinary catheterisation, and the use of ventilators and monitoring equipment, renders patients in ICUs very susceptible to hospital-acquired infections, particularly bloodstream infection (BSI), urinary tract infection (UTI) and ventilator-associated pneumonia (VAP).

A patient in an ICU often receives a number of different medications, administered for convenience via a central line. Studies have shown that intravenous cannulae are associated with a high risk of sepsis, which correlates with high rates of morbidity and mortality. Sepsis associated with peripheral vein cannulae can be reduced by adhering to strict infection control protocols. Control of sepsis in central lines is more difficult because the patient is usually seriously ill and the cannula may need to be in place for an extended period. The length of time the cannula is in place significantly affects the likelihood of infection. The organisms most commonly associated with BSI are *S. aureus*, coagulase-negative staphylococci, and Gram-negative enteric bacteria.

UTI is the most common infection acquired in ICU, with the vast majority occurring in patients with an indwelling urinary catheter. The predominant microorganisms are the enteric Gram-negative bacteria (e.g. *E. coli*, *Enterobacter*), enterococci, *Candida* spp. and *Pseudomonas aeruginosa*, and these organisms in ICU are often multi-drug-resistant. (See Chapter 21 for a more detailed description of UTI.)

VAP affects 10–20 per cent of patients receiving mechanical ventilation and results in significant morbidity and mortality. Because it is associated with mechanical ventilation, it occurs in patients with acute respiratory failure—that is, in people who already are suffering from a severe respiratory condition. This procedure predisposes the patient to infection because it involves the insertion of an endotracheal tube (a tube passed through the mouth and into the trachea), which bypasses important defences of the respiratory tract, such as mucociliary clearance and the cough reflex (see Chapter 9). The endotracheal tube also provides a source of microorganisms in contaminated secretions that accumulate around the tube. The cause of VAP is influenced by a number of factors, including the duration of ventilation, length of hospital and ICU stay, and prior antimicrobial therapy.

CASE HISTORY 13.4

Infection in a burns patient

A 32-year-old man was admitted to a major teaching hospital following a motor vehicle accident. He had sustained multiple fractures and severe burns to 65 per cent of his body. He was intubated and required operations to repair fractures, followed by multiple skin grafts. He acquired 13 separate bloodstream infections with different multi-resistant organisms including MRSA, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella* and *Enterobacter*. In all, he spent over four months in hospital, three months of it in ICU. Medically, he made a remarkable recovery. However, the number of separate bloodstream infections he sustained was an indication of the severity of his illness, due to his multiple operations and the difficulty, particularly in burns patients, of preventing serious infections. All of this contributed to his lengthy hospital stay and was associated with significant cost to both the patient and the hospital.

Questions

1. Why was this patient particularly susceptible to infection, especially by multi-resistant organisms?
2. Suggest ways in which the bacteria may have gained access to this patient.
3. What are the clinical signs of bacteraemia?
4. What extra precautions should be taken when caring for a burns patient?

Drug-resistant causes are common, including enteric bacteria, *S. aureus* and *Pseudomonas aeruginosa*. Good hand hygiene and appropriate use of PPE are critical for reducing the incidence of VAP.

The ICU is an ideal place for the development and spread of resistant organisms. High antimicrobial usage combined with seriously ill patients creates an environment in which resistant organisms are selected. These organisms have the potential to be spread to other parts of the hospital by colonised staff or by a patient when transferred to another ward. Good hand hygiene, the maintenance of strict aseptic technique and the use of appropriate protective clothing are essential to minimise the risk of spread of infection.

Transplant and oncology wards

Patients in transplant and oncology wards, in addition to being critically ill, are usually being treated with immunosuppressive drugs. These wards are thus important areas of the hospital where opportunistic infections and resistant organisms can flourish, and patients require special care to prevent infections being introduced into their environment.

Maternity wards

Patients in a maternity ward are often thought of as 'well' because their hospitalisation is not due to illness. However, there are significant infection risks associated with childbirth. These include infections that may occur at the site of a caesarean section, or in the perineal area where tearing or tissue damage may occur. A significant number of women carry potentially pathogenic microorganisms in the normal flora of the vagina and these can readily invade tissue damaged during childbirth. For example, Group B streptococci are part of the normal flora of some women and can cause puerperal fever (formerly called childbed fever), which is usually an infection of the placental site after birth. At-risk patients can be screened for these bacteria during pregnancy and administered antibiotics intra-partem. Faecal bacteria can also infect damaged tissue resulting from childbirth. Although these infections are usually caused by normal flora, there is a risk that hospital strains can colonise the mother before or after giving birth. Care of the patient includes education about personal hygiene and careful monitoring for any signs of infection (e.g. fever, inflammation, pus).

Newborn babies are highly susceptible to infection because of their immature immune system. Early in life a baby is largely dependent on antibodies it has received from its mother transplacentally, and in colostrum and breast milk after birth. Babies born to women carrying an infection at birth can be at risk of being infected via the placenta or during passage through the birth canal. Hepatitis B and HIV/AIDS are thought to cross the placenta in the final stages of pregnancy or during birth. Genital infections caused by microorganisms such as *Candida albicans* (thrush), herpes simplex virus (genital herpes), *Neisseria gonorrhoeae* (gonorrhoea) and *Treponema pallidum* (syphilis) can be spread from mother to child during birth. These infections can be very serious for a neonate. Organisms can enter through the eyes, umbilicus or breaks in the skin. Group B streptococci (*Streptococcus agalacticae*) are responsible for early onset sepsis, which has a high mortality rate. Some infections, such as herpes, are highly contagious and can be transmitted to other babies in the nursery, usually via the hands of hospital staff. Neonates are also at risk of acquiring infections caused by *Escherichia coli*, *Staphylococcus aureus* and many other hospital-derived pathogens.

Residential and aged-care facilities

An increasing number of elderly people in Australia are living in aged-care facilities, where the close proximity of residents creates an environment that allows microorganisms to be easily spread. Most of the residents in nursing homes have underlying or contributing illnesses that make them more susceptible to infection. They may have impaired mental function, impaired mobility, incontinence, or may be receiving multiple medications, including antimicrobial drugs. Respiratory pathogens, such as influenza viruses and respiratory syncytial virus, and gastrointestinal pathogens such as noroviruses, can spread easily and rapidly through these facilities. Scabies also spreads readily in nursing homes.

It is important to observe good infection control procedures in these settings, especially hand hygiene, use of gloves where appropriate, and maintenance of good cleaning and hygiene.

OCCUPATIONAL EXPOSURE FOR HOSPITAL STAFF

Healthcare work involves close bodily contact with patients, including those with infectious diseases. Thus, occupational exposure to pathogens is common, so it is important for health workers to understand the potential risks involved in their work. These risks will vary depending on the type of health facility. In general, hospital staff are healthy and thus have very low susceptibility to the many pathogens found in hospital, unless they have underlying illnesses. However, even if healthy, they may still be susceptible to some infections that a patient may have, especially respiratory, bloodborne, and skin and wound infections.

Transmission of vaccine-preventable diseases (e.g. measles, pertussis) can occur in healthcare facilities, so it is important that healthcare workers are up to date with all immunisations as recommended by the Department of Health in *The Australian Immunisation Handbook* (2008). This includes adequate cover for the common childhood diseases, such as measles, mumps, rubella, pertussis and chickenpox, as well as for other infections such as hepatitis B, to protect not only healthcare workers themselves, but also the patients they care for. Furthermore, appropriate immunisations provide some protection for the foetus of a pregnant worker. Annual influenza immunisations are recommended for staff in aged-care and residential facilities to protect elderly and immunosuppressed patients.

A major occupational risk factor for healthcare workers is needlestick or sharps injuries. As outlined earlier in this chapter, the *Australian Guidelines for the Prevention and Control of Infection in Healthcare* contain detailed information regarding the appropriate handling and disposing of sharps (see Figure 13.16). Hospital guidelines should also detail local procedures. Consistent adherence to Standard and Transmission-based Precautions is the best defence for workers to protect themselves from occupational infection.

CONCLUSION

The prevention of transmission of infection in healthcare facilities is the responsibility of all health workers. Each situation is unique—with different patients, procedures, risks and pathogens.

Health professionals must have a good understanding of the different types of pathogens—their habitat and properties and the way they are transmitted. It is important to be able to assess the risk to individual patients, and to identify reservoirs and sources of infection. In this way it is possible to implement the appropriate infection control procedures for each situation.

FIGURE 13.16

Sharps container

SUMMARY

- Healthcare-associated infections (HAIs) are infections acquired by patients in a hospital or other health facility. They are also called nosocomial infections.
- Infection is the most common adverse event affecting patient safety in hospitals worldwide.

THE IMPORTANCE OF HEALTHCARE-ASSOCIATED INFECTIONS

- The prevalence of hospital-acquired infection in developed countries ranges from 3.5 per cent to 12 per cent, and in Australia is around 7 per cent.
- Hospital-acquired infections increase patient suffering, prolong the length of hospital stay, increase the cost of medical care and increase the mortality rate.

TYPES OF HEALTHCARE-ASSOCIATED INFECTIONS

- The most common sites of HCAIs are the urinary tract, surgical sites, lower respiratory tract, skin and blood.
- HAI increase morbidity and mortality, especially bloodstream infections and surgical site infections.

ORGANISMS THAT CAUSE HEALTHCARE-ASSOCIATED INFECTIONS

- Bacteria are the most common cause of HAIs, followed by fungi and viruses.
- Hospital-derived microorganisms are often resistant to many antibiotics (multi-drug-resistant), and cause infections that are difficult to treat.
- Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of HAI and is endemic in most hospitals throughout the world.
- MRSA has classically been associated with hospitals and hospital infections (called HA-MRSA), but since the 1990s there has been recognition of MRSA strains that have originated in the community—community-associated MRSA (CA-MRSA).
- Coagulase-negative staphylococci (e.g. *Staphylococcus epidermidis*) are responsible for many cases of infection, especially associated with indwelling intravenous lines, prosthetic heart valves and other prostheses.
- In recent years there has been a re-emergence of virulent strains of *Streptococcus pyogenes* as a cause of HAIs.
- Vancomycin-resistant enterococci (VRE) can cause HAIs, although the incidence is relatively low. Patients in intensive care units are at greatest risk.
- Antibiotic-resistant Gram-negative organisms, such as *E. coli*, are prominent causes of HAIs.
- Infectious hospital-acquired diarrhoea, or *Clostridium difficile* infection, is a common HAI with a high fatality rate.
- In immunocompromised patients, organisms such as enterococci, mycobacteria, fungi and certain viruses frequently cause infections.

SOURCES OF HOSPITAL INFECTIONS

- Community strains are organisms that originate from outside the hospital and are generally sensitive to most antimicrobial drugs.
- Most hospital infections are caused by hospital strains.
- Hospital strains originate from within the hospital and can be difficult to treat because they are frequently resistant to antimicrobial drugs and disinfectants.

- Colonisation is the presence and growth of a microorganism on the skin or mucous membrane without infection.
- Colonisation with hospital strains can occur after admission and becomes more likely the longer the stay in hospital. Exposure to antimicrobial drugs also favours colonisation.
- Healthcare staff are frequently colonised by hospital organisms and are thus carriers of these potential pathogens.
- Patients with lowered resistance (e.g. surgical or burns patients) may be infected by a multi-resistant hospital strain, and are then important reservoirs of these organisms.
- Patients and staff can be sources of potential pathogens: infecting organisms, colonising organisms or the normal flora.
- Exogenous infections are derived from outside the patient's body (e.g. from other patients or staff or the hospital environment).
- Endogenous infections are derived from the patient's own body.
- Clean, dry surfaces are not a significant source of microorganisms unless freshly contaminated, but moist areas may be important sources of pathogens.

ROUTES OF TRANSMISSION OF MICROORGANISMS IN HEALTHCARE

- Contact transmission occurs when infectious microorganisms are transmitted to a susceptible host by touch or via contact with blood or other body substances.
- Droplet transmission occurs when a person with a respiratory infection coughs, sneezes or talks and expels large infectious droplets that directly infect the mucosal surfaces of another person.
- Airborne transmission occurs via small particles (aerosols) expelled during coughing, sneezing or talking, or when large droplets partially evaporate.

FACTORS CONTRIBUTING TO THE INCIDENCE OF HOSPITAL-ACQUIRED INFECTIONS

- Infections acquired in hospital are more serious and difficult to treat because they are frequently caused by drug-resistant strains of microorganisms.
- Many hospital patients have lowered resistance to infection due to age (young or old), immobility, underlying disease or infection, or surgical or traumatic wounds.
- Some medical treatments can lower a patient's resistance to infection, including cancer therapy, immunosuppressive drugs and some anti-inflammatory drugs.
- Diagnostic and therapeutic procedures involving the penetration of the skin or mucous membranes (e.g. urinary catheterisation, indwelling intravascular devices) can provide easy access for microorganisms to susceptible tissues.
- Infections that are brought about by medical procedures or treatments are called iatrogenic infections.
- Implanted prostheses are favoured sites of colonisation by microorganisms.
- Transfer of patients from one ward to another can spread infection.
- Physical contact between patients and staff in hospitals is frequent and varied, allowing easy transmission of microorganisms from person to person.

CONTROL OF INFECTION IN HEALTHCARE FACILITIES

- The Australian Commission on Safety and Quality in Health Care (ACSQHC) released the *Australian Guidelines for the Prevention and Control of Infection in Healthcare* in October 2010.
- 'Standard Precautions' refers to a number of work practices that are applied to all people, regardless of their infection status. The precautions are based on the principle that all people potentially harbour pathogenic microorganisms.
- Healthcare workers' hands are the most common vehicle for the transmission of potential pathogens from patient to patient and within the healthcare environment.
- The simplest and most effective method of preventing cross-infection is good hand hygiene.
- Personal protective equipment consists of the barriers used to protect the mucous membranes, airways, skin and clothing of the healthcare worker and/or patient from contamination with infectious microorganisms.
- A protective apron or gown should be worn when close contact with a patient or equipment could lead to contamination of the healthcare worker's skin or clothing.
- Face and eye protection reduces the risk of exposure to splashes or sprays of blood or body substances.
- Surgical masks cover the nose and mouth and are used to protect them from splashes of body fluids.
- Gloves should be used when hand contact with blood or other body substances, mucous membranes, damaged skin and other potentially infectious material is anticipated, or when handling potentially contaminated equipment and surfaces.
- Gloves do not remove the need for hand hygiene practices.
- An aseptic technique aims to prevent pathogenic organisms from being introduced into susceptible sites by hands or equipment, and protects patients during invasive clinical procedures.
- Transmission-based Precautions are applied in addition to Standard Precautions to patients infected (suspected or confirmed) with agents transmitted by the contact, droplet or airborne routes.

OTHER INFECTION CONTROL CONSIDERATIONS

- The health worker needs to evaluate the risks of transmission for each patient.
- An understanding of the nature of the infection is essential to 'break the chain of transmission'.
- Equipment should be disinfected or sterilised according to the risk involved for its use.

- Isolation of patients in a single room with correct ventilation is used to prevent transmission of infection.
- Patients whose immune system is compromised need to be protected from infection.

INFECTION CONTROL TEAMS

- In most large hospitals the policies and procedures for infection control are the responsibility of the Infection Control Committee.
- The infection control team monitors the incidence of infections within the hospital and conducts information sessions for hospital staff.

SPECIFIC PROBLEMS IN INFECTION CONTROL

- Patients in some units of the hospital are more susceptible to infection than others (e.g. surgical patients, because their defences have been breached).
- Other patients who are particularly susceptible to infection are those with burns, those in high-dependency units, the immunocompromised, and those who are susceptible because of the nature of the treatment they require.
- Infection risks in maternity and neonatal wards include post-partum infections of the mother as well as the baby.
- Infection control procedures should be implemented in long-term and aged-care facilities to protect residents who may be highly susceptible to infection.

OCCUPATIONAL EXPOSURE FOR HOSPITAL STAFF

- Hospital staff are at risk of becoming infected via needlestick injuries and exposure to respiratory diseases such as TB.
- Staff should be fully immunised.
- Pregnant staff should be aware of the infection risks associated with pregnancy.

STUDY QUESTIONS

1. Differentiate between hospital-acquired infections and community-acquired infections.
2. What was the major contribution of Ignaz Semmelweis to hospital infection control?
3. List the most common sites of hospital-acquired infections.
4. What is MRSA?
5. What is an important difference between hospital strains of bacteria and community strains?
6. What is meant by 'colonisation' in relation to microorganisms and the human body?
7. List the factors that can make a patient immunocompromised.
8. What is an iatrogenic infection? Give three examples.
9. Define the terms 'exogenous infection' and 'endogenous infection'.
10. What are the important sources of organisms that cause exogenous infections in hospitalised people?
11. Dry objects and surfaces do not generally support the growth of microorganisms, but can still act as a source of infection. Explain.
12. List the common ways that microorganisms can be spread in the hospital.
13. What are the major responsibilities of a hospital's Infection Control Committee?
14. Define the terms 'asepsis' and 'aseptic technique'.
15. Handwashing at the appropriate times is an extremely important means of reducing cross-infection in hospitals. When are the appropriate times? Explain your answer.
16. Describe the basic principles of Standard and Transmission-based Precautions, and explain why they are necessary.
17. What procedures are followed in Standard Precautions?
18. Describe the major ways in which the incidence of surgical wound infections can be reduced.
19. List the types of patients that are particularly susceptible to hospital-acquired infections.
20. What procedures are used to reduce the incidence of needlestick injuries?

TEST YOUR UNDERSTANDING

1. Why are hospital-acquired infections often more serious than those acquired in the community?
2. Explain why patients in the burns unit are particularly susceptible to infection.
3. Discuss how the use of Standard and Transmission-based Precautions is important to protect healthcare workers.
4. What special precautions should be used when caring for a patient who is immunocompromised?
5. Discuss the merits of the various types of protective clothing that can be worn by health workers.

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CHAPTER

14

Issues in public health

CHAPTER FOCUS

- What is the role of public health departments in the provision of health services in Australia?
- Which infectious diseases are notifiable in Australia?
- How is information about the incidence and prevalence of infectious diseases in Australia collected and distributed?
- Which aspects of primary healthcare are concerned with the prevention of infectious diseases?
- What infection control precautions are applicable to childcare centres?
- What are the particular challenges facing the provision of healthcare in rural and remote areas of Australia?
- How do public health issues in New Zealand differ from those in Australia?

INTRODUCTION—THE AUSTRALIAN HEALTH SCENE

The advances in medical knowledge and technology during the 20th century resulted in remarkable improvements in the standard of health and healthcare in the developed world. This was particularly true in countries such as Australia, which has one of the highest standards in the world. However, even in developed societies with a high standard of living, major inequalities in health status and quality of healthcare exist between different groups within the population.

One of the most dramatic changes has been in the number of deaths attributable to infectious diseases. At the beginning of the 20th century, more than 60 per cent of all deaths were due to infections but by the close of the century, less than 5 per cent of deaths in Western countries were directly caused by microorganisms. However, although the **mortality** rate has fallen, infectious diseases are still the cause of serious **morbidity** in our everyday lives and make up a significant proportion of the overall cost of the health system.

The decline in mortality due to infectious diseases can be attributed to three main factors.

1. The ability to isolate and identify the microorganisms responsible for causing infections has enabled scientists and health professionals to develop and implement procedures to prevent or control the spread of many diseases. Improvements in general standards of hygiene, sanitation facilities, housing and quality of water supply have all contributed to an improved standard of living and a decrease in the incidence of disease.
2. The discovery of antibiotics provided drugs that were able to selectively kill the bacteria responsible for many life-threatening illnesses and led to the development of other antimicrobial drugs (see Chapter 12).
3. The use of vaccines has dramatically reduced mortality from 'childhood diseases', and from 'adult' diseases such as influenza and pneumococcal disease. Vaccines are a valuable preventive measure against viral infections, which are difficult to treat with antibiotics (for the reasons discussed in Chapters 5 and 12). The use of vaccines and the value of immunisation are discussed more fully later in this chapter.

PUBLIC HEALTH

'Public health' is the term used to describe the promotion of health in the community rather than the individual, and the development of policies and guidelines to prevent or control disease. International agencies, such as the World Health Organization (WHO), carry out these functions at a global level, and individual government health departments in each country are responsible for national issues of public health. Electronic communication between health authorities in different countries enables rapid dissemination of information about disease outbreaks, the reporting of cases, and sharing of experience for treatment and control.

The responsibility for the delivery of healthcare in Australia rests with a number of government bodies at the Commonwealth, state and territory level, as well as with the private sector. At the local level, each state or territory is organised into area or district health services which report to the state health departments on matters of public health.

The Australian Department of Health and Ageing has the responsibility of providing guidelines and information in regard to health policies of national importance. The National Health and Medical Research Council (NHMRC) is a statutory authority within the portfolio of the Minister for Health. Its objectives are to:

- raise the standard of individual and public health throughout Australia
- foster the development of consistent health standards between states and territories
- foster medical research and training, and public health research and training, throughout Australia
- foster consideration of ethical issues relating to health.

The NHMRC publishes recommendations covering areas such as immunisation schedules, child health, communicable diseases, infection control, nutrition, mental health and other health issues. Another of its functions is to advise the government on the funding of competitive grants for medical and public health research.

An important function of the health authorities is to collect epidemiological data (see Chapter 8) and carry out surveillance of the occurrence of communicable diseases. Information supplied by doctors, laboratories, hospitals and health services is sent to local, state and Commonwealth authorities and is used to develop policies to control the spread of disease.

The control of infectious diseases is one area of public health for which there are intervention strategies of proven efficacy. Immunisation is available to prevent previously fatal childhood diseases and to protect against illnesses such as influenza and hepatitis B. Screening programs allow early detection of infectious diseases and the implementation of programs to prevent transmission as well as to reduce morbidity and mortality. Hospital infection control programs based on a knowledge of the aetiology (cause) and transmission of disease can reduce the incidence of hospital-acquired infections (see Chapter 13).

Today, when people can travel from one side of the world to the other in less than 24 hours, the possibility of

an infectious agent being introduced into an unsuspecting population is very high. Any major international event involving thousands of travellers from all parts of the world, often crowded into a relatively closed environment, creates a situation where infections can spread very easily. Increased medical services are required for the large number of people who attend the event, the environment must be continuously monitored, and the provision of safe food and water is essential.

An important aspect of the organisation of any major event involves the public health strategies put in place to protect the health of residents and visitors, and the coordination of health services so that swift action can be taken in the event of an outbreak of disease, or a natural or man-made disaster. If a serious outbreak of infection occurs among athletes or visitors to a city, it can spell disaster for an event that has taken years to plan and organise.

NOTIFIABLE DISEASES

In Australia a number of communicable diseases are required by law to be notified to the health authorities when diagnosed in a patient. The National Notifiable Diseases Surveillance System (NNDSS) was established in 1990 under the auspices of the Communicable Diseases Network Australia (CDNA). The NNDSS coordinates the reporting of notifiable diseases at a Commonwealth level, and at international level to the WHO. In 2007 the *National Health Security Act* provided a legislative basis for the exchange of health information, including personal details between jurisdictions and the Commonwealth, and established the National Notifiable Diseases list which specifies the diseases about which personal information can be provided. Sources of surveillance data include doctors, hospitals and laboratory notifications. The diseases or syndromes that are nationally notifiable are listed in Table 14.1. This list is updated from year to year as new diseases arise or become more important (e.g. SARS). Standard case definitions for all notifiable diseases have been developed and were adopted in 2004.

A number of additional diseases are required to be reported in the different states. These are conditions or diseases that occur in certain regions—for example, melioidosis in the Northern Territory—or that have significant implications for public health in that region. When the diagnosis can be made largely by symptoms, the responsibility for notification rests with the general practitioner. Diagnostic microbiology and serology laboratories are required

TABLE 14.1 Nationally notifiable diseases in Australia

Acquired immunodeficiency syndrome (AIDS)
Anthrax
Arbovirus infection—not otherwise classified
Australian bat lyssavirus
Barmah Forest virus infection
Botulism
Brucellosis
Campylobacteriosis (not in NSW)
Chlamydial infection
Cholera
Creutzfeldt-Jakob disease (CJD)
Creutzfeldt-Jakob disease—variant (vCJD)
Cryptosporidiosis
Dengue virus infection
Diphtheria
Donovanosis
Gonococcal infection
Haemolytic uraemic syndrome (HUS)
Haemophilus influenzae serotype b (Hib) (invasive only)
Hepatitis A
Hepatitis B
Hepatitis C/Hepatitis D
Hepatitis E
Human immunodeficiency virus (HIV) infection
Influenza laboratory-confirmed
Japanese encephalitis virus infection
Kunjin virus infection
Legionellosis
Leprosy (Hansen's disease)
Leptospiriosis
Listeriosis
Lyssavirus (NEC)
Malaria
Measles
Meningococcal infection
Mumps
Murray Valley encephalitis virus
Ornithosis
Pertussis (whooping cough)
Plague
Pneumococcal disease (invasive)
Poliomyelitis—wild type and vaccine-associated
Q fever
Rabies
Ross River virus infection
Rubella and congenital rubella syndrome
Salmonellosis
Severe acute respiratory syndrome (SARS)
Shiga toxin- and verocytotoxin-producing *Escherichia coli* (STEC/VTEC)
Shigellosis
Smallpox
Syphilis and congenital syphilis
Tetanus
Tuberculosis
Tularaemia
Typhoid
Varicella zoster (chickenpox)
Varicella zoster (shingles)
Viral haemorrhagic fevers (quarantinable)
Yellow fever

to notify any cases of communicable diseases that they identify. The chief executive officers of hospitals and general managers of health services have the responsibility of coordinating notifications of diseases in hospitalised patients, although, in practice, this often involves direct notification from a hospital doctor or laboratory to the state or territory surveillance centre.

The data collected for each notification include a unique identification number, the state or territory, the disease code, sex and age of the patient, date of onset, date of notification, and, where possible, Aboriginality or ethnicity and postcode of residence. This information is important for epidemiological studies and for identification of epidemics. The incidence of notifiable diseases is updated regularly on the *Communicable Diseases Intelligence (CDI)* website (go to <www.health.gov.au> and follow links to communicable diseases), and a quarterly review with case reports and comments on outbreaks of disease is published online. The data are analysed on the basis of age, sex, geographical distribution and seasonal variation, and provide a valuable resource for health practitioners.

Notification data compiled by the NNDSS should be interpreted with some caution, as they are influenced by a number of factors. For example, diagnostic laboratories are not always able to distinguish between incidence and prevalence statistics (see Chapter 8)—they may test the same patient more than once and record the disease each time they carry out the test, even though only one patient is involved. A disease that is rare or severe is more likely to be notified. A proportion of cases of some diseases, such as rubella or hepatitis C, may be asymptomatic or too mild to seek medical attention and so the number of cases reported may underestimate the true incidence. Gastrointestinal diseases are always underreported as many patients do not seek medical attention. Reporting procedures vary somewhat from state to state—for example, *Campylobacter* and chickenpox are not notifiable in New South Wales.

As well as the NNDSS, a number of other surveillance schemes are coordinated through *CDI*. Reports are published regularly on the health department website. They include:

- National Influenza Surveillance Scheme
- Australian Gonococcal Surveillance Program
- Sentinel Chicken Surveillance (for early detection of arbovirus outbreaks)
- Virology and Serology Laboratory Reporting Scheme (LABVISE), which records the incidence of diseases that are diagnosed by serological techniques
- Australian Paediatric Surveillance Unit (APSU), which carries out surveillance of rare childhood diseases
- National Enteric Pathogens Surveillance System
- Australian Tuberculosis Reporting System, conducted by the Australian Mycobacterial Reference Laboratory Network (AMRLN)
- National Neisseria Network, which examines cases of invasive meningococcal disease
- HIV and AIDS surveillance, coordinated by the Kirby Institute at the University of NSW (previously the National Centre for HIV Epidemiology and Clinical Research, NCHECR)
- Australian National Creutzfeldt Jakob disease registry (ANCJDR) (since 2003)
- OzFoodNet, which was established in 2000 as a collaborative project between federal, state and territory authorities, academic institutions, CDNA and the National Centre for Epidemiology and Population Health. Its aim is to improve surveillance and carry out research on food-borne diseases.
- The Australian Commission on Safety and Quality in Health Care (ACSQHC) was established in 2006 to improve safety and the quality of healthcare in Australia.
- In recent years there has been an increase in the community in the number of isolates of drug-resistant strains of *Mycobacterium tuberculosis* (TB), *Streptococcus pneumoniae* (pneumococcal disease) and *Staphylococcus aureus* (CA-MRSA). Surveillance and reporting of the occurrence of antimicrobial resistance is needed in order to control these infections, as well as monitoring the usage of and compliance with antimicrobial drugs (see Chapter 12).

It is important to be able to detect changes in the pattern of infectious diseases and implement control measures. The ability to collect data and disseminate information rapidly via an electronic medium (website) is an indication of the technological advances available for use in public health strategies in the 21st century.

INFECTIOUS DISEASES IN AUSTRALIA

Data in this section are extracted (with permission) from the *CDI* reports. Although the total numbers vary from year to year, reflecting seasonal outbreaks, the pattern of distribution is fairly constant. Table 14.2 shows the total number of notifications for the most common diseases in each state for 2009, together with the national five-year mean for each disease. The last column shows the ratio of incidence of the disease in 2009 to the five-year mean and is a useful guide to changes in incidence. For example, the ratio of chlamydia infections is 1.3, reflecting a continuing increase in chlamydia infections (see Figure 14.3, page 328). These figures provide an overview of the major infectious diseases and their distribution in Australia. However, 2009 was the most severe influenza season since recording started in 2001—the number of cases being ten times the average for the previous five years. This was due mainly to the epidemic of the new H1N1 strain of influenza ('swine flu'). The number of cases of pertussis was three times the average for the previous five years, reflecting a periodic epidemic cycle.

The most recent data and notification rates for diseases in various areas can be accessed on the health department website. The notification rates show wide differences in the occurrence of some diseases between different states and within different population groups, especially between

TABLE 14.2 Number of notifications of selected diseases received from state and territory health authorities, 2009

	ACT	NSW	NT	QLD	SA	TAS	VIC	WA	AUST-RALIA	LAST 5 YEARS MEAN	RATIO**
BLOOD-BORNE DISEASES											
Hepatitis B (newly acquired)	5	36	4	49	9	8	88	39	238	276	0.9
Hepatitis B (unspecified)	101	2 651	152	1 022	447	77	1 948	709	7 107	6 298	1.1
Hepatitis C (newly acquired)	7	41	5	NN	45	21	188	94	401	404	1.0
Hepatitis C (unspecified)	158	3 913	160	2 709	503	262	2 624	1 054	11 081	11 815	0.9
Hepatitis D	0	9	0	13	0	0	12	0	34	33	1.0
HIV*	12	351	6	242	41	10	280	101	1 043	1 035	1.0
GASTROINTESTINAL DISEASES											
Campylobacteriosis	357	NN†	205	4 610	1 755	626	5 838	2 582	15 973	16 004	1.0
Cryptosporidiosis	106	1 463	150	1 460	106	66	1 039	235	4 625	2 580	1.8
Haemolytic uraemic syndrome	0	4	0	2	4	0	2	0	12	20	0.6
Hepatitis A	6	98	1	56	59	5	303	35	563	274	2.1
Hepatitis E	0	17	0	3	0	0	8	5	33	29	1.1
Listeriosis	2	26	0	14	4	3	27	15	91	60	1.5
STEC, VTEC	0	21	1	23	62	0	16	6	130	83	1.6
Salmonellosis (NEC)	225	2 736	487	2 471	681	166	1 647	1 120	9 533	8 469	1.1
Shigellosis	8	156	85	115	51	2	85	120	622	645	1.0
Typhoid	2	47	0	13	2	1	42	8	115	79	1.5
OTHER BACTERIAL INFECTIONS											
Legionellosis	4	94	3	56	44	0	50	51	302	314	1.0
Meningococcal infection	2	96	6	60	22	3	42	28	259	341	0.8
Tuberculosis	23	488	28	218	58	9	419	112	1 355	1 135	1.2
SEXUALLY TRANSMISSIBLE INFECTIONS											
Chlamydial infection	941	14 948	2 115	16 721	3 757	1 453	13 889	8 836	62 660	47 069	1.3
Gonococcal infection	55	1 655	1 504	1 570	400	21	1 515	1 339	8 059	7 829	1.0
Syphilis—all	33	910	137	475	53	28	858	182	2 676	2 325	1.2
Syphilis < 2 years	11	522	38	179	53	10	390	88	1 291	977	1.3
Syphilis > 2 years or unspecified duration	22	788	99	296	NN	18	468	94	1 385	1 348	1.0
VACCINE-PREVENTABLE DISEASES											
Diphtheria	0	0	0	0	0	0	0	0	0	0	Nil
Haemophilus influenzae type b (Hib)	0	6	0	6	1	0	2	4	19	19	1.0
Influenza (laboratory confirmed)	1 259	12 393	1 967	18 363	10 752	1 305	6 990	5 533	58 562	5 904	10.0
Measles	1	19	1	32	3	2	36	10	104	51	2.0
Mumps	0	40	13	34	22	1	45	20	165	297	0.6
Pertussis	351	12 436	215	6 216	5 346	616	3 778	778	29 736	9 764	3.0
Pneumococcal disease (invasive)	29	477	86	270	145	35	368	149	1 559	1 726	0.9
Poliomyelitis	0	0	0	0	0	0	0	0	0	0	Nil
Rubella	0	7	0	6	3	0	6	5	27	38	0.7
Tetanus	0	2	0	0	0	0	1	0	3	3.6	0.8
Varicella zoster (chickenpox)	2	NN†	87	153	475	34	530	318	1 599	1 260	1.3
Varicella zoster (shingles)	12	NN†	112	25	1 045	117	575	539	2 659	1 242	2.1
Varicella zoster (unspecified)	66	NN†	3	38 359	280	80	1 847	866	6 977	3 130	2.2
VECTOR-BORNE DISEASES											
Arbovirus infection (NEC)	0	0	0	23	0	0	3	0	26	35	0.7
Barmah Forest virus infection	3	359	117	799	36	3	15	154	1 486	1 673	0.9
Dengue virus infection	17	132	27	1 036	17	2	38	133	1 402	327	4.3
Malaria	3	97	14	185	32	5	113	82	526	637	0.9
Murray Valley encephalitis virus infection	0	0	1	1	0	0	0	2	4	1.2	3.3
Ross River virus infection	2	912	427	2 154	326	29	85	851	4 786	4 428	1.1
ZOONOSES											
Brucellosis	0	4	0	22	2	0	3	1	32	43	0.7
Leptospirosis	2	18	4	110	0	0	11	1	146	134	1.1
Ornithosis	0	22	0	0	3	0	38	2	65	152	0.4
Q fever	0	139	3	131	9	0	25	2	309	409	0.8

Source: NNDSS, *Communicable Diseases Intelligence*. Kirby Institute report 2011, <www.med.unsw.edu.au/nchecrweb.nsf/>.
Notes: * HIV figures are for 2010 and include cases diagnosed in Australia and overseas. ** Ratio: The ratio of Australian total to the mean of previous five years, equivalent period.
† NN = Not notifiable.

Indigenous and non-Indigenous people. The highest *rate* of notifications (i.e. notifications per 100 000 population) comes from the Northern Territory, which highlights the high incidence of communicable diseases in the susceptible Indigenous population in that part of Australia. This is discussed later in this chapter. Changes from year to year in the number of notifications of certain diseases may reflect a change in reporting procedures rather than a true change in incidence. The introduction of a vaccine usually has a dramatic effect on the incidence of a disease (e.g. *Haemophilus influenzae*, or Hib—see Figure 14.11, page 337).

Australia is free of some of the diseases (such as malaria) that present major problems in other parts of the world. There are a number of reasons for this. In some cases, it is because there are no reservoirs of infection for the microorganisms, or because the appropriate vector is not found in Australia. In addition, Australia has strict quarantine laws aimed at keeping infections, especially exotic diseases, out of the country. This situation may change with increasing global travel. For example, the number of cases of dengue fever has varied from year to year (see Figure 14.1), but in each outbreak the index case was identified as an overseas traveller, usually from South-East Asia. In 2000, there were several cases of dengue fever in soldiers returning from peacekeeping duties in East Timor. In February and March of 2003 there were nearly 500 confirmed cases of dengue fever around Cairns. The index case was a woman who had been to Papua New Guinea. In 2009 there was a fourfold increase in notifications compared to the five-year average, 66 per cent of which were locally acquired. Local transmission mainly occurs in Far North Queensland. Recent data show that all four types of dengue virus have now been identified in Australia. There was one death from dengue fever in 2009.

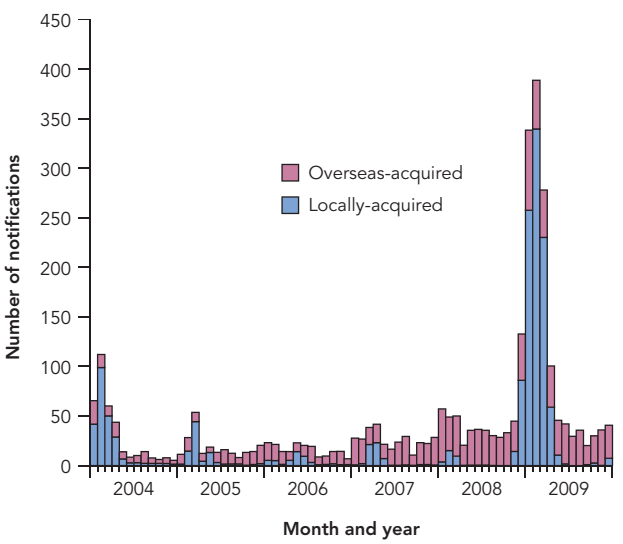

FIGURE 14.1

Notifications of dengue virus infections, Australia, 2004–09, by month and year of diagnosis

Source: National Notifiable Diseases Surveillance System (NNDSS) 2011, *Communicable Diseases Intelligence* 35(2): 65.

Notifications of malaria usually occur in people who have contracted the disease outside Australia. However, in 2002, an outbreak of malaria affecting ten people in Far North Queensland involved local transmission of *Plasmodium vivax*. The index case was identified as an overseas traveller. A few cases of Japanese encephalitis and chikungunya virus have been reported, all acquired overseas. These occurrences highlight the need for good preventive measures. The appropriate mosquito vector for each of these diseases occurs in Australia, so they have the potential to become established here. Of the 115 cases of typhoid reported in 2009, 88 per cent were acquired overseas, with India being responsible for 62 per cent of cases. There have been no reported cases of rabies, plague, botulism, yellow fever or other haemorrhagic fevers in Australia in recent years. However, the first case of poliomyelitis since 1978 was reported in Melbourne in 2007 in a student from Pakistan. This event reinforces the need for everyone to be vaccinated. (See the section on herd immunity later in this chapter.)

The *CDI* data reveal marked differences between the incidence of various diseases in different parts of Australia. Health workers should be aware that, if they choose to work in rural or remote areas, the diseases and health problems they are likely to encounter will be quite different from those in a big city hospital. For example, the Northern Territory has diseases such as melioidosis and donovanosis, which are rarely seen in the southern states. Diseases such as hydatids (dog tapeworm) are seen mainly in rural areas where dogs are fed raw offal; and Q fever, a rickettsial disease carried by ticks, occurs mainly in cattle-raising areas. Leptospirosis occurs mainly in rural Victoria, Queensland and the Northern Territory (see Figure 14.23, page 353).

The distribution of diseases is also influenced by climate and environmental factors. Where a vector such as a tick or mosquito is required for transmission, the disease is confined to areas where the vector is found. In addition, seasonal variations occur, often linked to breeding seasons for vectors such as mosquitoes. For example, Ross River fever occurs in most parts of the country, with the highest rates in north Queensland, and is most common in late summer or after a period of heavy rain and flooding when the mosquito population is high (see Spotlight box: Arboviruses endemic to Australia, page 355).

ANALYSIS OF NOTIFICATION RATES

Figure 14.2 illustrates the incidence of the major groups of notifiable diseases. In 2009, 43 per cent of notifications were for vaccine-preventable diseases. This unusually high percentage was due to the fact that there was a tenfold increase in the incidence of influenza, following the appearance of the new H1N1 'swine flu' strain of influenza A. Generally, the highest numbers of notifications are due to sexually transmitted diseases.

Vaccine-preventable diseases

A number of diseases are preventable by vaccination, including the so-called childhood diseases and diseases such

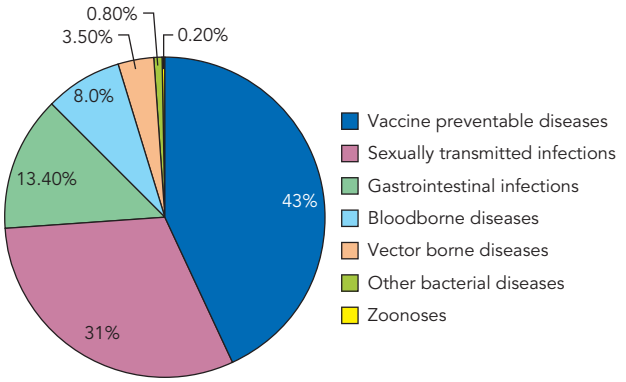

FIGURE 14.2

Notifications to the National Notifiable Disease Surveillance System, Australia, 2009, by disease category

Source: Figure derived from NNDSS 2009, *Communicable Diseases Intelligence* 35(2): 65, Table 3.

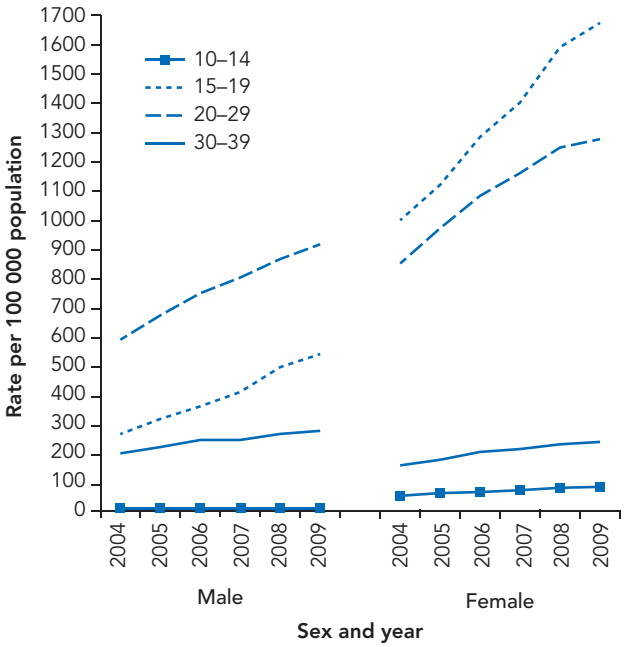

FIGURE 14.3

Trends in notification rates of chlamydial infections in persons aged 10–39 years, Australia, 2004–09, by age group and sex

Source: NNDSS 2011, *Communicable Diseases Intelligence* 35(2): 93, Figure 26.

as influenza, but a significant number of cases still occur, sometimes with serious outcomes. The actual incidence of vaccine-preventable diseases varies from year to year, but it is unacceptably high in a country like Australia where a free childhood vaccination program is available. The addition of meningococcal, pneumococcal, chickenpox and rotavirus vaccines to the recommended immunisation schedule for children has led to a lowering of the number of cases of these diseases (see Figures 14.8 and 14.10, pages 336 and 337). However, a number of people refuse to have their children vaccinated, which puts not only their own children at risk, but also other children who are too young to have been vaccinated. (See the section on herd immunity, below.)

Sexually transmitted infections (STIs)

Usually the highest number of notifications of infectious diseases in Australia is for the sexually transmissible infections (STIs), chlamydia, gonorrhoea and syphilis. There has been an apparent increase in STIs in recent years. This may reflect the use of more sensitive detection methods (for chlamydia) and also improved reporting procedures. Nationally, the trend in rates of notification for chlamydia shows a steady increase in all age groups: there were over 62 000 notifications in 2009, up from 52 000 in 2007 (see Figure 14.3). The increase in chlamydia infections, especially in young women, is a cause for concern as it is associated with pelvic inflammatory disease (PID), which can result in blockage of the fallopian tubes and infertility.

The highest *rate* of STI notifications is in the Northern Territory and the Kimberley region of Western Australia. In these regions, as in previous years, the recorded incidence of chlamydia is about four times the average for the whole of the Australian population; gonorrhoea is 18 times, and syphilis about 5 times, the national average. STIs in the Indigenous population are a contributing factor to other illnesses (see page 351).

Blood-borne diseases

Incidence of HIV/AIDS

HIV testing began in Australia in 1985. From 1985 to 2000 the number of newly diagnosed HIV infections in Australia declined from about 1700 in 1985 to about 700 in 2000 (see Table 14.2 and Figure 14.4). By 2010 a total of 30 486 cases of HIV had been diagnosed in Australia. After reaching a low in 2000, there was a gradual increase in the number of cases, which has now steadied at around 1000 new cases each year. The change in numbers occurred only among men; the incidence in females remains constant at 60–80 per year (8 per cent). Men with a history of sexual contact with other men account for the majority of HIV infections (see Figure 14.5). Transmission in the heterosexual community is usually associated with injecting drug use. More than 50 per cent of heterosexual transmissions in Australia occur when the partner or sexual contact is from a high-incidence country—for example, sub-Saharan Africa. In the Indigenous community, heterosexual contact is responsible for about 20 per cent of transmissions, injecting drug use for 19 per cent, and 21 per cent of infections are in women (see Figure 14.21). Current data on the incidence of HIV infections and AIDS are accessible on the National Centre for HIV Epidemiology and Research website: <www.med.unsw.edu.au/nchecr>.

A massive public education campaign and the introduction of needle exchange programs has controlled the spread of HIV in Australia up until now. The availability of a number of new anti-HIV drugs, which have been effective in

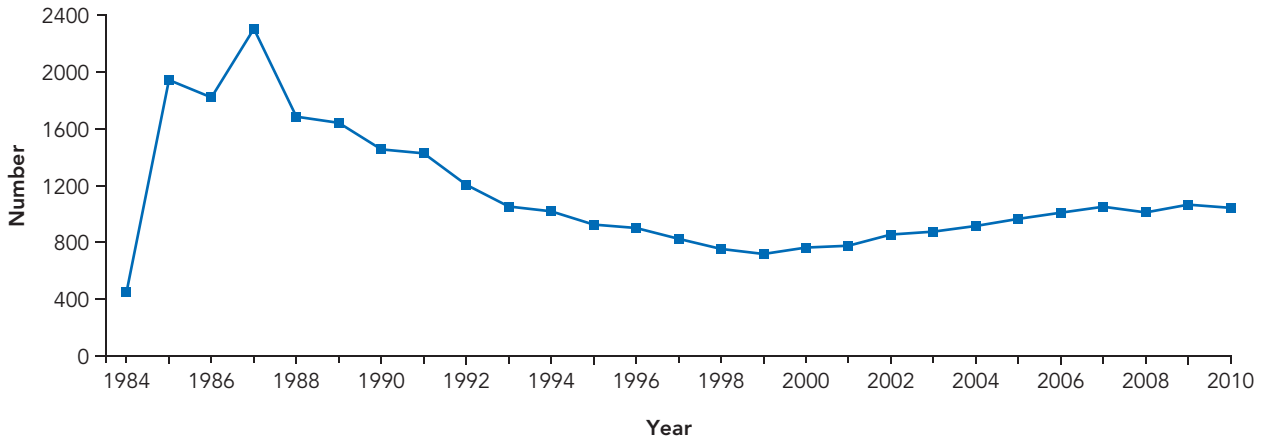

FIGURE 14.4

Diagnoses of HIV infection and AIDS in Australia

Source: The Kirby Institute. HIV, *Viral Hepatitis and Sexually Transmissible Infections in Australia Annual Surveillance Report 2011*. The Kirby Institute, University of New South Wales, Sydney, NSW.

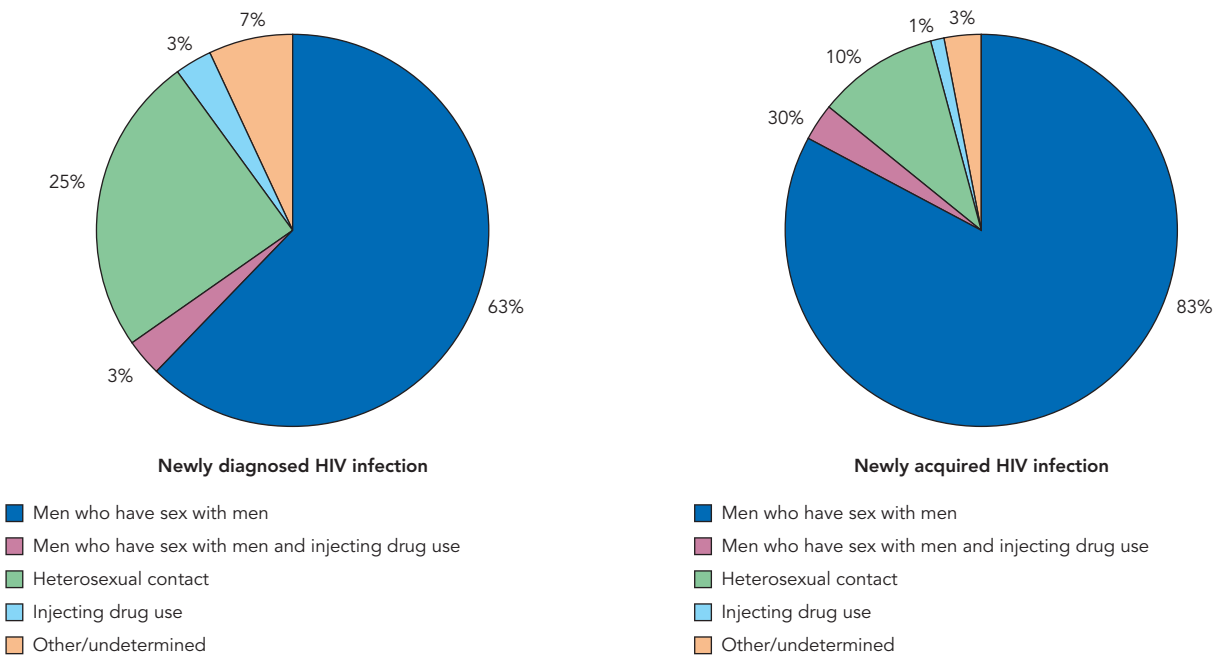

FIGURE 14.5

Newly diagnosed HIV infection and newly acquired HIV infection, 2006–10, by HIV exposure category

Source: The Kirby Institute. *HIV, Viral Hepatitis and Sexually Transmissible Infections in Australia Annual Surveillance Report 2011*. The Kirby Institute, University of New South Wales, Sydney, NSW.

reducing viral load and illness in people with HIV infection, has also contributed to a reduction in the progression of the disease to AIDS. The annual number of AIDS diagnoses in Australia declined from 395 in 1997 to 213 in 2001, and has remained relatively stable over the past five years at around 240 per year.

Although HIV is a notifiable disease, people who are infected are often asymptomatic for a long time and testing is voluntary, so the true incidence is not known. A worrying aspect of the spread of HIV is that the incidence in surrounding Asian countries is much higher than in Australia and heterosexual transmission is common in the sex industry there. Holidaymakers and travellers risk importing the disease to Australia (see Figure 14.6).

Viral hepatitis

Hepatitis B is transmitted in a similar way to HIV—in blood, body secretions, sexually, by vertical transmission mother to baby, and through parental exposure such as intravenous drug use and tattooing. A vaccine for hepatitis B has been

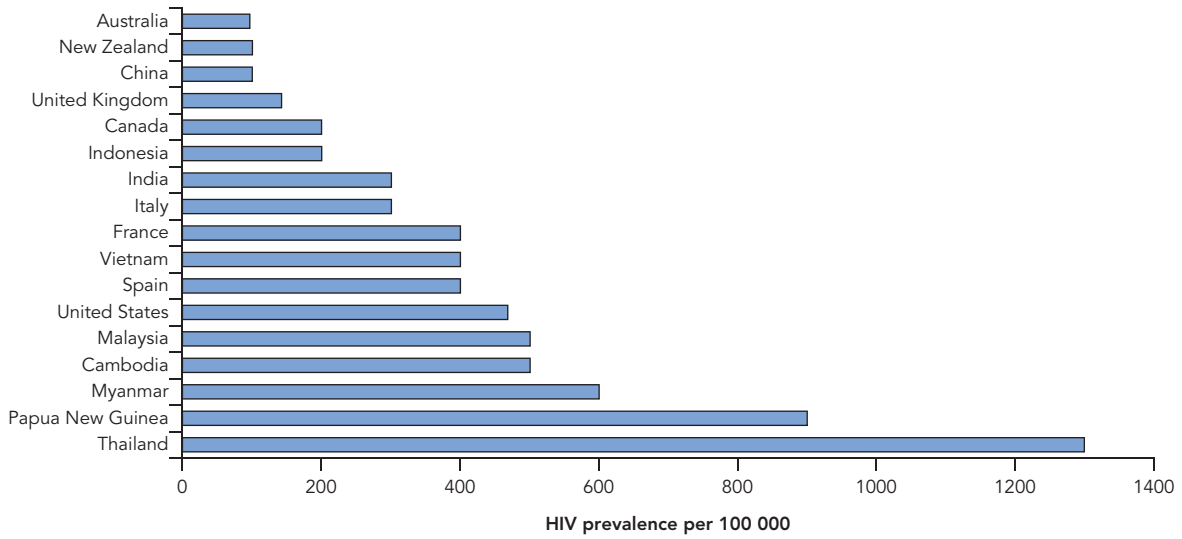

FIGURE 14.6

HIV prevalence in the population aged 10–49 years, in selected countries

Source: The Kirby Institute. *HIV, Viral Hepatitis and Sexually Transmissible Infections in Australia Annual Surveillance Report 2011*. The Kirby Institute, University of New South Wales, Sydney, NSW.

available for some years and is included in the childhood immunisation schedule.

Hepatitis C is a different virus that also attacks the liver (see Chapter 18, page 470). There is no vaccine for the disease. It is acquired through exposure to contaminated blood, via intravenous drug use, tattooing or needlestick injuries. The risk of sexual or vertical transmission is low. The vast majority of diagnoses of newly acquired hepatitis C infection occur among injecting drug users. The rate has declined to 52 per 100 000 of population and is highest in adult injecting drug users in the 20–29 age group. In 2010, 297 000 people in Australia had been exposed to hepatitis C, 76 000 had cleared the infection, and 168 000 had chronic infection and early liver disease. The needle exchange program to prevent the spread of AIDS in Australia has the added benefit of providing protection against hepatitis B and C.

Food-borne illness and gastrointestinal infections

The WHO recognises the surveillance of food-borne disease as being an essential tool to help reduce illness related to food. OzFoodNet publishes regular reports of outbreaks of food-borne disease on the CDI website with the aim of improving our understanding of the epidemiology of food-borne disease, through surveillance and special studies of food-borne pathogens. It also is responsible for assessing the efficacy of current and proposed food hygiene standards and their enforcement. Analysis of data on food-borne diseases in Australia enables the network to identify outbreaks linked to particular foods, and advise health departments on the detection of problems with food or water safety. Food safety also depends on the implementation of quality assurance and control programs in the food industry, based on Hazard Analysis Critical Control Point (HACCP) principles.

It is estimated that over 5 million people in Australia experience a food-borne illness every year. Although most cases of infection are not reported, the cost to the economy is significant in terms of lost work days. Health departments use surveillance to detect outbreaks, and to monitor trends and the effect of intervention strategies. An outbreak is defined as two or more people with a particular infection or illness associated with a common food or meal. Gastrointestinal infections notified to authorities represent only a small proportion of those occurring in the community, as most infections are mild and self-limiting. Food-borne illnesses due to *Salmonella*, *Campylobacter*, hepatitis A, *Shigella*, *Listeria* and toxigenic *Escherichia coli* are notifiable. *Campylobacter* is the most commonly reported food-borne pathogen in Australia, but OzFoodNet reports that *Salmonella* is responsible for most outbreaks of **gastroenteritis**. *S. typhimurium* is the most common serotype reported in Australia; *S. enteriditis* is mainly acquired overseas, especially in the United States. The highest incidence of shigellosis is in Indigenous people, especially in the Northern Territory. *Listeria* infections are not common but can have serious outcomes. Most cases of non-pregnancy-associated listeriosis occur in people who are elderly or immunocompromised, and some of these are fatal. In 2006 there were eight cases of listeriosis in pregnant women; in two cases the infant died.

A wide variety of different foods have been implicated in food-borne illness—very often transmission of infection is due to poor hygiene practice by a food handler who is carrying an infection (e.g. norovirus). However, some foods are more likely to be contaminated naturally—for example, chicken and egg products (such as raw egg custards and mayonnaise) with *Salmonella*; unpasteurised cheese and meat

products with *Listeria.* Food-borne illnesses are increasing with the growing use of ready prepared or takeaway food. Health departments set standards for food preparation and monitor food outlets to prevent outbreaks of gastroenteritis. An increasing number of outbreaks are associated with fresh food and salad vegetables (cooking destroys most pathogens). Fruit and vegetables need to be properly washed before eating and not allowed to come in contact with uncooked meats and chicken. In 2009, food prepared in restaurants was most commonly associated with outbreaks (39 per cent of cases), followed by aged-care facilities (12 per cent) and commercial caterers (12 per cent).

There is also the potential for food-borne diseases to be imported and to spread nationally. For example, in 2006 three cases of cholera occurred in elderly women who ate raw whitebait imported from Indonesia; in 2007 there was an outbreak of shigellosis associated with baby corn from Thailand (see Case History 18.3, page 451).

PRIMARY HEALTHCARE

Primary healthcare, in its broadest sense, is a philosophy of promoting equal healthcare at all levels of the social system. It involves the provision of services on the basis of the needs of the population, the provision of health education, and the development of a balanced system of health promotion, disease prevention and treatment of illness.

Despite an overall high standard of health in Australia, there are disadvantaged groups of people in the community whose health status is poor. For example, Aborigines and Torres Strait Islanders have a much lower general standard of health than other Australians. The infectious diseases that affect Indigenous people are examined later in this chapter.

Many of the factors that influence health are socioeconomic. Factors such as marital status, sex, ethnicity, level of education and place of dwelling (urban or rural) can have a significant impact on health. Social circumstances that can cause inequalities in health status include:

- level of education
- level of economic resources
- living conditions, including quality of housing, clean water supply, air pollution, hazardous environments
- working conditions
- availability of social support.

Numerous studies show that people in the lowest socioeconomic groups, with low levels of education, have the poorest health. They tend to delay seeking treatment, so their illnesses have often become more severe by the time they present to the hospital or health service. Other untreated, underlying illnesses often contribute to morbidity. They are also less likely to seek out preventive services such as screening and immunisation.

Two aspects of primary healthcare that can directly affect the level of infectious diseases in the community are immunisation and preventive screening programs. Other activities of primary healthcare are beyond the scope of this text, but should be kept in mind as being essential for good health. They include:

- basic education, including health education
- provision of adequate food and nutrition
- provision of a safe water supply and proper sanitation
- control of endemic diseases
- provision of maternal and child care, and family planning information
- provision of appropriate medication and treatment.

The changing lifestyle in Australia, with many women in the workforce, has meant that about 1 million children aged 0–5 years are in pre-school and long-daycare centres. These pose special problems in infection control and are discussed in a later section of this chapter.

SCREENING PROCEDURES

An important aspect of primary healthcare is the implementation of screening programs for the detection of infectious diseases. Early detection often allows intervention, which can reduce or prevent morbidity and mortality.

Antenatal screening tests

Among the most useful screening programs are antenatal screening tests. Some infections that are transmitted from mother to baby are not apparent in the mother and can affect the outcome of the pregnancy. If infection can be detected before or during pregnancy, appropriate treatment can be given. For most diseases, the presence of specific IgG antibodies alone indicates past infection, and the absence of IgG antibodies indicates susceptibility. The presence of specific IgM antibodies suggests recent infection.

Routine screening is only recommended if there is a suitable sensitive test available and there is significant risk of damage to the foetus or infant *that is preventable*.

One of the most important screening procedures is the investigation of pregnant women to determine their status with respect to immunity to rubella. Rubella is a mild disease in adults, but is known to cause serious congenital defects if contracted during the first trimester of pregnancy. Serological screening before pregnancy to detect the presence or absence of rubella antibodies is used to determine the woman's susceptibility to infection so that vaccination can be offered. It identifies women who are not immune and who should therefore be advised to avoid exposure to people who have the disease. Measurement of the woman's immune status also enables the risk to the foetus from exposure to rubella during pregnancy to be determined. If exposure occurs during pregnancy, the measurement of IgG and IgM antibodies can assist in counselling. Vaccination of pregnant women is not advised, but postnatal immunisation is offered to women who are not immune. Mass immunisation of the whole population with MMR vaccine at 12 months and again at 4 years of age has greatly reduced the incidence of congenital rubella syndrome.

Screening for syphilis is also recommended. If syphilis infection is detected early during pregnancy, treatment

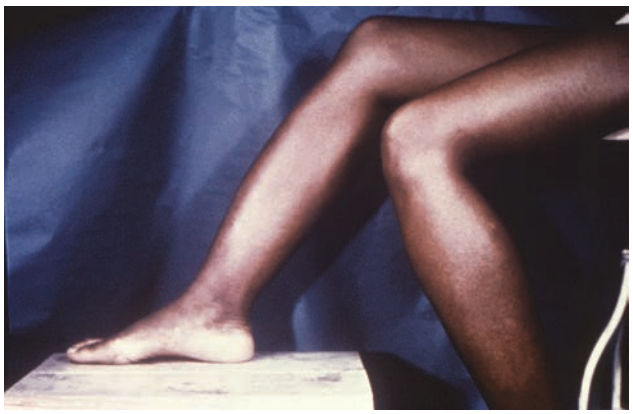

FIGURE 14.7

Bone deformity due to congenital syphilis because mother did not receive treatment in pregnancy

Source: CDC/Susan Lindsley.

with antibiotics (penicillin) can be given to the mother to prevent birth defects, which do not usually occur until after 20 weeks' gestation (see Figure 14.7).

In some cases the presence of antigens or antibodies indicates chronic infection—for example, hepatitis B (HBV). Mothers who are chronic hepatitis B carriers have a high risk of their baby being infected at term or during delivery. Babies who are likely to be exposed to the virus can be identified by maternal screening during pregnancy, allowing prophylactic treatment to be given. The baby is given anti-hepatitis B immunoglobulin at birth, followed by a course of vaccination. This treatment breaks the chain of vertical transmission.

Mothers infected with human immunodeficiency virus (HIV) have a high risk of passing the infection to the baby. However, treatment with combined antiretroviral therapy (HAART) can lower the viral load in the blood and reduce the risk of transmission to less than 2 per cent.

Babies are also at risk of infection during passage through the birth canal from organisms present in the vagina. Of these, Group B streptococci (GBS) are the most common cause of neonatal sepsis (about 1–2 cases per 1000 live births). Detection of GBS in the vagina by screening at 26–28 weeks allows for intra-partum antibacterial prophylaxis for high-risk women (administration of antibacterials just before and during labour). Genital herpes infection can cause serious problems if passed to the neonate during birth. This can be avoided if the mother is screened for STIs and given appropriate treatment (antiviral medication and/or caesarean section).

Tuberculosis

The **Tuberculin skin test** (**TT**), or **Mantoux test** (**Mx**), consists of a simple skin test to determine whether a person has been exposed to and infected by the tubercle bacillus. It does not always reveal the state of the infection but allows follow-up investigations to be carried out. The test looks for a delayed hypersensitivity reaction to the intradermal injection of a small dose of purified protein from *Mycobacterium tuberculosis*. The injection site is examined after 48–72 hours and the diameter (in mm) of the resulting **induration** is measured as an estimate of the strength of the reaction.

A Mantoux test may be positive if the person has a latent infection or active disease, has recovered from active disease, or has been vaccinated against tuberculosis with the BCG (Bacille Calmette et Guerin) vaccine. It may also give a positive result if the person has been infected with a non-tuberculous mycobacterium (e.g. leprosy). Most Mantoux-positive people have a latent infection and do *not* have active disease when investigated further.

Preventive treatment may be offered to those with a latent infection. Individuals who are infected with the human immunodeficiency virus have a high risk of reactivation of a latent TB infection.

Screening of blood products

In Australia all blood and blood products supplied by the Red Cross blood bank, as well as organs donated for transplant, are screened for the presence of infectious agents. These include the human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV), cytomegalovirus (CMV), *Treponema pallidum* (syphilis) and human T cell leukaemia virus I (HTLVI). Blood is not accepted from donors who are classified as 'high-risk' on the basis of their medical history or sexual preference, who are intravenous drug users or who have had previous blood transfusions. A ban has been placed on blood or organ donation by people who have lived for a cumulative period of more than six months in Britain between 1980 and 1996, because of fears of transmission of variant Creutzfeldt-Jakob disease. A period of exclusion applies to donations from people with a recent history of travel to countries where diseases such as malaria are endemic.

Although there is always the possibility of as yet undiscovered viruses or infectious agents being present, current screening procedures ensure that the supply of blood and blood products is as safe as possible.

Cervical cancer detection

Sexually mature women are advised to have regular cervical smear tests (Pap smears) to detect any changes in the cells of the cervix which may lead to invasive carcinoma. Although this is not strictly an infection, most cases of cervical cancer have been associated with prior infection by certain types of the human papillomavirus (HPV). Regular screening can detect early cellular changes so that treatment can be given to minimise the risk of progression to cancer (see Case History 5.2: Cervical cancer, Chapter 5, page 100). A vaccine for HPV is now recommended for 13-year-old girls and should eventually reduce the risk of cervical cancer due to HPV. However, older women should continue to have regular Pap tests.

IMMUNISATION

The development of vaccines and the implementation of immunisation programs were among the major reasons for the improvement in health during the 20th century. One

of the great success stories of vaccination campaigns is the worldwide eradication of the dreaded disease, smallpox; and in many developed countries, polio has been virtually eliminated. During the polio epidemics in Australia in the 1940s and 1950s, there were up to 1000 cases of paralytic polio disease and 100 deaths each year. The introduction of the **Salk vaccine** (a killed preparation) and later the **Sabin vaccine** (an attenuated virus) saw a dramatic fall in the occurrence of the disease. The last recorded case of polio acquired in Australia was in 1976, and in 2000 Australia was declared free of endemic polio. Until the widespread introduction of childhood immunisation, many children died from infectious diseases. However, the last 50 years has seen a dramatic decrease in infant mortality due to vaccine-preventable diseases (see Table 14.3).

The principles behind the development of immunity to an infectious disease are fully described in Chapter 9. To summarise: when a person is exposed to a foreign organism, the body responds by producing antibodies, activated lymphocytes and long-term memory cells against that organism. If the person is subsequently exposed to the same organism, the preformed antibodies and memory cells (which produce more antibodies and activated T lymphocytes) combine to kill or remove the invading organism before disease can occur. The person is then said to be *immune* to that disease. Immunity is specific for each disease and sometimes even for each different strain of a pathogenic organism. Thus, if the organism is slightly different (due to a mutation, such as occurs with the influenza virus), then the person may not be immune to the altered form of the pathogen.

The possibility of producing immunity by artificial means was recognised as far back as 1796 when Edward Jenner noticed that milkmaids who had suffered from cowpox, a mild disease with lesions similar to smallpox, appeared to be immune to smallpox. He first used a preparation derived from cowpox (caused by *Vaccinia* virus, hence the word 'vaccine') to immunise people successfully against smallpox. The milder cowpox virus induced immunity to smallpox.

Jenner's work laid the basis for our modern immunisation programs. In practice, the terms 'vaccination' and 'immunisation' are used interchangeably. However, **vaccination** is used by some people in a general sense to denote the administration of any vaccine without regard to whether the recipient is made immune. **Immunisation** is a more specific term, denoting the process of inducing or providing immunity artificially. It may be active or passive.

Active immunisation is the administration of a vaccine in order to stimulate the body's immune system to produce both specific antibodies and cellular immunity to the antigens contained in the vaccine. It takes several days for this process to occur and immunity to be developed.

Passive immunisation is the provision of temporary immunity by the administration of preformed antibodies derived from another person or animal. They may be in the form of pooled, non-specific human immunoglobulin, specific antitoxins (e.g. tetanus) or specific antibody preparations (e.g. hepatitis B immunoglobulin). These products are used mainly when a person has been accidentally exposed to a serious disease and requires immediate protection because they have no immunity. The antibodies circulate only for a short time (weeks to months) before being degraded by normal body processes.

Maternal antibodies that cross the placenta in the late stages of pregnancy are a natural type of passive immunisation which protects the infant from infection during the first months of life. At birth the neonate has a range of maternal antibodies which represent the exposure of the mother to previous infectious diseases. Premature infants (less than 32 weeks) have not yet received maternal antibodies transplacentally and so are at a significantly higher risk of infection.

TABLE 14.3 Number of deaths from diseases commonly vaccinated against, Australia, 1926–2004, by decade

PERIOD	DIPHTHERIA	PERTUSSIS	TETANUS	POLIOMYELITIS	MEASLES*	POPULATION ESTIMATED YEARLY AVERAGE (millions)
1926–35	4 073	2 808	879	430	1 102	6.6
1936–45	2 791	1 693	655	618	822	7.2
1946–55	624	429	625	1 013	495	8.6
1956–65	44	58	280	123	210	11.0
1966–75	11	22	82	2	146	13.75
1976–85	2	14	31	2	62	14.9
1986–95	2	9	21	0	32	17.3
1996–2004	0	17	6	0	0	19.2

Indicates decade in which community vaccination started for the disease.

Sources: B. Feery 1997, One hundred years of vaccination. *Public Health Bulletin* 8: 61–63; B. Feery 1981, Impact of immunisation on disease patterns in Australia. *Medical Journal of Australia* 2: 172–76. Deaths recorded for 1966–75 and 1996–2004 updated with data provided by AIHW Mortality Database.

Further passive protection is afforded by the antibodies present in colostrum and breast milk, especially against gastrointestinal infections.

As the immune system develops during the first year of life, the maternal antibodies are destroyed and the infant builds up its own immunity to the various microorganisms to which it is exposed. The development of protective immunological memory in early childhood can be stimulated by the administration of appropriate vaccine preparations, before significant risks of exposure to the infectious agents occur. This is called **artificial active immunity**.

Types of vaccines

Obviously, it would not be safe to administer live pathogens in order to promote an immune response. It has been necessary to develop suitable preparations which contain the specific immunogenic groups (antigens) of the pathogen but which are unable to cause disease (see Chapter 9). Table 9.4 lists the properties of vaccines in common use. Some (e.g. measles, mumps, rubella, Sabin polio, OPV) consist of live, attenuated (weakened) forms of the pathogen. They have the advantage of being able to multiply in the host, producing a high level (titre) of antibody formation from a single dose of vaccine, without producing disease. There is always a very slight risk that these preparations may revert to a virulent (disease-producing) strain, but this is extremely rare.

Toxoids are inactivated forms of bacterial toxins that retain their ability to induce immunity. Toxoids are used when the disease symptoms are due mainly to the formation of a toxin; for example, tetanus toxoid and diphtheria toxoid.

The safest vaccines are preparations that use only part of the pathogenic organism to induce effective immunity. They do not contain any nucleic acid, so replication (and disease) is not possible. For example, the vaccine for hepatitis B consists of the surface antigen HBsAg from the outer layer of the virus. It is produced by placing the viral gene for HBsAg into cells of the yeast *Saccharomyces cerevisiae*, inducing the synthesis of large quantities of the antigen, which is then extracted and purified for use in the vaccine.

The vaccine against *Haemophilus influenzae* type B (Hib) is a **conjugate vaccine**, consisting of the antigenic polysaccharide from Hib, bound to a protein carrier. The new pneumococcal vaccine suitable for children under 2 years of age is a polyvalent conjugated vaccine consisting of seven of the polysaccharide antigens from *Streptococcus pneumoniae* bound to a protein carrier. A cell-free pertussis (whooping cough) vaccine, which has fewer side effects than the old 'killed whole cell' preparation, is now used in Australia.

Vaccines containing non-replicating antigens require the administration of several doses to build up adequate levels of immunity. Theoretically, live vaccines should require only one dose, but some of the newer attenuated viral vaccines have only been available since the 1970s and are still being evaluated to determine how long the artificially acquired immunity will last. In some cases (e.g. rubella, hepatitis B), antibody titres are known to fall over time. Measurements of antibody titre at various time intervals after immunisation sometimes show wide variations among individuals, and it is not known whether this represents a lowered level of immunity.

At present, it is recommended that women intending to become pregnant should have their rubella antibody level checked; if seronegative they should be vaccinated or, if their antibody titre is low, have a booster dose of vaccine. This should be done at least two months before becoming pregnant. Since it is a live vaccine, its use close to or during pregnancy is not advised, although there are no reports of congenital abnormalities due to vaccine use.

Vaccination schedules

The Australian Health Department provides free vaccination for most childhood diseases. The current recommended childhood vaccination schedule is shown in Table 14.4. This is now the standard schedule Australia-wide. Different vaccine preparations are licensed in the various states and territories. For details of approved preparations and updates to the schedule, go to <www.immunise.health.gov.au>.

Highlights of the schedule

Some of the changes in the last few years include the replacement of whole cell pertussis vaccine with an acellular preparation, the inclusion of hepatitis B for all infants, and the introduction of meningococcal vaccine in 2003, pneumococcal vaccine and chickenpox (varicella) in 2005, rotavirus in 2007, and HPV for girls aged 12–13 in 2008.

Meningococcal disease

Meningococcal infections are caused by *Neisseria meningitidis*. There are 13 known serogroups distinguished by differences in the surface polysaccharides of the outer membrane capsule. Globally, serogroups A, B, C, W135 and Y most commonly cause disease. In Australia, serogroups B and C occur most frequently—approximately 32 per cent of cases are serogroup C and most of the remainder are Group B. The overall notification rate of meningococcal disease of both serogroups B and C to the National Notifiable Diseases Surveillance System increased continuously between 1991 and 2002. Since 2003 there has been a sustained decrease in the number of notifications, hospitalisations and deaths from meningococcal group C following the introduction of routine and catch-up vaccination programs for infants and those born after 1983 (see Figure 14.8). The new meningococcal vaccine, MenCCVs, is a conjugate vaccine that confers protection against serogroup C only, but it is more effective than previous vaccines and can be administered to children under 2 years of age. Approximately 70 per cent of notifications are now due to serogroup B. A vaccine for group B is currently being trialled overseas.

Pneumococcal disease

Streptococcus pneumoniae is a leading cause of otitis media, pneumonia, bacteraemia and meningitis, and is responsible for significant morbidity and mortality, especially in infants, the elderly and people with a predisposing illness. Invasive pneumococcal disease (IPD) occurs when the bacteria infect a normally sterile site. IPD is usually a disease of the very

TABLE 14.4 National Immunisation Program routine schedule of vaccines

AGE	VACCINE
Birth	■ Hepatitis B (hepB)[a]
2 months	■ Hepatitis B (hepB)[b] ■ Diphtheria, tetanus and acellular pertussis (DTPa) ■ *Haemophilus influenzae* type b (Hib)[c,d] ■ Inactivated poliomyelitis (IPV) ■ Pneumococcal conjugate (7vPCV) ■ Rotavirus
4 months	■ Hepatitis B (hepB)[b] ■ Diphtheria, tetanus and acellular pertussis (DTPa) ■ *Haemophilus influenzae* type b (Hib)[c,d] ■ Inactivated poliomyelitis (IPV) ■ Pneumococcal conjugate (7vPCV) ■ Rotavirus
6 months	■ Hepatitis B (hepB)[b] ■ Diphtheria, tetanus and acellular pertussis (DTPa) ■ *Haemophilus influenzae* type b (Hib)[c] ■ Inactivated poliomyelitis (IPV) ■ Pneumococcal conjugate (7vPCV)[e] ■ Rotavirus[j]
12 months	■ Hepatitis B (hepB)[b] ■ *Haemophilus influenzae* type b (Hib)[d] ■ Measles, mumps and rubella (MMR) ■ Meningococcal C (MenCCV)
12–24 months	■ Hepatitis A (Aboriginal and Torres Strait Islander children in high risk areas)[f]
18 months	■ Varicella (VZV)
18–24 months	■ Pneumococcal polysaccharide (23vPPV) (Aboriginal and Torres Strait Islander children in high-risk areas)[g] ■ Hepatitis A (Aboriginal and Torres Strait Islander children in high-risk areas)
4 years	■ Diphtheria, tetanus and acellular pertussis (DTPa) ■ Measles, mumps and rubella (MMR) ■ Inactivated poliomyelitis (IPV)
10–13 years[h]	■ Hepatitis B (hepB) ■ Varicella (VZV)
12–13 years[i]	■ Human papillomavirus (HPV)
15–17 years[i]	■ Diphtheria, tetanus and acellular pertussis (DTPa)
15–49 years	■ Influenza (Aboriginal and Torres Strait Islander people medically at-risk) ■ Pneumococcal polysaccharide (23vPPV) (Aboriginal and Torres Strait Islander people medically at-risk)
50 years and over	■ Influenza (Aboriginal and Torres Strait Islander people) ■ Pneumococcal polysaccharide (23vPPV) (Aboriginal and Torres Strait Islander people)
65 years and over	■ Influenza ■ Pneumococcal polysaccharide (23vPPV)

a Hepatitis B vaccine should be given to all infants as soon as practicable after birth. The greatest benefit is if given within 24 hours, and must be given within 7 days.

b Total of three doses of hepB required following the birth dose, at either 2m, 4m and 6m or at 2m, 4m and 12m.

c Give a total of 4 doses of Hib vaccine (2m, 4m, 6m and 12m) if using PRP-T Hib containing vaccines.

d Use PRP-OMP Hib containing vaccines in Aboriginal and Torres Strait Islander children in areas of higher risk (Queensland, Northern Territory, Western Australia and South Australia) with a dose at 2m, 4m and 12m.

e Medical at-risk children require a fourth dose of 7vPCV at 12 months of age, and a booster dose of 23vPPV at 4 years of age.

f Two doses of hepatitis A vaccine are required for Aboriginal and Torres Strait Islander children living in areas of higher risk (Queensland, Northern Territory, Western Australia and South Australia). Contact your state or territory Health Department for details.

g Contact your State or Territory Health Department for details.

h These vaccines are for one cohort only within this age range, and should only be given if there is no prior history of disease or vaccination. Dose schedules may vary between jurisdictions. Contact your state or territory Health Department for details.

i This vaccine is for one cohort only within this age range. Contact your state or territory Health Department for details.

j Third dose of vaccine is dependent on vaccine brand used. Contact your state or territory Health department for details

Source: National Immunisation Program Schedule, <www.immunise.health.gov.au>. Reproduced with permission of the Australian Government.

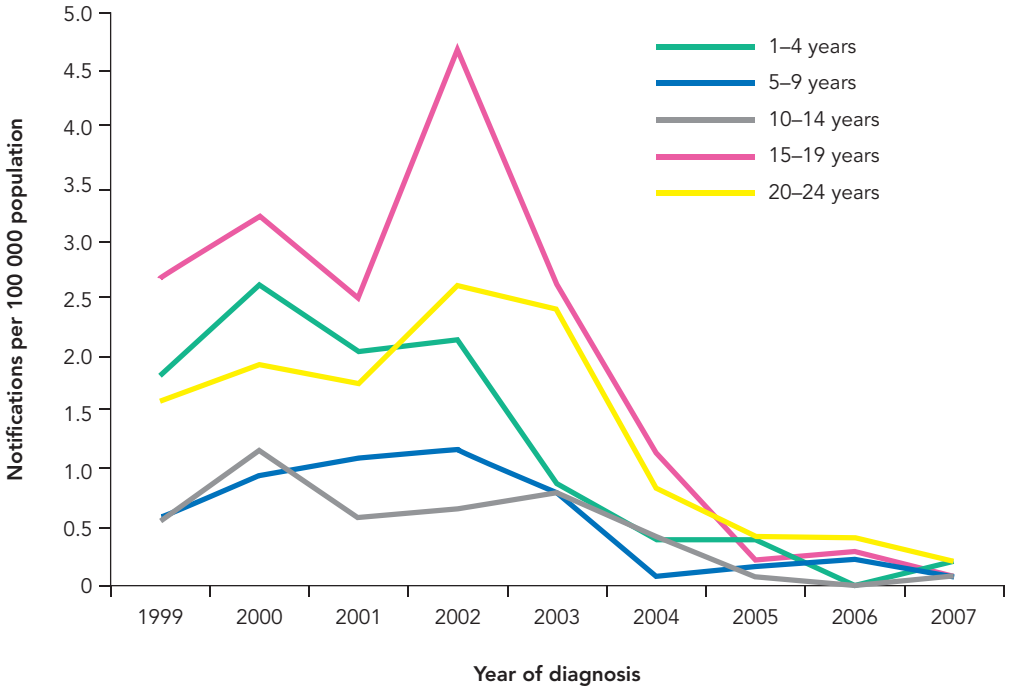

FIGURE 14.8

Meningococcal serogroup C disease notification rates, Australia, 1999–2007, by age group and year of diagnosis

Source: NNDSS 2010, *Communicable Diseases Intelligence* 34 (Supplement): S53, Figure 3.7.5.

young and very old. Ninety different serotypes are identified by the polysaccharides in the capsule of *S. pneumoniae* and this has made vaccine development difficult. A 23-valent vaccine suitable for adults has been funded for Aboriginal and Torres Strait Islanders since 1999. In 2001, the 7-valent pneumococcal conjugate vaccine against *Streptococcus pneumoniae*, which is suitable for young children, was added to the schedule for all Aboriginal and Torres Strait Islander infants and young children, and in 2005 it was included in the national schedule (Figure 14.9). In addition, a free program of immunisation against invasive pneumococcal disease with a 23-valent vaccine is available to all adults over age 65. The highest rates of invasive disease occur in Indigenous people in the Northern Territory. Notification rates have decreased since the introduction of the vaccines (see Figure 14.10).

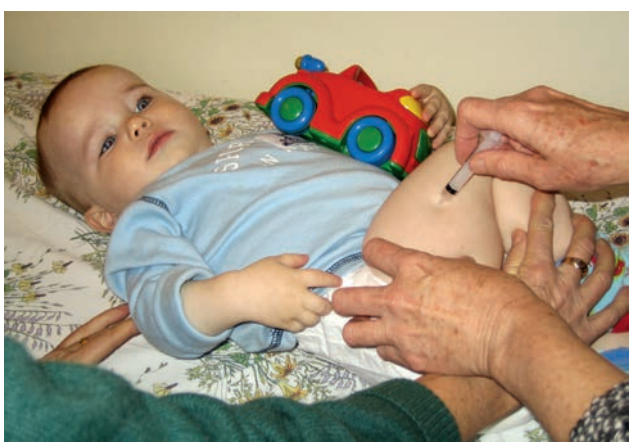

FIGURE 14.9

Baby receiving 12-month vaccinations

Source: © Christine Bishop.

In the latest schedule (see Table 14.4), the inactivated injectable vaccine IPV (Salk) is recommended instead of the live oral polio vaccine OPV (Sabin) in order to prevent the very slight risk of vaccine-associated disease. This is now included in the schedule of free vaccines. The fourth dose of DTPa, which was previously given at 18 months of age, is no longer required. Instead, the fourth dose of DTPa is now recommended at 4 years of age with a fifth dose at 15–17 years. The purpose of this last dose is to increase immunity in older age groups and so minimise the risk of transmission to unvaccinated babies.

To improve protection against measles, a two-dose vaccination schedule (given in the combined measles-mumps-rubella, MMR, preparation) is now recommended at 12 months and 4 years. In addition, a 'catch-up' campaign of measles vaccination for school-age children was carried out in 1998. A campaign is under way to increase measles vaccine coverage in susceptible young adults born since 1966 who do not have evidence of two doses of the vaccine in the past.

Haemophilus influenzae type b (Hib) is responsible for the invasive diseases meningitis, pneumonia and epiglottitis in young children, with an overall fatality rate of 2–5 per cent. Sequelae to Hib meningitis, ranging from mild hearing loss to neurological impairment, occur in 20–30 per cent of cases. Hib vaccines suitable for infants of two months were introduced in

1993 in Australia and are now included in the routine immunisation schedules. The result has been a dramatic decrease in the incidence of Hib (see Figure 14.11). In children under the age of 5 years, there has been a marked reduction in the number of notifications to 0.1 per 100 000. The rate is higher in Indigenous than non-Indigenous children. The impact of herd immunity following the vaccination of young children has led to a reduction in infections across all age groups.

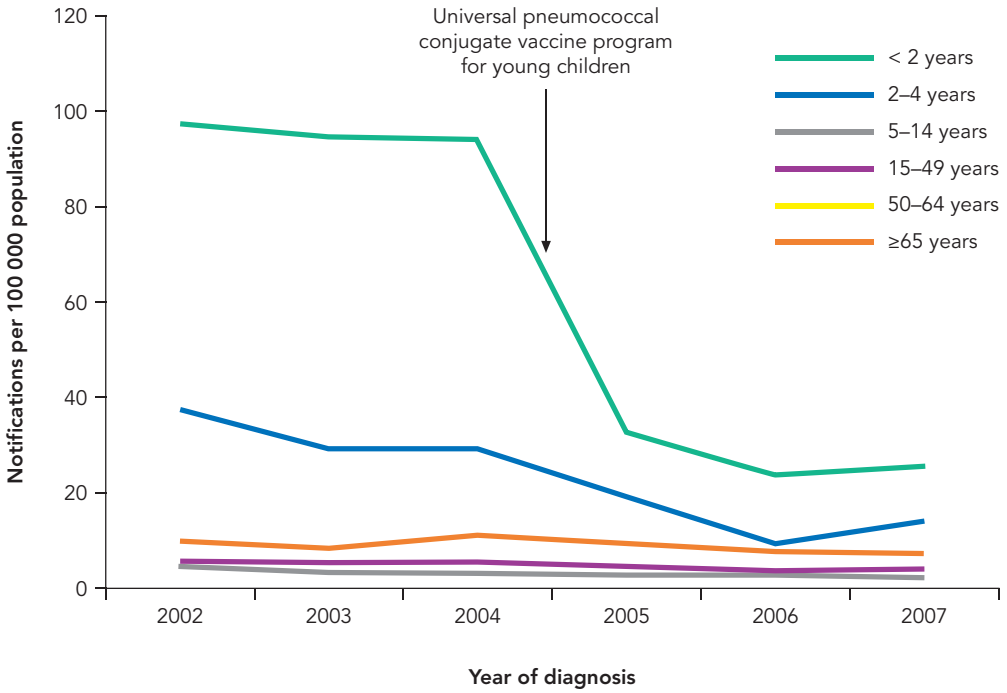

FIGURE 14.10

Pneumococcal disease notification rates, 2002–07, by age group and year of diagnosis

Source: NNDSS 2010, *Communicable Diseases Intelligence* 34 (Supplement): S74, Figure 3.10.2.

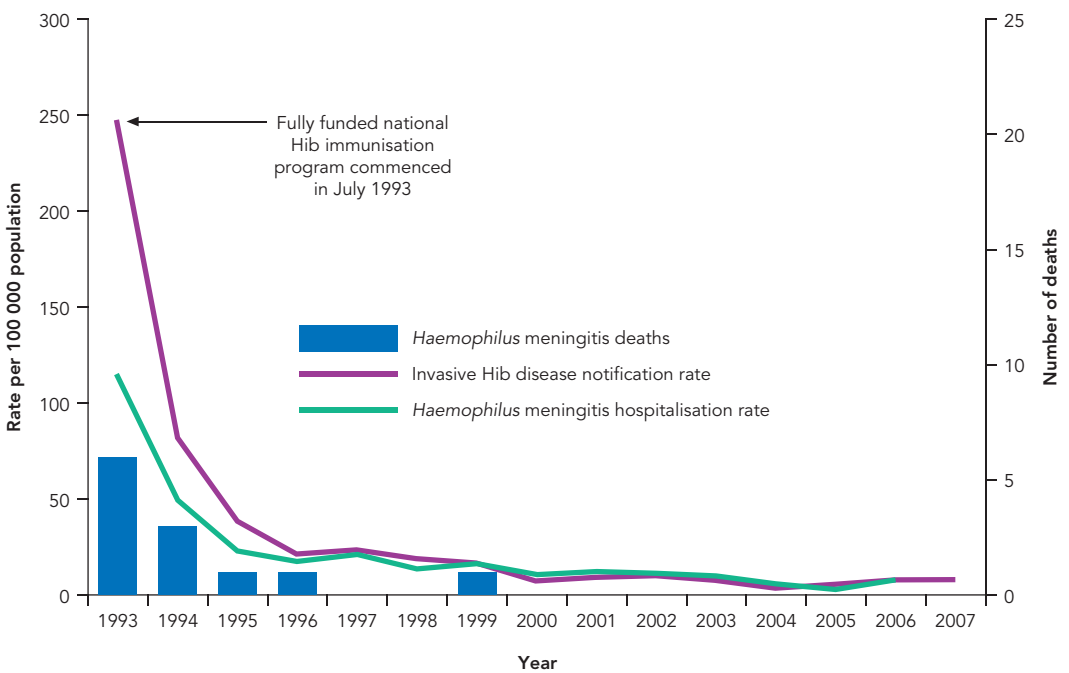

FIGURE 14.11

Incidence of Hib

Hib notification, Hib meningitis hospitalisation rates and numbers of deaths of children aged 0–4 years, Australia, 1993 to June 2007.

Source: NNDSS 2010, *Communicable Diseases Intelligence* 34 (Supplement): S17, Figure 3.2.2.

Indigenous Australians have a high risk of acquiring tuberculosis, so BCG vaccine, which is most effective in children, is recommended for neonates in regions of high incidence. There are differences in schedules used in Australia and other countries (e.g. New Zealand; see page 360). The schedules are continually modified to include new vaccines and to take into account new knowledge and changes in the prevalence of various diseases.

Procedures for vaccination

The NHMRC publishes up-to-date comprehensive information on immunisation procedures and recommendations for administration in the *Australian Immunisation Handbook*. Recommendations change from time to time and updates are available at the website <www.immunise.health.gov.au>. Vaccines should be administered by the correct route and at the optimal site (for injections). The age at which vaccines are administered is also important. Maternally derived antibodies (IgG) seem to lower the infant's response to live (attenuated) vaccines, but do not interfere with the response to the non-replicating (killed) vaccines. For this reason, DTP (triple antigen: diphtheria, tetanus, pertussis) can be given as early as 2 months of age, but the live MMR (measles, mumps, rubella) vaccine is better delayed to 12 months. Since premature infants are lacking in maternal antibodies, immunisation should begin at the chronological age and not be delayed because of prematurity.

Adult immunisation

Although the major emphasis in all vaccination campaigns is on childhood vaccination, there is a large number of adults who, for various reasons, are not fully immunised. This may be because they are recent immigrants from countries where vaccination is not readily available, or it may be because of their age and the previous unavailability of vaccines. There is significant morbidity and mortality among adults from vaccine-preventable diseases and there is a need to develop a national strategy aimed at immunising adults. A vaccine against seasonal influenza is available and recommended for susceptible groups. The vaccine is prepared each year to reflect the viral types that are circulating in the community. Currently, influenza vaccine and pneumococcal vaccine are available free to all Australians 65 years and older. Immunity to pertussis is known to decline with age. Since many grandparents are involved with caring for young children, older Australians are also being encouraged to have a booster dose of the pertussis vaccine.

Aboriginal people and Torres Strait Islanders have a high incidence of invasive pneumococcal disease. The Northern Territory has developed an adult immunisation schedule which, in addition to influenza vaccine, includes:

- influenza and pneumococcal vaccines for Aboriginal people 50 years and older; and for those 15 years and older who fall into high-risk groups
- measles-mumps-rubella vaccine for adults 18–40 years
- adult diphtheria and tetanus (ADT) every 10 years.

Immunisation against other diseases

As discussed above, it is important that adults as well as children are fully immunised against the vaccine-preventable diseases. There are other, less common diseases that are also preventable by vaccines, but immunisation is usually only recommended for high-risk groups. Vaccines are available to protect against influenza, pneumococcus, Q fever, meningococcal meningitis and tuberculosis.

Other vaccines (e.g. for cholera, typhoid, yellow fever and Japanese encephalitis) are required only by travellers to countries where the disease is endemic. Travellers should consult their state health department for current recommendations for vaccination or refer to the *Australian Immunisation Handbook* <www.immunise.health.gov.au>. Information about infectious diseases in other countries is available online from the WHO website <www.who.int/ith/> or the Centers for Disease Control and Prevention <www.cdc.gov/travel/index>.

Immunisation for health professionals

Due to the nature of their work, health professionals are at significantly higher risk of encountering infectious diseases. Health workers are advised to ensure they are vaccinated against hepatitis B. The value of the BCG vaccine in offering partial protection against tuberculosis is a controversial area. Some states recommend vaccination for all health workers, while others favour regular Mantoux testing, followed by antibiotic prophylaxis if required. In institutional and child-care facilities, vaccination for hepatitis A is advisable.

All healthcare workers, especially those working with children, should ensure they have adequate immunisation against all the 'childhood diseases', especially as some of these diseases—for example, measles and chickenpox—are much more severe in adults. Immunisation is also necessary to protect susceptible children (and adults) such as those suffering from leukaemia, who are particularly vulnerable to infection. A vaccine for chickenpox is available and should be given to all non-immune paediatric staff and those working with immunocompromised patients. A booster immunisation for pertussis is also important to protect susceptible infants.

COMPLIANCE WITH IMMUNISATION

Herd immunity

The success of immunisation programs in developed countries has meant a dramatic reduction in infant morbidity and mortality (see Table 14.3, page 333). However, to maintain this situation it is essential that parents continue to have their children immunised. An important factor in the maintenance of a community free of a particular disease is the proportion of individuals in the community who are immune to that disease. This is described as group or **herd immunity**. As we saw in Chapter 8, in order to control the spread of disease the reservoirs of infection must be eliminated, in this case by immunisation, since if the number of immune individuals is high, the likelihood of exposure of an unimmunised individual to the disease—and therefore the

risk of an outbreak of the disease—is low. *Thus, a high group immunity protects not only the immunised members but also the susceptible ones* (see Figure 14.12).

This is particularly important for a disease such as pertussis (whooping cough). Immunity to pertussis declines with age, so older children and adults may suffer a severe but usually non-fatal illness. However, they can act as a reservoir of infection for unimmunised babies under 6 months for whom pertussis may be fatal. If the older children and adults are immunised, they do not contract the disease and so the susceptible babies are protected because they have less chance of being exposed to an infected person.

It is necessary to understand the concept of herd immunity in order to realise the importance of continuing vaccine

FIGURE 14.12

Herd immunity

The greater the percentage of immune persons in a population, the less likely a susceptible individual is to be exposed to the disease.

Eradication of polio: the importance of herd immunity

SPOTLIGHT ON

In April 2007 an overseas student returned to Australia from a visit to his home in Pakistan and was admitted to hospital in Melbourne with a diagnosis of poliomyelitis. This was the first case of polio diagnosed in Australia since 1976.

Polio is a highly infectious disease caused by a virus. It invades the nervous system, and one in 200 infections leads to irreversible paralysis. The virus is transmitted by the faecal–oral route—it enters the body through the mouth and multiplies in the intestine. About 90 per cent of infections are asymptomatic; in the other 10 per cent, initial symptoms are fever, fatigue, headache, vomiting, stiffness in the neck and pain in the limbs (usually in the legs). Among those paralysed, 5–10 per cent die when their breathing muscles become immobilised. Between 1912 and 1956, over 30 000 cases of paralytic polio were reported in Australia. It was not until the Salk vaccine was developed and licensed in 1956 that the number of cases decreased, the last known case of wild polio virus transmission in Australia being reported in 1972. Australia and the Western Pacific region were officially declared polio free in 2000.

The WHO had set a target of worldwide eradication of polio by 2000. Polio cases decreased from an estimated 350 000 cases in 1988, to about 2000 reported cases in 2006. The reduction was the result of the global effort to eradicate the disease by vaccination.

However, between 2002 and 2005 there was a resurgence of the importation of polio into 21 countries that had previously been declared free of polio. This was mainly due to the decision by the Nigerian government to suspend its vaccination program, thus reducing the level of herd immunity and allowing the virus to spread to neighbouring countries. Civil unrest in West Africa made the situation worse and the virus spread to the Middle East (carried by pilgrims to the *Haj*). Of the 21 countries affected, four had sustained outbreaks of polio (Indonesia, Somalia, Sudan and Yemen). The countries where transmission occurred had only a 52 per cent rate of vaccination coverage, compared with over 80 per cent in the other countries where transmission of the wild polio virus was able to be controlled.

Despite an extensive information and vaccination campaign, by 2008 there were still four countries in the world where polio is considered to be endemic: Afghanistan, Pakistan, India and Nigeria. An additional ten countries reported cases of polio that had been imported by travellers who had visited these endemic regions.

These cases highlight the need to maintain the level of herd immunity in our community. As long as the polio virus is endemic anywhere in the world, there is the risk of importation of the virus into Australia. The high number of Australian travellers, visitors and refugees from countries where polio is endemic poses a significant risk to unvaccinated members of the community. Since transmission is by the faecal–oral route, contamination of food or water could enable the virus to spread.

Continued high levels of vaccination coverage in the Australian population and for people travelling overseas will prevent outbreaks of the virus.

programs after the incidence of a disease has declined—for example, polio. If a person with polio (e.g. a traveller from a country where the disease is still prevalent) enters a country which has been declared 'polio free', and has discontinued its vaccination program, there is the potential for an epidemic to occur. This will continue to be the case until the disease is eradicated worldwide (see Spotlight box on previous page).

Importance of the MMR vaccine

An individual's response to a vaccine preparation depends on their immune system. Not all vaccines are 100 per cent effective. For example, the measles vaccine produces immunity in only 90–95 per cent of people vaccinated, but that is usually high enough to protect everyone because of herd immunity. If there is low compliance with immunisation programs, then the level of protection can fall as low as 50 per cent in the community. The lack of full immunisation coverage greatly increases the risk of epidemics, as is shown by the outbreaks of measles in Australia in the 1990s (see Figure 14.13).

Measles is a highly contagious, vaccine-preventable and potentially deadly disease that affects vulnerable children around the world—many of whom do not have access to healthcare. More than 17 million children are affected each year. Tragically, more than 600 children die each day. The WHO has identified measles as a disease which, theoretically, could be eradicated worldwide because, like smallpox, humans are the only reservoir and the disease is transmitted directly from person to person.

A monovalent (single) measles vaccine was introduced in Australia in 1968, and from 1989 the recommended vaccination schedule included the combined measles-mumps-rubella vaccine (MMR) for infants aged 12–15 months.

Despite the availability of this vaccine, low uptake levels resulted in serious outbreaks of measles in Australia. In 1989–90 in Australia, only 52.9 per cent of children under 6 years of age were *fully* immunised. An epidemic of measles that began in 1992 continued into 1994, with 4895 cases being reported. Measles was responsible for 2223 hospitalisations and seven deaths in Australia between 1993 and 1998. The measles epidemic of 1993–94 prompted the introduction of a second dose of MMR vaccine, which is now given at 4 years of age. A highly successful 'catch-up campaign' for 1.7 million school children was carried out between August and November 1998. From 2002 onwards the number of notifications of measles in Australia progressively decreased.

However, in Australia there is still a pool of young adults born between 1966 and 1989 who are susceptible to measles because they were born before the MMR vaccine was introduced for infants and who missed out on catching the wild-type virus because the incidence of the disease had dropped after the monovalent vaccine was first introduced. These people make up a susceptible population who are not only at risk of catching the disease themselves but can also transmit it to unimmunised infants. If they travel to countries where the disease is endemic they have the potential to transport the disease back to Australia and infect susceptible individuals. In 2001 there was an outbreak of measles in Melbourne, resulting in 31 people being infected, 17 of whom required hospitalisation. The index case was a 19-year-old male who

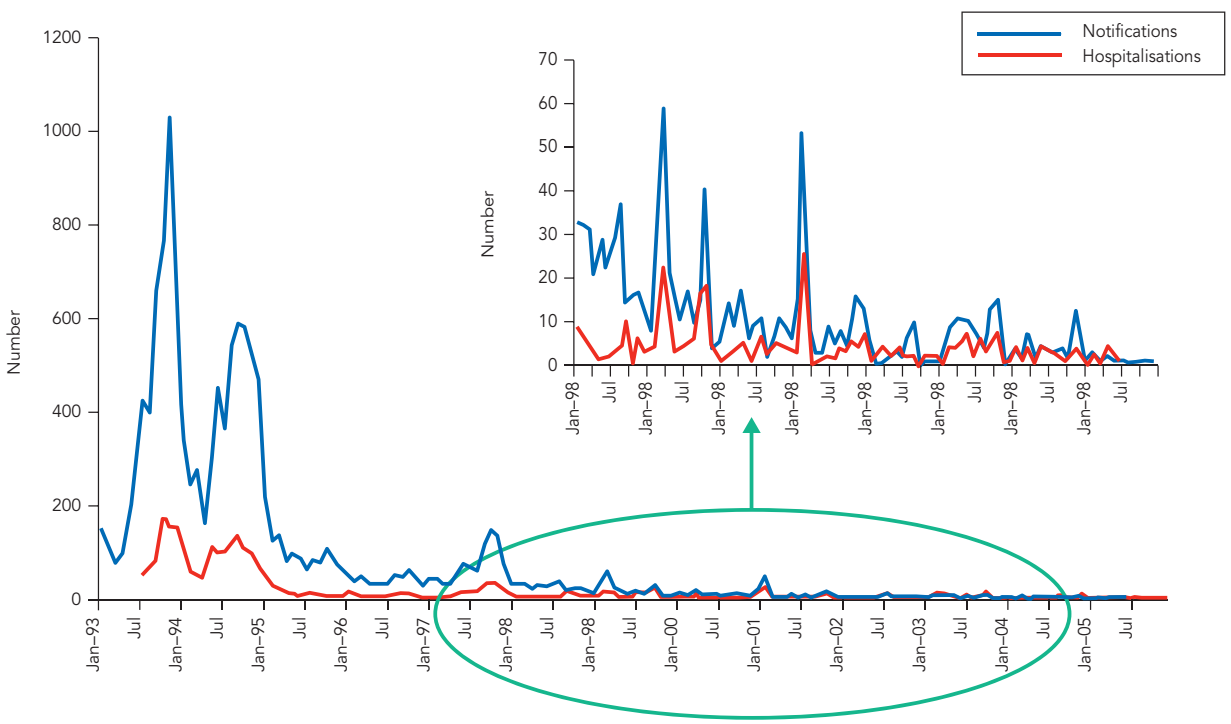

FIGURE 14.13

Notifications and hospitalisations of measles, Australia, 1999–2005, by month of diagnosis or admission

Source: *Vaccine Preventable Diseases and Vaccination Coverage in Australia 2003 to 2005*, 31 (Supplement), Figure 12. Reproduced with permission of the Australian Government.

MMR vaccine and autism

SPOTLIGHT ON

In 1998 a group of British researchers reported the occurrence of an apparently new syndrome of an unusual type of inflammatory bowel disorder (IBD) in a small group of 12 children. The researchers suggested that the measles-mumps-rubella (MMR) vaccine had caused the IBD, which then resulted in decreased absorption of essential vitamins and nutrients through the intestinal tract. They proposed that this could result in developmental disorders such as autism.

There were a number of faults in this study. It was a very small group of children who had been referred to the clinic for a particular problem (i.e. not a random sample), no controls (unvaccinated children) were included, and some of the children had behavioural problems before they were vaccinated. MMR vaccination and the onset of autism may coincidentally appear associated in time, because the average age at which parents report concerns about child development is 18–19 months, and in the United Kingdom over 90 per cent of children received the MMR vaccine before their second birthday.

Since then, more thorough, large epidemiological studies by the WHO have found no evidence of an association. The Global Advisory Committee on Vaccine Safety (GACVS) concluded that:

> *no evidence exists of a causal association between measles, mumps, and rubella (MMR) vaccine and autism or autistic disorders.**

In addition, more recent reviews by the American Academy of Pediatrics, the British Chief Medical Officer, the UK Medical Research Council, Canadian experts, and numerous other scientific experts have stated that there is no link between autism or IBD and the measles vaccine.

However, many parents in the United Kingdom decided not to vaccinate their children with MMR and the overall coverage rate has fallen to less than 50 per cent. This creates a potential risk for outbreaks of measles and mumps and the possibility of more cases of congenital rubella. In 2007–08, outbreaks of measles and mumps occurred in the United Kingdom and across Europe, and many of the patients were not vaccinated. Measles has now been declared endemic again in the UK, 14 years after the local transmission of measles was halted.

The level of coverage is now too low for herd immunity to protect unvaccinated or partially vaccinated people in the community. Outbreaks of measles have been reported in the United States where measles had been officially declared to be eliminated since 2002. The index case had been infected in another country. Many people have had only one dose of MMR, which was given at 15 months. The booster at 4 years of age is necessary to ensure protection.

Some parents in Australia have also opted not to vaccinate their children, thus decreasing the protective effect of herd immunity for other susceptible people.

* WHO 2003, 2002 Global Advisory Committee on Vaccine Safety, *Weekly Epidemiological Record*, 16–17 December, 78: 17–18, <www.who.int/wer/pdf/2003/wer7804.pdf>.

had travelled to India. In a previous outbreak that infected 44 people, the index case had returned from a holiday in Bali. A catch-up program of MMR vaccination aimed at these susceptible young adults was conducted in 2001, but the uptake has not been high. This age group still has the highest rate of measles notifications. In 2009, 34 per cent of notifications were reported as being acquired overseas and most of the remaining cases were epidemiologically linked to the imported cases.

The WHO target of eradication is being affected by the number of parents who are not having their children vaccinated with MMR because of concerns about the safety of the vaccine (see Spotlight box).

Despite overwhelming evidence as to the safety and efficacy of the vaccine, vaccination rates have dropped to a critical level in some countries, so that there are now increasing numbers of cases of measles (and mumps) being reported in countries where the level of herd immunity is now too low to protect unimmunised people (see Spotlight box). Another concern is that there may also be a corresponding decrease in protection against rubella, which could see an increase in congenital rubella syndrome when the present cohort reach child-bearing age.

Compliance with vaccination schedules

There are various reasons why people do not comply with immunisation recommendations. About 3 per cent of Australians fall into the category of 'conscientious objectors' for one of the following reasons:

- ignorance or complacency about the serious nature of vaccine-preventable diseases
- fear of complications of vaccination
- lack of correct information about side effects and/or contraindications
- ethical or religious beliefs.

In 1996 the Australian Childhood Immunisation Register was established, and in 1997 a seven-point 'Immunise Australia' plan was devised to increase immunisation levels to a target coverage of 90 per cent. Various initiatives were introduced, including incentive payments to parents. The level of vaccine coverage is now monitored by the National Centre for Immunisation Research and Surveillance of Vaccine Preventable Diseases (NCIRS) and has increased steadily, especially in the Northern Territory.

Trends in vaccine coverage have been monitored by NCIRS at three-monthly intervals from 1996. There has been a steady increase in vaccine uptake towards the target of 90 per cent coverage at 12 and 24 months. The 2009 figures show an average 91.8 per cent uptake of recommended vaccines at 12 months, and 92.2 per cent at 24 months. However, only 82.7 per cent of children had full coverage at 5 years. The rates are consistent across all states and territories. Areas of low coverage have been identified in many remote areas, and in areas containing a higher proportion of conscientious objectors (see Figure 14.14).

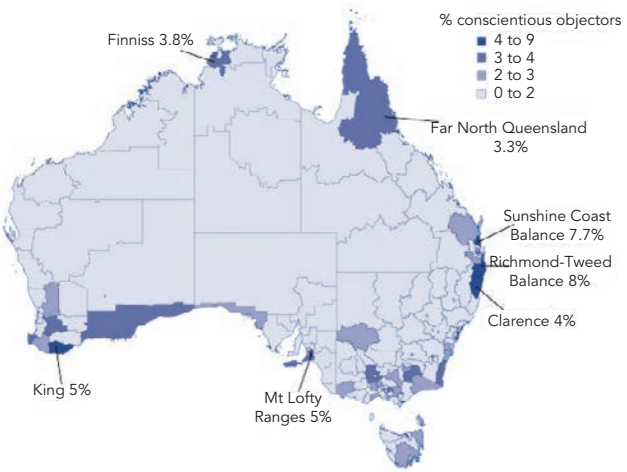

FIGURE 14.14

Proportion of conscientious objectors to immunisation, Australia, 2009

Source: NNDSS 2011, *Communicable Diseases Intelligence* 35(2): 145, Figure 17.

Risks and complications of vaccination

In the past there have been instances of adverse reactions, even some deaths, following administration of vaccines. The standard of vaccine production and administration is now very high. Health professionals should be familiar with the contraindications for vaccination. Children with immunodeficiencies require special consideration. The *Australian Immunisation Handbook 2008* (9th edition) is available online at <www.immunise.health.gov.au/handbook.htm> and contains complete information about the use of the recommended vaccines, together with a description of any adverse reactions that might be expected and contraindications for use (see Table 14.5).

An adverse reaction is defined as 'a serious uncommon or unexpected event following administration of a vaccine'. Such an event *may or may not* be caused by the vaccine (it may be caused by chance after the vaccination). Any vaccine may cause an adverse event.

TABLE 14.5 **Comparison of the effects of diseases and the side effects of vaccines**

DISEASE	EFFECTS OF DISEASE	SIDE EFFECTS OF VACCINATION
Diphtheria—contagious bacteria spread by droplets; causes severe throat and breathing difficulties.	About 1 in 15 patients dies. The bacteria release a toxin, which can produce nerve paralysis and heart failure.	DTPa/dTpa vaccine—about 1 in 10 has local inflammation or fever. Booster doses of DTPa may occasionally be associated with extensive circumferential swelling of the limb, but this resolves completely within a few days. Serious adverse events are very rare.
Hepatitis A—contagious virus spread by contact or ingestion of faecally contaminated water/food or through contact with the faecal material of a person infected with hepatitis A.	Jaundice (yellowing of the skin and eyes), fever, anorexia, nausea, vomiting, hepatic (liver) pain and malaise (tiredness). It may take up to 1 month for patients to recover, and some patients may require hospitalisation. Young children may not show any symptoms but are still infectious. Patients are infectious for up to 2 weeks before the onset of jaundice and for approximately 1 week after the jaundice appears.	About 1 in 5 will have discomfort or local inflammation at the site of injection.
Hepatitis B—virus spread mainly by blood, sexual contact or from mother to newborn baby; causes acute hepatitis or chronic carriage.	About 1 in 4 chronic carriers will develop cirrhosis or liver cancer.	About 1 in 15 will have injection site pain, and 1 in 100 will have fever. Anaphylaxis occurs in about 1 in 600 000.
Haemophilus influenzae type B (Hib)—contagious bacteria spread by respiratory droplets; causes meningitis, epiglottitis (respiratory obstruction), septicaemia and osteomyelitis.	About 1 in 20 meningitis patients dies, and about 1 in 4 survivors will have permanent brain or nerve damage.	About 1 in 100 epiglottitis patients die. About 1 in 20 has discomfort or local inflammation. About 1 in 50 has fever.
Human papillomavirus—virus spread mainly via sexual contact.	About 1 in 2 cervical cancers worldwide have been associated with HPV16, and 1 in 10 with HPV18.	About 8 in 10 will have pain, and 2 in 10 will have swelling/redness at the site of injection. Very occasionally, headache, fever and nausea may occur.

Comparison of the effects of diseases and the side effects of vaccines *cont'd*

DISEASE	EFFECTS OF DISEASE	SIDE EFFECTS OF VACCINATION
Influenza—contagious virus spread by respiratory droplets; causes fever, muscle and joint pains, pneumonia.	Causes increased hospitalisation in the elderly. High-risk groups include the elderly, diabetics and alcoholics.	About 1 in 10 has local reactions. Guillain-Barré syndrome occurs in about 1 in 1 million.
Measles—highly infectious virus spread by droplets; causes fever, cough and rash.	About 1 in 15 children with measles develops pneumonia and 1 in 1000 develops encephalitis (brain inflammation). For every 10 children who develop measles encephalitis, 1 dies and 4 have permanent brain damage. About 1 in 100 000 develops SSPE (brain degeneration) which is always fatal.	About 1 in 10 has discomfort, local inflammation or fever. About 1 in 20 develops a rash, which is non-infectious. Fewer than 1 in 1 million recipients may develop encephalitis (inflammation of the brain).
Meningococcal infections—bacteria spread by respiratory droplets. Cause septicaemia (infection of the blood stream) and meningitis (infection of the tissues surrounding the brain).	About 1 in 10 patients dies. Of those that survive, 1 in 30 has severe skin scarring or loss of limbs, and 1 in 30 has severe brain damage.	Conjugate vaccine—about 1 in 10 has local inflammation, fever, irritability, anorexia or headaches.
Mumps—contagious virus spread by saliva; causes swollen neck and salivary glands and fever.	About 1 in 200 children develops encephalitis, and 1 in 5 males past puberty will develop inflammation of the testes. Occasionally, mumps causes infertility or deafness.	About 1 in 100 vaccine recipients may develop swelling of the salivary glands. About 1 in 3 million recipients will develop mild encephalitis.
Pertussis—contagious bacteria spread by respiratory droplets; causes whooping cough and vomiting lasting up to 3 months.	About 1 in 200 whooping cough patients under the age of 6 months dies from pneumonia or brain damage.	DTPa/dTpa vaccine—about 1 in 10 has local inflammation or fever. Booster doses of DTPa may occasionally be associated with extensive circumferential swelling of the limb, but this resolves completely within a few days. Serious adverse events are very rare.
Pneumococcal infections—bacteria spread by respiratory droplets; causes septicaemia, meningitis and occasionally other infections.	About 1 in 10 meningitis patients dies.	7vPCV—about 1 in 10 has local reaction or fever. 23vPPV—about 1 in 2 has a local reaction.
Polio—contagious virus spread by faeces and saliva; causes fever, headache and vomiting and may progress to paralysis.	While many infections cause no symptoms, about 1 in 20 hospitalised patients dies and 1 in 2 patients who survive is permanently paralysed.	Local redness, pain and swelling at the site of injection are common. Up to 1 in 10 has fever, crying and decreased appetite.
Rotavirus—virus spread by faecal–oral route; causes gastroenteritis which can be severe.	In children <5 years of age, rotavirus infections in Australia account for approximately 10 000 hospitalisations every year, approximately 115 000 children visit a GP, and approximately 22 000 children require an Emergency Department visit. Illness may range from mild, watery diarrhoea of limited duration to severe dehydrating diarrhoea and fever which can result in death.	About 1–3 in 100 vaccine recipients may develop diarrhoea or vomiting in the week following vaccine administration.
Rubella—contagious virus spread by droplets; causes fever, rash and swollen glands, but causes severe malformations in babies of infected pregnant women.	About 5 in 10 patients develop a rash and painful swollen glands; 5 in 10 adolescents and adults have painful joints; 1 in 3000 develops thrombocytopenia (bruising or bleeding); 1 in 6000 develops inflammation of the brain; 9 in 10 babies infected during the first 10 weeks after conception has a major congenital abnormality (including deafness, blindness or heart defects).	About 1 in 10 has discomfort, local inflammation or fever. About 1 in 20 has swollen glands, stiff neck or joint pains. About 1 in 20 has a rash, which is non-infectious. Thrombocytopenia (bruising or bleeding) occurs after a first dose of MMR at a rate of about 1 in 30 500.
Tetanus—caused by toxin of bacteria in soil; causes painful muscle spasms, convulsions, lockjaw.	About 3 in 100 patients dies. The risk is greatest for the very young or old.	DTPa/dTpa vaccine—about 1 in 10 has local inflammation or fever. Booster doses of DTPa may occasionally be associated with extensive circumferential swelling of the limb, but this resolves completely within a few days. Serious adverse events are very rare.
Varicella (chickenpox)—highly contagious virus; causes low-grade fever and vesicular rash. Reactivation of the virus later in life causes herpes zoster (shingles).	About 1 in 100 000 patients develop encephalitis (brain inflammation). About 3 in 100 000 patients die. Infection during pregnancy can result in congenital malformations in the baby. Onset of infection in the mother from 5 days before to 2 days after delivery results in severe infection in the newborn baby in up to one-third of cases.	About 1 in 5 has a local reaction or fever. A mild varicella-like rash may develop in 3–5 in 100 recipients.

Source: *Australian Immunisation Handbook* 2008, 9th ed. © Australian Government. Available at: <www.immunise.health.gov.au/internet/immunise/publishing.nsf/Content/Handbook-home>.

Reports of suspected adverse events following immunisation are collated in a central database by the Adverse Drug Reactions Advisory Committee. NCIRS carries out an analysis of the reports which is published regularly in the *CDI* bulletins. The most commonly reported adverse reactions are low-grade fever, pain or redness at the injection site. There is a very low reporting rate of serious events, which demonstrates a high level of safety in Australian vaccines.

Reactions to the combined 'triple antigen' DTPa have decreased since the introduction of acellular pertussis vaccine. Much publicity was focused on the side effects of the old whole cell pertussis vaccine. It was common for infants to be feverish and crying after the DTP vaccination. These effects have been greatly reduced with the use of the new vaccine. Although the old whole cell DTP vaccine was blamed for serious brain damage, several large controlled case studies failed to show any permanent neurological damage that was directly attributable to the pertussis vaccine.

On the other hand, pertussis (whooping cough) is a serious, sometimes fatal, respiratory infection that is particularly serious in infants under 6 months of age. Encephalopathy occurs in 1.1 per cent of all infants under 6 months of age who contract pertussis. Acute neurological complications from an attack of whooping cough occur in 2–7 per cent of unimmunised individuals. Worldwide, about 250 000 babies are infected with pertussis each year and many of them suffer brain damage. The vaccine has been very effective in reducing the incidence of the disease in Australia (see Table 14.3, page 333). There are periodic epidemics of pertussis every three to five years, usually in the winter months, with a low level of endemic cases in between (see Figure 14.15). Many mild cases occur in older people (60–65), reflecting a decrease in immunity with increasing age. These people pose a risk to unvaccinated infants.

Lack of compliance with vaccination by some parents puts babies who are too young to be immunised at risk. In the five years between 1993 and 1998, nearly 35 000 cases of pertussis were notified in Australia, and nine babies died in the 1996–97 outbreak. Figure 14.15 shows the increase in notifications between 2007 and 2009 and illustrates the importance of maintaining herd immunity to prevent epidemics.

Vaccination with the combined measles-mumps-rubella (MMR) vaccine is recommended at 12 months and again at 4 years. Measles is a serious, highly infectious viral illness. Acute encephalitis occurs in 2–10 people per 10 000 reported cases of measles, with a mortality rate of 10–15 per cent. About 15–40 per cent of survivors have permanent brain damage. Mumps is an unpleasant but less serious infection and permanent adverse sequelae are rare. Orchitis may occur in up to 20 per cent of postpubertal males, but subsequent sterility is rare. Rubella is a mild, often subclinical infection. However, when contracted during the first 8–10 weeks of pregnancy it results in foetal damage in up to 90 per cent of pregnancies. Malaise, fever and a rash may occur 7–10 days after MMR vaccination. Febrile convulsions occur in about 0.1 per cent of children. Symptoms can be alleviated with paracetamol. Reports of the vaccine being linked to autism are unfounded (see Spotlight box: MMR vaccine and autism, page 341).

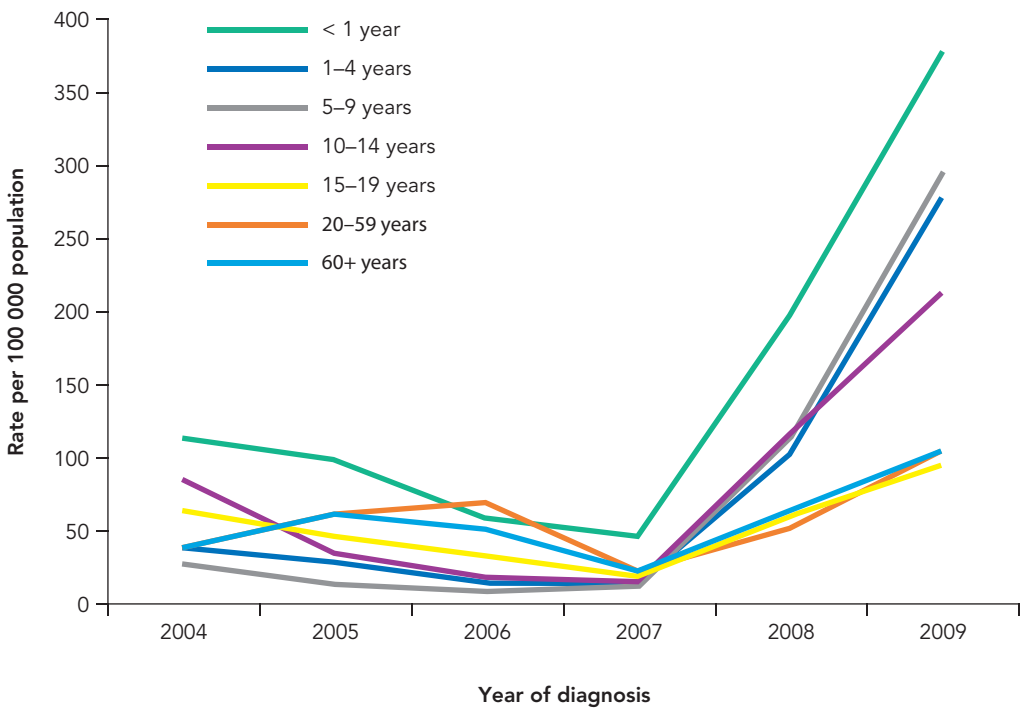

FIGURE 14.15

Notification rate of pertussis, Australia, 2004–09, by age group

Source: NNDSS 2011, *Communicable Diseases Intelligence* 35(2): 107, Figure 56.

The *Australian Immunisation Handbook* states that:

> *There is epidemiological evidence which indicates that there is **NO** causal association between immunisation and the following events:*
> - *sudden infant death syndrome (SIDS) and any vaccine,*
> - *autism and MMR vaccine,*
> - *multiple sclerosis and hepatitis B vaccine,*
> - *inflammatory bowel disease and MMR vaccine,*
> - *diabetes and Hib vaccine,*
> - *asthma and any vaccine.*
>
> (*Australian Immunisation Handbook*, 9th ed., p. 61)

Contraindications to vaccination

The handbook contains detailed instructions for the administration of vaccines to all different groups. In a very few cases, vaccination is NOT recommended. Because there is a small group of individuals in the community for whom vaccination would be unsafe, it is even more important to maintain herd immunity to protect them from infection.

> *There are only 2 absolute contraindications applicable to all vaccines:*
> *(i) anaphylaxis following a previous dose of the relevant vaccine, and*
> *(ii) anaphylaxis following any component of the relevant vaccine.*
> *There are 2 further contraindications applicable to live (both parenteral and oral) vaccines:*
> *(iii) Live vaccines should not be administered to individuals with impaired immunity, regardless of whether the impairment is caused by disease or treatment. The exception is that, with specialist advice, MMR can be administered to HIV-infected individuals in whom impaired immunity is mild.*
> *(iv) In general, live vaccines should not be administered during pregnancy, and women should be advised not to become pregnant within 4 weeks of receiving a live vaccine.*
>
> (*Australian Immunisation Handbook*, 9th ed., p. 20)

It is obvious that the risks associated with immunisation are much less than the risks from having the disease. More importantly, as explained before, a high level of immunisation in the community protects not only immunised individuals, but also the infants who are not old enough to be immunised. Thus, the process of immunisation has wider implications for the community than merely protecting the health of the individual.

The NHMRC has issued recommendations regarding the attendance at school and daycare centres of children who are not adequately immunised. Victoria, the ACT and New South Wales have enacted legislation requiring the provision of immunisation certificates prior to school entry. Children who are not vaccinated against particular diseases are excluded from school when an outbreak of one of the diseases occurs.

Homoeopathic immunisation

Some natural health practitioners advocate homoeopathic immunisation as a safe alternative to the recommended schedule. However, the NHMRC childhood immunisation schedule has been shown to prevent tetanus, diphtheria and poliomyelitis and give a high level of protection against whooping cough, measles, mumps and rubella. 'Homoeopathic "immunisation" has *not* been shown to give protection against infectious diseases; only conventional immunisation procedures produce measurable immune response' (*Australian Immunisation Handbook*, 9th ed., p. 359).

The Council of the Faculty of Homeopathy, London, issued a statement in 1993, which reads:

> *The Faculty of Homeopathy, London, strongly supports the conventional vaccination program and has stated that vaccination should be carried out in the normal way, using the conventional tested and proved vaccines, in the absence of medical contraindications.*
>
> (*Australian Immunisation Handbook*, 9th ed., p. 359)

INFECTIOUS DISEASES IN CHILDCARE CENTRES

About a million children in Australia attend preschool or long-daycare centres. This is a reflection of changing lifestyles, with many more mothers in the workforce than in previous years. The centres cater for children from a few weeks of age up to 5 years. Babies, whose immune system is not mature, and toddlers who have not been fully immunised are therefore exposed to a community of people who may be carrying a range of infectious diseases. There is considerable evidence that children attending these centres suffer from more infectious diseases than children cared for at home. Some of these diseases are minor, but others can be life-threatening, especially in unimmunised babies. Staff employed in childcare centres are also at risk of contracting some of these infections.

It is important that childcare workers have a thorough knowledge of the important diseases of childhood and understand the principles of transmission of microorganisms and control of infection. These principles are similar to those applied in any health facility. Many trained nurses and health workers are employed in childcare centres and can contribute to the general standard of health and hygiene.

The childcare centre is a unique environment, populated by a large number of people (and microorganisms) from a range of diverse backgrounds. It is different from the child's own home where the population of microorganisms is similar in all members of the family. At home, infants do not come into contact with a large number of people and those they meet are usually immune to childhood diseases and so cannot transmit them.

A feature of this type of facility is the close personal contact between babies, infants and carers, allowing for easy transmission of microorganisms. It is not possible to prevent the spread of all infections, but certain measures can be taken to minimise outbreaks of serious illness. The

ways in which pathogens are transmitted are described in Chapter 8.

Special precautions therefore need to be used in these settings to minimise the risk of infection. The NHMRC's 2005 publication, *Staying Healthy in Child Care: Preventing Infectious Diseases in Child Care*, deals with all aspects of infection control in childcare centres. It is available online at <www.nhmrc.gov.au/guidelines>. Here, we look briefly at some of the most important points.

Modes of transmission

Aerosol droplets

One of the most common methods of transmission is via aerosol droplets. Pathogens may be shed in secretions from the nose and mouth and transmitted to other children by direct inhalation or by contamination of an object which is handled or placed in the mouth. The diseases transmitted in this way include colds and influenza, diphtheria, whooping cough, measles, meningitis, rubella, respiratory syncytical virus (RSV), viral gastroenteritis and chickenpox, as well as bacterial infections such as *Streptococcus pneumoniae* and *Haemophilus influenzae* (Hib). It is difficult to prevent airborne transmission in confined areas. Secretions from the nose and mouth can be contained by using tissues for nose-blowing, by careful handwashing, and by regular washing and disinfection of utensils and toys.

Faecal–oral transmission

A significant part of the daily activities of childcare involves toileting. Babies and infants require nappies to be changed; older children need to be toilet trained and are prone to accidents. The potential exists for the microorganisms present in the faeces to be liberally spread around. Viruses, bacteria and parasites may be present not only in the faeces of ill children with obvious signs of diarrhoea, but also in the faeces of infected but asymptomatic children. It is very easy for the microorganisms to be transferred from one child to the next, via hands contaminated in the toilet area being put in the mouth. They may also be present on other objects or equipment such as toys, cups, toilets, tap handles, toilet flush buttons, or in nappy-changing areas, on the floor or on table tops.

The types of infection transmitted in this way include those responsible for viral or bacterial gastroenteritis: *Shigella*, *Campylobacter*, *Salmonella*, norovirus and rotavirus, as well as hepatitis A, hand, foot and mouth disease (coxsackie virus), and the protozoa *Giardia* and *Cryptosporidium*.

Suspected outbreaks of gastroenteritis should be notified to the relevant health authorities so that investigations and control measures can be implemented. Children with diarrhoea should be excluded.

Children need to be taught how to wash their hands after going to the toilet. Staff also need to be thoughtful about handwashing. The spread of organisms around the centre can be minimised by following correct procedures for nappy-changing areas and use of the toilet. Contaminated areas should be cleaned and disinfected promptly, but care must be taken that infants do not come into contact with harsh disinfectant solutions. The use of disposable nappies, and the wearing of pants and other clothing, can also reduce the spread of faecal microorganisms.

Skin infections

Children are very prone to skin infections, associated usually with minor scratches, abrasions or mosquito bites. The infections are often caused by staphylococci or streptococci; they can easily develop into impetigo and, in severe cases, cause systemic infections. It is important that infected skin sores are treated with antibiotics and the sore is covered to prevent shedding of the bacteria on to floors, carpets or bedding where they can be transmitted to other children. Children sitting on the floor when they have uncovered infected sores on their legs is a common way of spreading these skin infections. In serious cases of impetigo, the child may be excluded from the centre unless the sores can be adequately covered.

A common problem is infestation with head lice. Although the lice do not carry any disease, the irritation and constant scratching can break the skin. Lice spread easily and lay eggs on the hair shafts. Parents should be informed and advised about the best methods of treating the lice. Many lice are now resistant to chemicals that were previously effective.

Pathogens in blood and body fluids

Hepatitis B, hepatitis C and human immunodeficiency virus (HIV) are transmitted by direct contact with blood or body secretions. Contact with urine and saliva can be responsible for the transmission of mumps and cytomegalovirus. Staff should be aware of the use of Standard Precautions.

Prevention of cross-infection

Various strategies can be employed to prevent cross-infection. Many of these are derived from similar practices in hospitals and health facilities. They are based on a knowledge of the different types of microorganisms responsible for infectious diseases and an understanding of the method of transmission of each pathogen.

Handwashing

Regular handwashing before and after each task and contact with children (especially with respiratory secretions) is the most effective method of infection control. Gloves should be worn when handling body substances, and hands should be washed after removing the gloves. Good hygiene, thorough cleaning and disinfection procedures all play an important part in preventing the transmission of infections. Children should also be encouraged to wash their hands when they arrive and before they leave the centre, after toileting or nappy changing, before eating and after playing outside (see Figure 14.16).

Separation of tasks

It is important that childcare workers are aware of the importance of separate areas for different tasks. For example, staff who are involved with food preparation should not also

FIGURE 14.16

Well-designed toilet and handwashing facilities encourage good hygiene

Source: Dr Penny Bishop.

change nappies. There is always the possibility of contamination of their clothing as well as their hands, so it is preferable that these duties are performed by different personnel. If this is not possible, staff should use protective clothing and be aware of the importance of thorough handwashing between tasks. If any of the surfaces, taps or door handles in the kitchen are contaminated, there is the potential for the organism to contaminate the food or eating utensils and thus be spread to all the children.

Food preparation is an important area where infection can easily be spread. Staff should be aware of regulations regarding the handling of food. Hands should be washed before preparing food and all surfaces and utensils involved in food preparation kept scrupulously clean. Children's hands should be washed before eating and they should not share food or utensils or anything else they may put in their mouths (e.g. dummies, toothbrushes). The food should be well heated and served at once or else covered and refrigerated. It should not be allowed to stand at room temperature for any length of time before eating (see Figure 14.17).

FIGURE 14.17

The use of separate plates and utensils can minimise transmission of infection

Source: Dr Penny Bishop.

Sharing of toys is a common way for pathogens to be transmitted from one child to another. Toys should be washable and cleaned or disinfected at least once a week to minimise the transfer of microorganisms. Children should have their own personal items such as toothbrushes.

Sometimes the most effective way of preventing the spread of disease is to place children in separate groups or play areas depending on their level of susceptibility (i.e. age and immune status).

Protection against disease

Immunisation was discussed earlier in this chapter. It is the most effective method of protection against many childhood diseases. Legislation allows schools and childcare centres to exclude unimmunised children when there is an outbreak of an infectious disease for which a vaccine is available. The recommended schedule for childhood immunisations is given in Table 14.4, page 335.

Immunisation not only protects the person who has been immunised, but also children who are too young to be immunised or those who have been vaccinated but did not respond. As discussed earlier in this chapter, it is essential to establish a high level of **herd immunity** in these susceptible communities. Parents who refuse to have their children immunised are placing at risk all children who are too young to be fully immunised. This is very important in the childcare setting, where close contact allows for easy transmission of pathogens, especially by the respiratory route. Serious, vaccine-preventable diseases such as diphtheria and whooping cough can be fatal in small infants.

Exclusion of sick children

To prevent the spread of disease in a centre, it is sometimes necessary to exclude sick children and their contacts from the centre until they are no longer infectious to others. Exclusion of infectious children is an important way of breaking the chain of infection in a centre. The NHMRC (2005) has published guidelines for the recommended minimum periods of exclusion of infected children *and their contacts* from school, preschool and childcare centres.

Occupational risks for childcare workers

Staff in childcare centres are also exposed to the infectious diseases that affect the children. Scrupulous attention to handwashing can prevent the transmission of some of these infections to staff. All staff should make sure they are fully immunised, especially as some childhood diseases, such as chickenpox, can be more serious in adults. Additional vaccines that are appropriate for childcare workers are hepatitis A, hepatitis B and chickenpox.

Children who are born overseas are sometimes infected with tuberculosis (as indicated by a positive Mantoux or Tuberculin test) and their dormant infection may become active after their migration to Australia. Childcare workers should be aware of the increased risk of disease in children from countries with a high prevalence of TB. Regular Mantoux testing of staff may be advised in centres with a high migrant population.

Childcare workers should be aware that some infectious diseases can have serious consequences if contracted during pregnancy. It is especially important for women to be protected against rubella, for which a vaccine is available. If the mother suffers from **rubella** during the first trimester, the baby may be born deaf or blind, or with heart or lung damage. Women intending to become pregnant can have a blood test to determine their rubella antibody titre from previous disease or vaccination, and a booster vaccination may be advisable before becoming pregnant.

Cytomegalovirus (**CMV**) is a mild disease that is very common in young children. Infection in early pregnancy can have serious effects on the unborn child. Staff can be tested before becoming pregnant to ascertain their level of previous exposure. No vaccine is available. If there is a suspected case in the childcare centre, pregnant women should avoid contact with urine and saliva and have a blood test to determine their immune status and thus assess the risks involved.

Chickenpox infection during pregnancy has been shown to lead to a slightly increased risk of congenital damage. Infections with **human parvovirus**, *Erythema infectiosum* (fifth disease), causes miscarriage in a small percentage of women.

HEALTHCARE IN RURAL AND REMOTE AREAS

The challenges of the provision of healthcare in remote areas of Australia are often quite different from those encountered in large city hospitals. Various factors influence the type and severity of the infectious diseases encountered. They include:

- climate and environmental conditions that favour some microorganisms that are not commonly found in other parts of Australia
- socioeconomic factors such as education, unemployment and lifestyle, as well as availability of good housing, sanitation and clean water supplies
- availability and access to health services, and the level of compliance with public health measures such as immunisation.

In many areas of rural Australia, certain diseases occur with a frequency above the national average. This is particularly true in the unique tropical northern regions of Australia, which include the Northern Territory, north Queensland and the Kimberley region of Western Australia. The range and incidence of infectious diseases vary between regions and are significantly different from the rest of Australia. These areas have a wide range of vegetation and a tropical climate, and are the habitat for organisms not usually found in other parts of Australia. When humans disturb the natural ecology of these regions they may be exposed to new microorganisms.

The special health problems faced by people in the northern parts of Australia are the subject of a number of ongoing projects at the Menzies School of Health Research in Darwin. As well, the Northern Territory Department of Health and Community Services collects and coordinates the publication of reports of infectious diseases in the quarterly *Northern Territory Disease Control Bulletin*, which is also available online at <www.nt.gov.au/health>.

Aboriginal health

The northern tropical areas of Australia have a larger proportion of Aborigines and Torres Strait Islanders than other regions. There have been numerous studies and reports dealing with the health status of Indigenous people. They highlight the disparity in general health and incidence of infectious diseases that exists between Indigenous and non-Indigenous people (see Australian Bureau of Statistics Report 2008 and Australian Health Ministers' Advisory Council. *Aboriginal and Torres Strait Islander Health Performance Framework Report 2008* at <www.health.gov.au>).

Aboriginal and Torres Strait Islander people are disadvantaged in terms of most socioeconomic indicators (income, education, employment, housing). The burden of both communicable and non-communicable disease is 2.5 times that of non-Indigenous people and they have a lower life expectancy (up to 20 years less) than other Australians. This is most noticeable in the high mortality rate among young adults (20–40 years). The overall mortality rate for Indigenous Australians is at least twice the rate for the Australian population as a whole. The largest difference is in the middle age group (35–54), where the ratio is five to six times that for non-Indigenous Australians. Although the mortality rates and infant mortality rates have decreased in recent years, the gap between Indigenous and non-Indigenous people has not changed. Many deaths are due to circulatory diseases, hypertension, chronic heart disease, respiratory diseases, diabetes, mental and behavioural disorders, alcohol abuse and trauma, but there are also significant differences in the morbidity and mortality rates for infectious diseases. Underlying diseases contribute to the severity and morbidity of infectious diseases (see Figure 14.18).

Approximately 25 per cent of Aborigines and Torres Strait Islanders live in remote areas, mainly the Northern Territory, including the Top End and Central Australia. While many Aborigines living in urban environments experience poorer health and lower socioeconomic conditions than other Australians, Aborigines in the north are also exposed to a number of unique tropical diseases. The overall notification rate for communicable diseases from the Northern Territory is six times the national average, and mortality rates are three to four times greater than the rate for non-Aboriginal people in the Territory (see Table 14.6, page 352).

At all stages of their lives, Aboriginal people in remote areas experience poorer health than non-Aboriginals. Despite improvements in recent years, the infant mortality rate (in the first year of life) is still two to three times higher than the rest of Australia. Disorders of growth and nutrition are prevalent among Aboriginal people. There has been an increase in the numbers of low birth weight babies, and infants are often malnourished and vulnerable to a range of infections and other diseases such as pneumococcal

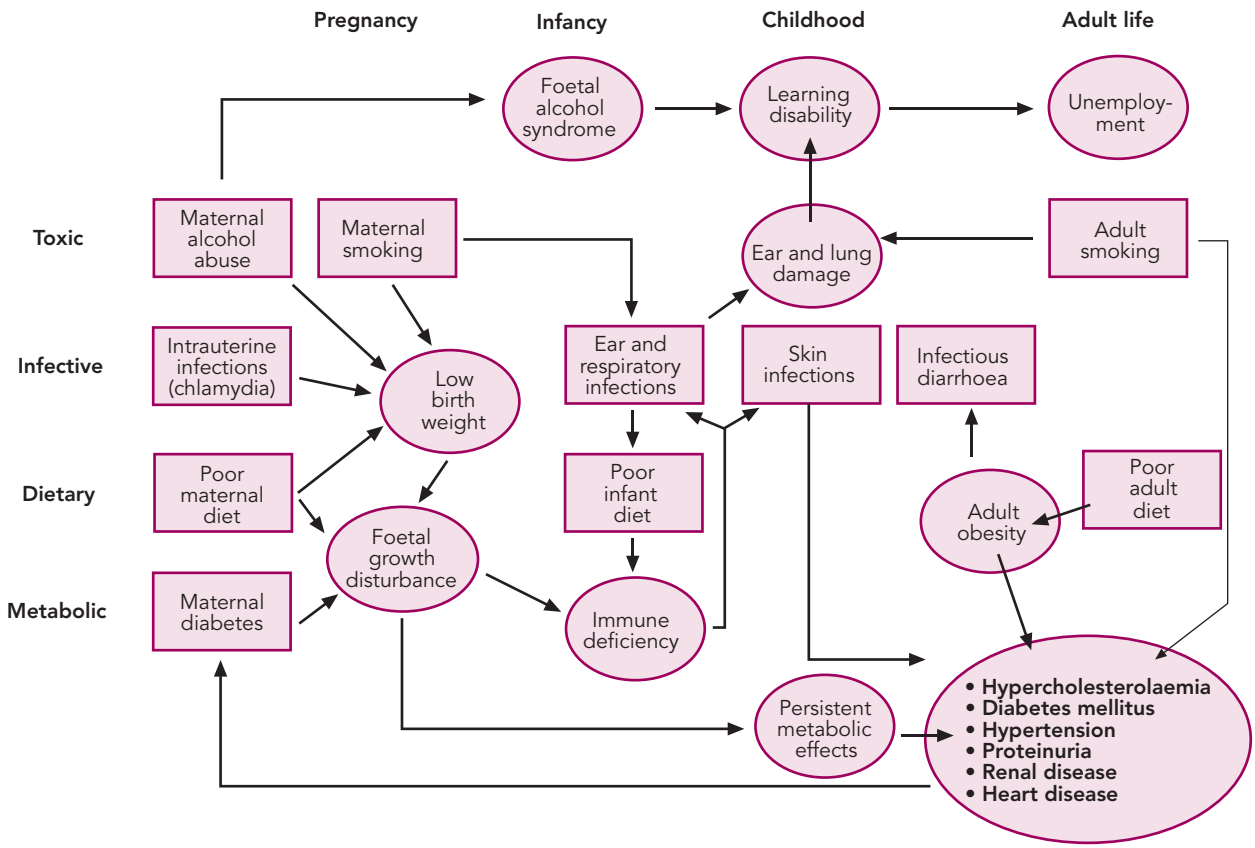

FIGURE 14.18

Diagram of Aboriginal health

Source: J. Mathews et al. 1995, Aboriginal Health, Menzies School of Health Research, Darwin. Reproduced with permission from *Today's Life Science*, 7(8): 24.

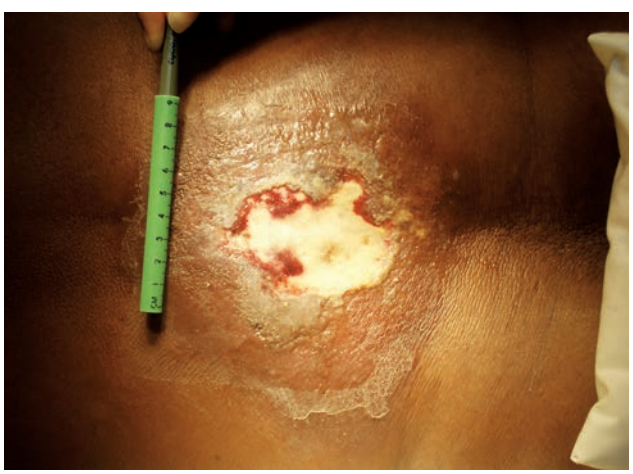

FIGURE 14.19

Severe staphylococcal abscess resulting from a delay in seeking medical treatment

Source: Dr Christine Bishop.

meningitis. In all age groups, there are serious diseases that occur more commonly in Aboriginal than in non-Aboriginal people. They include diabetes, circulatory disorders, eye and ear infections, and other communicable diseases. Many of these diseases are directly related to deficiencies in nutrition, lifestyle and housing. Many Aboriginal people living in remote areas delay seeking medical treatment—often resulting in a more serious outcome (see Figure 14.19).

Among the communicable diseases, Aborigines have a much higher incidence of sexually transmitted infections and tuberculosis than the rest of Australia. The risk of death from cervical cancer for Aboriginal women is much higher than in non-Aboriginals, especially in rural and remote areas. To improve Aboriginal health, there is general agreement on the need to coordinate services at the community level, with community-controlled health services and Aboriginal health workers taking a prominent role in the delivery of primary healthcare and the implementation of specific programs. It is also essential that health professionals have a good understanding of the culture of the people for whom care is being provided. Compliance with vaccination used to be below the national average but has improved in recent years and is now similar to the national average.

Infectious diseases in the Aboriginal population

Overcrowded living conditions and poor hygiene in many Aboriginal communities lead to a high rate of bacterial

colonisation. The carriage of bacteria by infants and young children provides a reservoir of infection and allows cross-infection and reinfection to occur. As well as a high incidence of notifiable disease, bacterial infections are responsible for chronic morbidity (illness) and mortality in many Aboriginal children. Complications (sequelae) of bacterial infections include chronic bronchitis, blindness, infertility, deafness, rheumatic heart disease and renal failure in adult life.

Group A *Streptococcus pyogenes* (GAS)

Streptococcal impetigo is frequently seen in Aboriginal children. A number of factors contribute to the existence and persistence of skin sores. Skin lesions due to minor trauma, mosquito bites and scabies infestations (see Figure 14.20) can become infected with bacteria. Scabies is endemic in most Aboriginal communities and transmission occurs via close body contact, bed linen, etc. Continuous itching and scratching of scabies allow secondary bacterial infections with GAS to occur.

Various sequelae (outcomes) are attributed to infection with GAS, including **rheumatic fever** and **acute post-streptococcal glomerulonephritis** (**APSGN**). These diseases are commonly seen in Aboriginal communities, attributed largely to overcrowded living conditions. Such diseases are rarely seen in developed countries if streptococcal infections are well managed.

Rheumatic fever may cause damage to the aortic and mitral valves of the heart due to immunogenic reactions to streptococcal antigens. The weakened heart valves are susceptible to further infection and disease later in life (see Chapter 20).

Epidemics of acute post-streptococcal glomerulonephritis occur at regular intervals in Aboriginal communities in the Northern Territory and Far North Queensland. However, **glomerular haematuria** and/or **proteinuria** (blood and protein in the urine) are also common in non-epidemic circumstances (the incidence is 20 per cent in school-age children) and patients with infected skin sores are more likely to have evidence of renal impairment. The lack of access to renal dialysis machines is another problem, as many Aborigines are unwilling or unable to travel long distances to large centres where facilities are available. The incidence of end-stage renal failure is about 100 times greater for Bathurst and Melville Islanders than for other non-Indigenous Australians, and rates for other Northern Territory Aborigines are about ten times the national average. The contribution that persistent childhood infections with GAS makes to chronic renal disease is uncertain, as other factors—including diabetes, obesity and hypertension—also play a role.

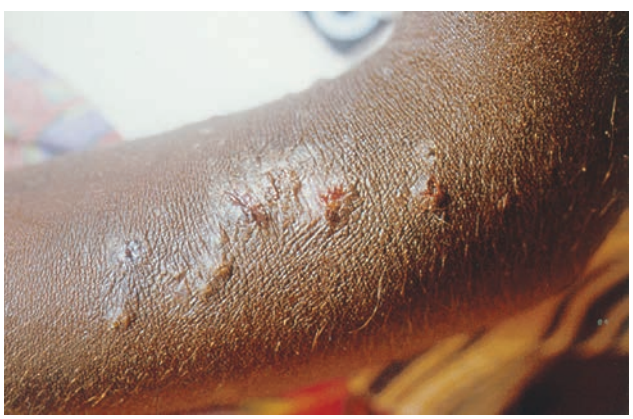

FIGURE 14.20

Streptococcal skin infection associated with scabies infestation

Source: © Professor Bart Currie, Menzies School of Health Research, Darwin.

Meningococcal disease

The incidence of meningococcal disease in Indigenous Australians is still nearly five times that in non-Indigenous people. Serogroup B, for which there is no vaccine, accounts for nearly 90 per cent of infections in Aborigines and Torres Strait islanders. Serogroup C causes 15 per cent of disease in Indigenous people, compared with about 30 per cent in non-Indigenous people.

Otitis media

Chronic **otitis media** (**OM**) (middle ear disease) and associated hearing loss are prevalent among Australian Aborigines. About 50–80 per cent of school-age children have been shown to be affected, as well as a significant proportion of adults. Perforated eardrums are present in up to 40 per cent of infants aged less than 12 months. Recent studies in the Northern Territory have shown that otitis media develops in Aboriginal infants below the age of 3 months, with a high prevalence of non-inflamed but immobile eardrums, **otitis media with effusion** (**OME**). This can progress to **acute otitis media** and develop into **chronic suppurative otitis media** (**CSOM**) with associated loss of hearing.

The rapid and early onset of otitis media in Aboriginal children is associated with early nasal colonisation by a number of bacteria—in particular, *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella (Branhamella) catarrhalis*, which are able to spread to the middle ear. It was hoped that the introduction of the 7vPCV vaccine might decrease the incidence of otitis media, but studies have shown no effect as the infections are often established by 3 months of age, well before the vaccine is administered.

Ear infections leading to hearing impairment have serious educational outcomes for the affected individuals. Students with fluctuating hearing loss are likely to exhibit poor behaviour patterns and to experience learning difficulties. Lack of hearing and subsequent poor communication skills may cause children to perform poorly or to stop attending school altogether.

Trachoma

Trachoma is a chronic conjunctivitis caused by repeated episodes of infection with *Chlamydia trachomatis*. If left untreated, trachoma can lead to blindness. It begins as conjunctivitis, called 'follicular' or inflammatory trachoma, seen mainly in young children. This can lead to cicatricial

trachoma. Scarring of the eyelids causes the eyelashes to turn inwards (trichiasis), the cornea becomes opaque and blindness results. The disease is spread person to person and within the family unit.

Although the prevalence has decreased over the last 20 years, the *Australian Trachoma Surveillance Report* identifies 243 remote Aboriginal communities considered to be at risk. Screening in 150 of these communities has identified a follicular trachoma rate in 1- to 14-year-olds of 19 per cent in South Australia, 12 per cent in the Northern Territory and 9 per cent in Western Australia. The occurrence of trichiasis increases with age. Screening also measures the percentage of children with 'clean faces', a program aimed at reducing exposure to infection.

The recommended treatment for trachoma is azithromycin, a macrolide antibiotic that is effective when used as a single dose. It replaces prolonged courses of tetracycline ointments or drops. In areas where it has been used, a 95 per cent success rate has been recorded.

Haemophilus influenzae type b (Hib) infections

As well as giving rise to otitis media, infections due to invasive *Haemophilus influenzae* type b (Hib) cause meningitis, pneumonia, epiglottitis, septicaemia, osteomyelitis, septic arthritis and cellulitis. Children under 5 years of age are particularly susceptible, and children in the Northern Territory and Far North Queensland, especially Aboriginal children, used to have among the highest rates of Hib infection in the world. The introduction of Hib vaccine has seen a 98 per cent fall in the incidence of Hib disease in the age groups targeted by vaccination programs in the Northern Territory as well as the rest of Australia (see Figure 14.11, page 337).

Streptococcus pneumoniae

As well as causing otitis media in children, this organism is a significant cause of pneumonia and meningitis in children and adults. In the Northern Territory, invasive pneumococcal disease is responsible for 40 per cent of community-acquired bacterial pneumonia in adult Aborigines admitted to hospital, with a mortality rate of 21 per cent, especially in older people. The 23vPPV pneumococcal vaccine was introduced for adult use in 1999 and a polyvalent conjugated vaccine, 7vPCV, became available in 2001 for use in children under 2 years of age. The incidence of invasive disease is still four times higher in the Northern Territory than in other parts of Australia, but since the introduction of the vaccination programs overall notification rates have decreased and the incidence of serious pneumococcal disease is similar in Indigenous and non-Indigenous children.

Respiratory syncytial virus

Respiratory syncytial virus (RSV) is a major cause of lower respiratory tract infection in young children, causing bronchiolitis, pneumonia and tracheobronchitis, which often require hospitalisation. Data from Western Australia and north Queensland show that the rate of hospital admissions for bronchiolitis was at least three times higher for Indigenous children than for non-Indigenous children.

Gastrointestinal infections

The rates in the Northern Territory for enteric diseases caused by *Shigella*, *Salmonella*, *Campylobacter* and hepatitis A are all significantly higher than in other parts of Australia. The highest incidence occurs in children under 5 years of age, and the ratio of Aboriginal to non-Aboriginal infections is as high as 30:1. Shigellosis is the most infectious of the bacterial enteric diseases. Seasonal outbreaks of gastroenteritis due to the protozoan *Cryptosporidium parvum* have been reported from Alice Springs.

Hepatitis

A seroprevalence study of Aboriginal children in the Top End has shown a high burden of exposure to hepatitis A by the age of 5 years. Similar data are not available for non-Aboriginal children in the Northern Territory. The high incidence of hepatitis B carriers in the Aboriginal population has been recognised for some time and the Northern Territory was the first to introduce hepatitis B vaccination into the recommended childhood schedule.

Tuberculosis

The Northern Territory has the highest incidence of notifications of new cases of tuberculosis in Australia, at about six times the national average. Although there is a high level of TB in the Aboriginal population, two-thirds of all notifications in Australia are for foreign-born individuals.

Leprosy

A few cases of leprosy are notified each year, spread among all states. Early diagnosis and use of the WHO-recommended multi-drug therapy can help to prevent serious neurological damage.

HIV

The pattern of HIV infection in Aboriginal and Torres Strait Islanders varies from that in non-Indigenous Australians. Data for 2006–10 (see Figure 14.21) show that, whereas the main route of transmission in both groups is sexual contact between men, heterosexual contact was the reported route of exposure in 18.5 per cent of Indigenous cases compared to 16 per cent in non-Indigenous cases; 19.4 per cent of infections were related to injecting drug use, compared to 2.5 per cent in the non-Indigenous population; and 21.4 per cent of infections occurred in women, compared with 8 per cent of non-Indigenous cases.

Sexually transmitted infections

Sexually transmitted infections (STIs) account for over 50 per cent of all notifications of communicable diseases from the Northern Territory. They occur mainly in the 15–50 age group, with a higher frequency in Central Australia than in the Top End, which may reflect the higher proportion of Aboriginal people in Central Australia. Syphilis, gonorrhoea and chlamydia are the most commonly notified STIs,

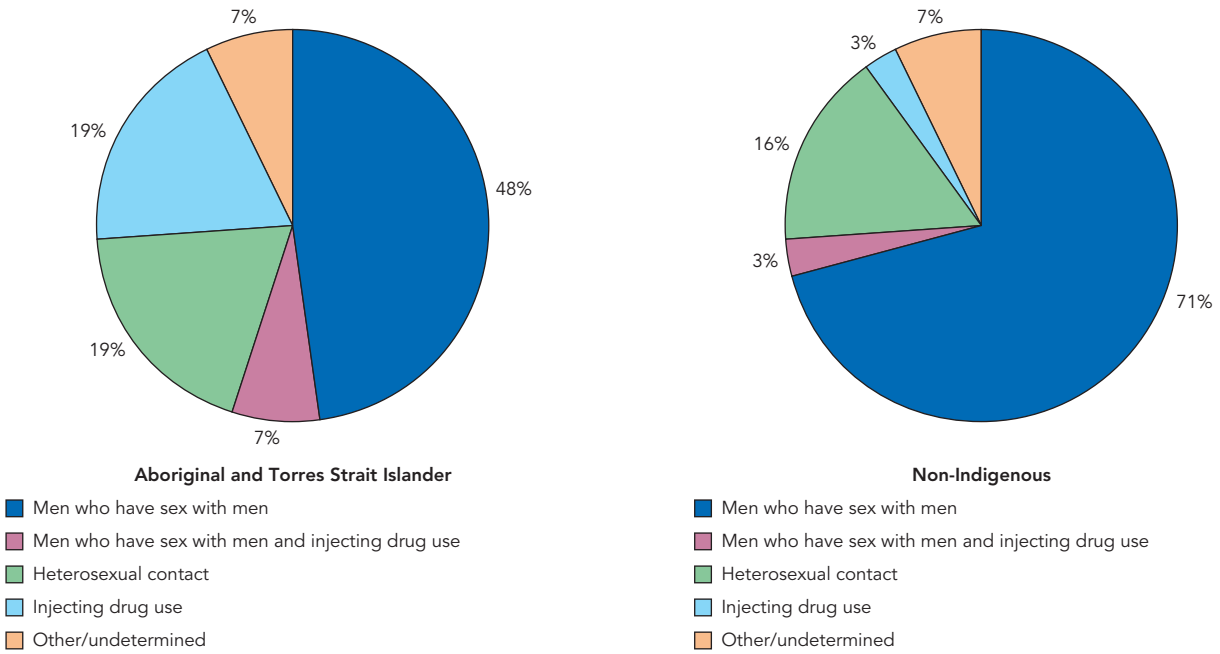

FIGURE 14.21

HIV diagnoses, 2006–10, by Aboriginal and Torres Strait Islander category and HIV exposure

Source: Kirby Institute, *Annual Surveillance Report 2011*: 20, Figure 22. The Kirby Institute, University of New South Wales, Sydney, NSW.

followed by genital herpes, non-specific urethritis (NSU) and donovanosis. Donovanosis is now much less common due to programs of treatment with azithromycin. It is rarely seen in the southern parts of Australia.

The high level of STIs in the Northern Territory is of concern because of the possibility of the spread of AIDS. STIs that have ulcerative lesions, such as syphilis and donovanosis, provide an increased risk factor for transmission of the AIDS virus.

Table 14.6 shows the notification rates of selected diseases that occur more frequently in rural and remote areas. The data for the Northern Territory, Western Australia

TABLE 14.6 **Notification rates per 100 000 population for selected notifiable diseases by state or territory, 2009**

DISEASE	ACT	NSW	NT	QLD	SA	Tas	Vic	WA	AUST. AVERAGE
Hepatitis B	28	37	67	23	27	15	35	36	32
Cryptosporidiosis	30	20	66	33	6	13	19	10	21
Salmonella	64	38	216	56	42	33	30	50	43
Shigella	2	2	38	3	3	0.4	2	5	2.8
Chlamydia	27	210	940	379	231	290	255	395	286
Gonorrhoea	16	25	669	36	25	4	28	60	37
Syphilis	9	13	61	11	3	6	16	8	12
Invasive pneumococcal disease	8	7	38	6	9	7	7	7	7
Chicken pox	0.6	NN†	39	3	29	7	10	14	11
Tuberculosis	6	7	12	5	3	2	8	5	6
Barmah Forest virus	1	5	52	18	22	0.6	0.3	7	7
Ross River fever	0.6	13	190	49	20	6	2	38	22
Dengue fever	5	2	12	24	1	0.4	0.7	6	6

† NN = Not notifiable.

Source: Australia's Notifiable Disease Status, 2009, Annual Report of the National Notifiable Diseases Surveillance System, NNDSS Annual Report Writing Group, 2011, *Communicable Diseases Intelligence* 35(2), Table 5, p. 70.

and Queensland reflect not only the tropical climate but also the increased burden of disease in the Indigenous population in those areas.

Unusual diseases of rural and remote areas

As discussed above, the remote areas of Australia provide a unique environment for the occurrence of diseases that are not commonly seen in more settled areas.

Melioidosis

Melioidosis is the most common cause of fatal, community-acquired bacterial pneumonia in the Northern Territory. It is caused by a soil organism, *Burkholderia pseudomallei* (formerly *Pseudomonas pseudomallei*), which is endemic in northern Australia. After heavy rain during the wet season the bacteria are found in surface water and mud, and can become airborne. It usually gains entry to the body through breaks in the skin during exposure to soil or water. Percutaneous exposure may be followed by the bacterium spreading to the lungs and causing pneumonia. Melioidosis can cause abscesses in the skin or in deep tissue and body organs (Figure 14.22).

There is a spectrum of presentations, from fulminant sepsis with a 25 per cent fatality rate to subclinical infections that may reactivate years later. Healthy people are less susceptible to the disease, but those with risk factors including diabetes, alcohol-related problems and renal disease are particularly vulnerable. Prompt diagnosis and treatment is required to prevent fatal outcomes. Environmental factors such as rainfall contribute to variations in incidence. Heavy rainfall and flooding in 2010–12 contributed to a higher-than-average incidence of melioidosis. In the Northern Territory's Top End in 2010 there were 91 cases and 11 people died, and in 2011 there were 70 cases and 9 died. Cases were also reported from Central Australia.

Leptospirosis

Leptospirosis is a zoonosis with worldwide distribution and is now recognised as a re-emerging disease. The disease is likely to be underdiagnosed in visitors or people returning to Australia and may need to be considered among the many causes of travel-associated febrile illness.

In Australia, leptospirosis occurs mainly in rural Victoria, Queensland and the Northern Territory. It is caused by a spirochaete, *Leptospira interrogans*, serovar *australis* being the one most commonly associated with serious infections in Australia. Symptoms include headache, chills, myalgia, rash and, sometimes, meningitis and jaundice; in severe cases there may be pulmonary haemorrhage and liver and kidney failure. The disease is usually a result of occupational exposure to animals and animal urine (see Figures 14.23 and 14.24).

Transmission to humans occurs by skin contact (especially if broken) with the tissues or urine of infected animals or with contaminated water, soil or vegetation. It can also be contracted by recreational exposure to contaminated water (e.g. in national parks). Reservoirs include domestic and native rodents, bandicoots, rabbits, cattle and feral pigs. The incidence is highest in north Queensland, especially among workers in the banana industry. The significant flooding that

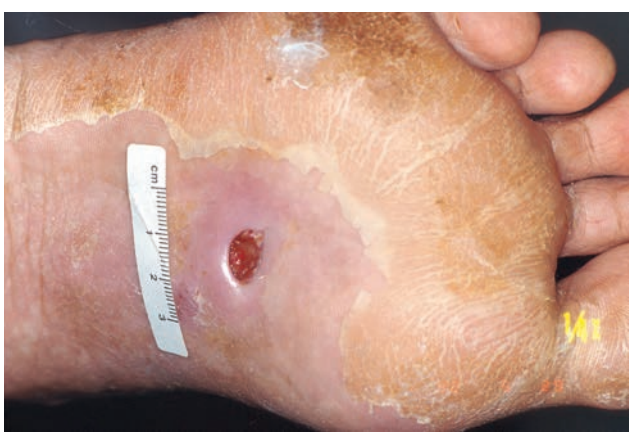

FIGURE 14.22

Lesion of melioidosis

Source: © Professor Bart Currie, Menzies School of Health Research, Darwin.

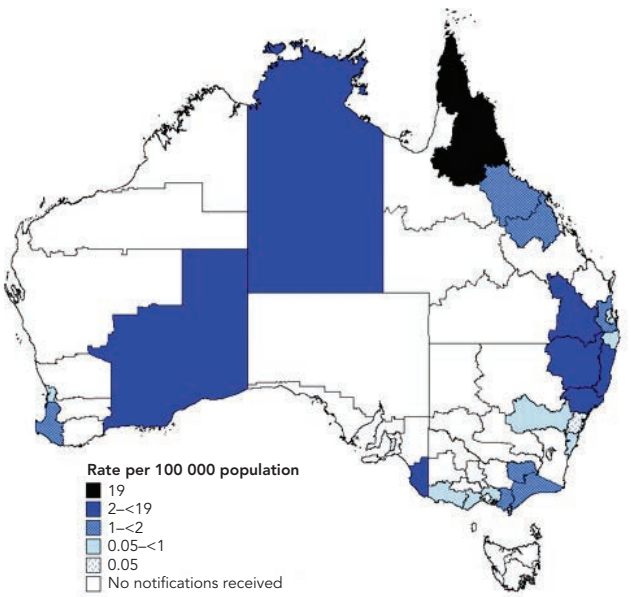

FIGURE 14.23

Distribution of leptospirosis in Australia, 2003

Source: NNDSS 2005, *Communicable Diseases Intelligence* 29: 51.

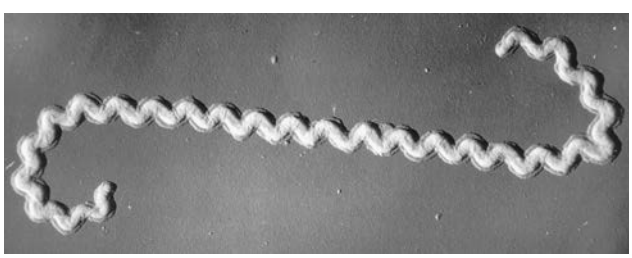

FIGURE 14.24

Scanning electron micrograph of *Leptospira interrogans*

Source: Electron micrograph taken by Annabella Chang and provided by Ben Adler, Monash University.

occurred in southern Queensland in 2010–11 contributed to the spread of the bacteria, resulting in an increased rate of leptospirosis notifications.

Rickettsial diseases

Scrub typhus is caused by the rickettsia *Orientia tsutsugamushi* and is transmitted to humans by the bite of a mite that lives on rodents. It occurs wherever the host mite is found and is widely distributed throughout areas of South-East Asia, the north-west of Western Australia, Far North Queensland, the Torres Strait Islands and in Litchfield Park in the Northern Territory.

It is difficult to diagnose as symptoms are non-specific headache, fever, myalgia and a maculopapular rash affecting the trunk. An eschar may be visible at the site of the bite (see Figure 14.25). Scrub typhus can be successfully treated with doxycycline or azithromycin but has a 50–60 per cent mortality without antibiotic treatment.

Queensland tick typhus (**spotted fever**) is caused by *Rickettsia australis* that is transmitted to humans by a tick bite. The disease occurs along the east coast of Australia, including Queensland. It is usually a mild disease with symptoms of fever, headache and myalgia, although there have been reports of cases with complications including renal failure and pneumonia.

Q fever is caused by the rickettsia *Coxiella burnetii* and occurs mainly in cattle-raising areas, although it is not seen in the Northern Territory. Transmission is by the airborne dissemination of the organism in dust particles, or by contact with contaminated animals. Abattoir workers, meat packers and stockyard workers are most at risk. A vaccine is available.

Arboviruses

Arboviruses, including Ross River virus, Barmah Forest virus, Kunjin virus and Murray Valley encephalitis virus, are carried by mosquitoes and tend to occur mainly in rural areas where mosquitoes breed in the wet season (see Spotlight box: Arboviruses endemic to Australia). The tropical climate of northern Queensland favours the mosquito *Aedes aegypti*, which can act as a vector for **dengue fever**. Although dengue virus is not endemic in Australia, cases of the disease imported by travellers from overseas can cause an epidemic if the mosquitoes are not controlled.

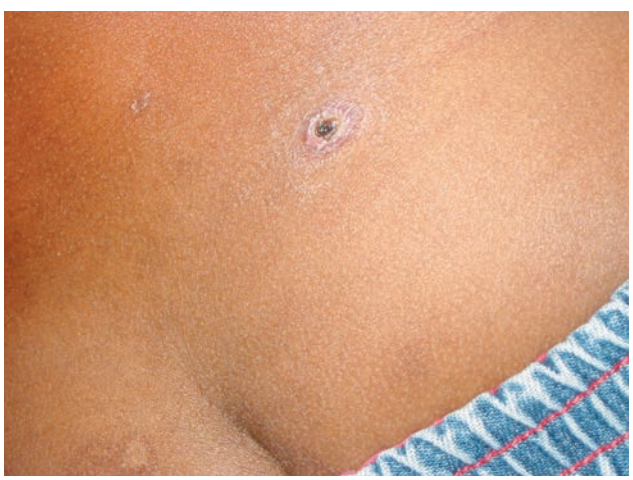

FIGURE 14.25

Typical eschar of scrub typhus due to infection by the rickettsia *Orientia tsutsugamushi*

Implications for healthcare

The range and severity of infectious diseases seen in remote areas, together with the socioeconomic conditions that prevail, pose special challenges to health workers. Because of the distances involved, many people living in these areas often do not seek medical care until the disease is well advanced (see Figure 14.19, page 349). In addition, underlying illnesses may contribute to the severity of the disease and make diagnosis more difficult. Health workers also need to be aware of the customs and taboos of Indigenous people.

Protocols have been developed specifically for these areas, and health workers should be aware of their existence and use them to optimise the treatment and control of infectious diseases. They include:

- specific immunisation schedules
- additions to the list of notifiable diseases
- treatment protocols for use by health workers
- input into the national Antibiotic Guidelines.

INFECTIOUS DISEASES FROM OUTSIDE AUSTRALIA

Australia is fortunate in that, being an island, it is free from some of the infectious diseases that occur in other parts of the world. However, there is always the risk that some of these diseases may be imported into Australia, carried by travellers, tourists, students or immigrants. Government authorities have regulations to prevent the importation of disease into Australia. These involve screening of migrants for diseases such as TB and quarantine of infected animals and goods. Sometimes these procedures fall down, as was shown by the outbreak of equine influenza (EI) that occurred in 2007. As more people undertake overseas travel and more tourists and students visit Australia, the potential for the spread of microorganisms and/or their vectors is increased (see Chapter 1). The entry of illegal immigrants who have not undergone health screening prior to their arrival poses an additional health risk. In this section, we look briefly at some of the diseases that pose potential health problems.

Occasionally, a person suffering from one of these diseases may enter Australia but the disease does not spread because of the lack of an appropriate vector, or does not become established because an intermediate host or animal reservoir is lacking. Diseases such as tuberculosis already occur in Australia, but the incidence is much lower than in other parts of the world and the multi-drug-resistant strains of *Mycobacterium tuberculosis* that are found in other countries are still not common in Australia. Australian residents returning from overseas sometimes present to their doctors with unusual symptoms. Unless a full history, including overseas travel, is obtained, the correct diagnosis may be

SPOTLIGHT ON

Arboviruses endemic to Australia

The wet season in Australia usually begins in November with heavy rains and flooding in Central and Northern Australia and continues throughout the summer, providing extensive breeding grounds for mosquitoes. Health departments in Western Australia, South Australia and the Northern Territory issue warnings to residents and tourists, especially campers, about the risks of being bitten by mosquitoes that might be carrying Ross River virus, or other mosquito-borne diseases. Outbreaks of disease caused by arboviruses (**ar**thropod-**bo**rne virus) have been increasing in recent years, causing significant morbidity.

Ross River virus (RRV) is the most common of a number of arboviruses that are endemic in Australia. Other endemic viruses that cause significant morbidity and occasional mortality are Barmah Forest virus, Murray Valley encephalitis virus and Kunjin virus. Japanese encephalitis and dengue fever occur in Australia but the index case is usually imported.

Ross River virus infection occurs in cycles dependent on environmental conditions such as tides and rainfall. The virus is carried by a number of mosquitoes, the most common being *Aedes vigilax* or 'salt marsh' mosquito, which bites during the day and night, and the common banded mosquito, *Culex annulirostris*, which is found throughout Australia and feeds mainly in the evening and at night. Mosquitoes spread the virus after feeding on an infected animal or person and then biting another human (see Figure 14.26).

The highest incidence of the disease is usually in North Queensland, with the peak season extending from February until May, but in recent years large outbreaks have occurred throughout the summer in other parts of Australia, including the north coast of New South Wales, Western Australia, throughout the Northern Territory and as far as Kangaroo Island in South Australia.

The main preventive measure is to avoid being bitten by mosquitoes. People should wear long-sleeved shirts, loose-fitting protective clothing, use insect repellents, ensure that insect screening is adequate in homes, and avoid known mosquito areas and wetlands, especially at dusk and dawn. Residents should control mosquito breeding around homes by emptying water from containers, pools and guttering. Macropods, especially kangaroos, are the main vertebrate hosts for RRV.

Symptoms of Ross River fever and Barmah Forest viral infection are flu-like chills, fever, pain in joints and muscles, chronic fatigue and headaches. The joints may be swollen and stiff and a rash may occur that lasts for 7–10 days. Most people recover in a few weeks but in some the symptoms of polyarthritis persist for months and even up to a year. The disease causes significant morbidity and loss of earning capacity in rural areas. Tourists returning from endemic areas can spread the disease to the city if the appropriate mosquito vector is present.

The mosquito-borne flaviviruses that cause encephalitis are Murray Valley encephalitis (MVE), Kunjin virus (KV; related to West Nile virus) and Japanese encephalitis (JE). Of these, MVE is the most serious. The symptoms are severe headache, high fever and drowsiness, which in serious cases can lead to coma and death. Mild cases make a full recovery, but serious non-fatal cases can display long-term neurological sequelae, such as paraplegia. There was a serious outbreak of MVE in 1974 and sporadic cases have occurred since then located mainly in the northern regions of Australia. Three cases were reported in 2001, two in Alice Springs and one in Mt Isa, and there was one case in Central Australia in 2004. Following heavy rains and flooding in 2011, there were 16 cases across Australia and three deaths. February to May is the peak risk period for the virus in the Northern Territory. People living, visiting or camping overnight within 5 kilometres of swamp, creek and river systems are at greatest risk.

Kunjin virus causes encephalitis, but the disease is usually milder. MVE and Kunjin are transmitted by the common banded *Culex* mosquito, which bites only after sundown. The major vertebrate hosts for both viruses are waterbirds.

Cases of Japanese encephalitis have been imported to the Torres Strait Islands from Papua New Guinea and the virus could become established in Far North Queensland.

FIGURE 14.26

(a) Flooded river systems provide breeding grounds for mosquitoes, (b) which are the vectors for a number of arboviral infections.

Source: (b) J.J. Harrison on Wikimedia.

missed. A number of cases of unusual diseases are diagnosed each year in Australian travellers returning from overseas.

Tuberculosis

According to the WHO, **tuberculosis** (**TB**) affects one-third of the world's population and 50 per cent of all refugees. It is estimated that there are more than 8 million new cases worldwide each year, and that over 2 million deaths annually are attributable to TB. Infection with HIV increases susceptibility to TB, and it is estimated that HIV will account for an extra 1.5 million cases of TB per year. TB thrives in areas of poverty, malnutrition, natural disasters and political instability. More than half of all notifications of TB come from China, India and South-East Asia, but the highest *rate* of notifications is from South Africa and sub-Saharan Africa (see Figure 14.27).

Prior to 1945 the incidence of active tuberculosis in Australia was more than 45 per 100 000 population. A national TB campaign of mass chest X-rays, TB (BCG) vaccinations and treatment resulted in a large decline in the rate of notifications. Despite the regional threat of disease, the number of notifications for the whole country now averages less than 1000 cases per year, or a *rate* of 5 per 100 000 population. Although this rate compares favourably with other countries, it is still a cause for concern. The rate varies from about 12 per 100 000 in the Northern Territory to 2 per 100 000 in Tasmania.

Over 80 per cent of all notifications are in foreign-born migrants and displaced persons, mainly from Indochina. Notifications in Australia are monitored by the National Mycobacterium Surveillance System (NMSS), which reports in *CDI*. The incidence of TB in Indonesia and Papua New Guinea is 250 per 100 000, which is a cause for concern given their proximity to Northern Australia and the movement of people across the Torres Strait (see Figure 14.28).

Tuberculosis is a highly infectious disease that is readily transmitted from person to person on aerosol droplets. The bacterium lodges in the body, usually in the lungs. It may remain latent for many years or it may develop into active TB (see Chapter 17). Although immigrants are screened prior to entry to Australia, it is possible they may develop TB by reactivation of an earlier infection. They also have a high rate of lymphatic TB.

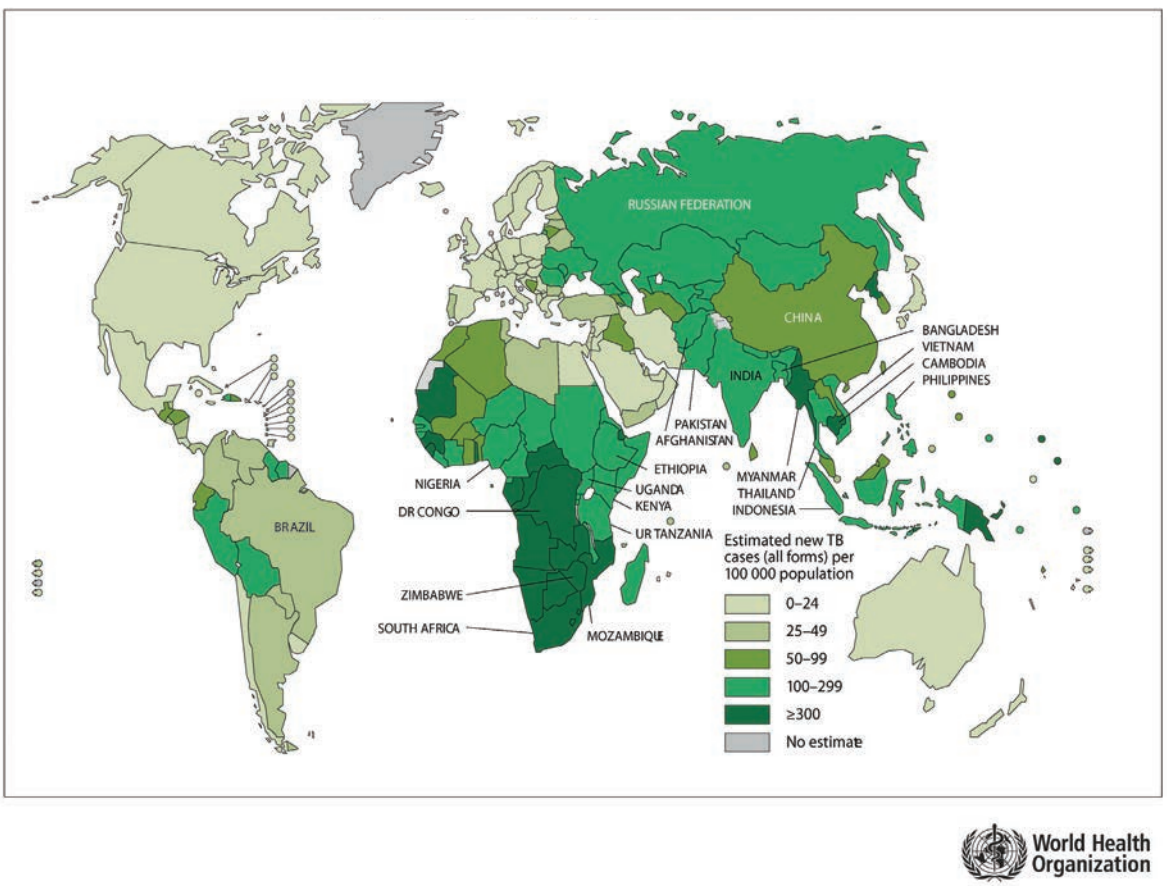

FIGURE 14.27

Estimated global incidence of tuberculosis, 2010

Source: Global TB Control, 2011, World Health Organization. Reproduced with permission.

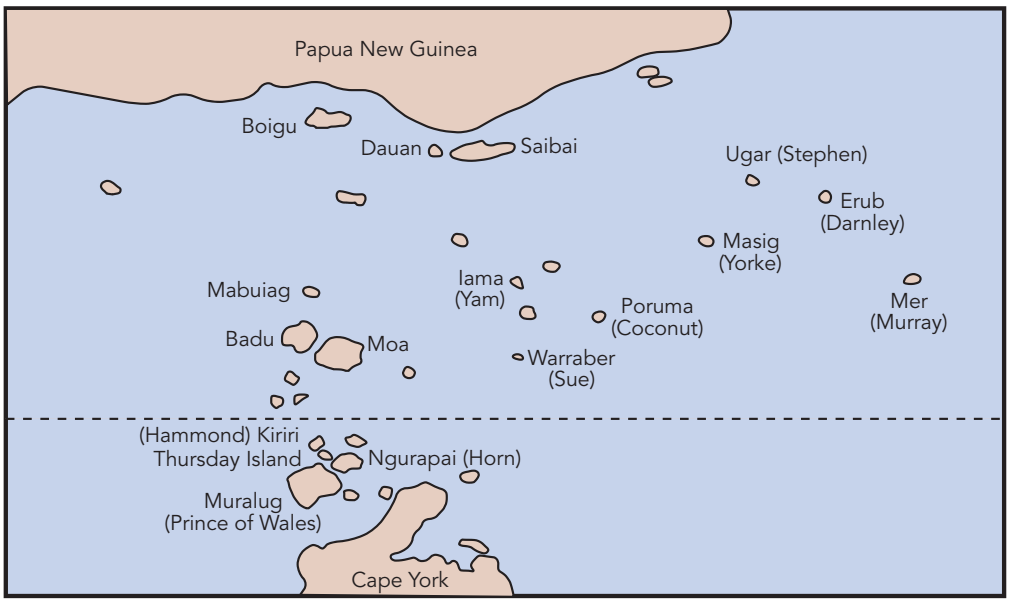

FIGURE 14.28

Map of Northern Australia and Torres Strait Islands showing proximity to Papua New Guinea and South-East Asia

People infected with HIV are particularly susceptible to reactivation of latent TB infection as well as to new infections. There is concern that all these factors may combine to cause a serious epidemic in Australia in a population that is largely non-immune.

The NHMRC working party has identified a number of issues that affect the incidence and management of TB in Australia. These include:

- the pattern of immigration and travel
- the increased worldwide incidence of TB
- the emergence of multi-drug-resistant strains
- the interaction of TB and HIV infection.

The BCG vaccine offers some protection against infection by *Mycobacterium tuberculosis*, but does not guarantee immunity. It is currently recommended for healthcare workers in some states, for children in high-risk groups (e.g. Northern Territory Aborigines) and for young travellers to high-risk areas. A disadvantage of BCG vaccination is that it interferes with the interpretation of the Mantoux or Tuberculin test, which is used to identify whether exposure to TB has occurred and whether preventive treatment is necessary.

Multi-drug-resistant strains of TB (MDR-TB) have emerged in many countries, due mainly to poorly supervised and partially treated TB infections. MDR-TB is defined as being resistant to two antimicrobial drugs. Extremely drug-resistant strains of TB, XDR-TB, emerged in South Africa and have now been reported from nearly 30 other countries. These strains are resistant to isoniazid, rifampicin and one or more of the other drugs that are usually used to treat TB. In South Africa many of the TB cases are co-infected with HIV. At present, MDR-TB strains are not common in Australia, but it is possible they can be transported to Australia by returning travellers.

Malaria

Malaria is the most important of the diseases carried by insect vectors (see Chapter 20). It is endemic in the tropical regions north of Australia, especially South-East Asia, Papua New Guinea (PNG) and the Pacific Islands (see Figure 14.28). The cause of malaria, the protozoan *Plasmodium*, is carried by the *Anopheles* mosquito, which is found in many parts of Australia, especially in the north.

The Torres Strait Islands to the north of Queensland are close to Papua New Guinea. There were four locally acquired cases of *P. falciparum* in the 1990s and two more cases in 2001, which were thought to have been introduced by visitors from PNG. For the disease to spread there has to be a reservoir, or pool, of infected people and conditions suitable for the appropriate vector to breed and transmit the disease.

There is a real possibility of the reintroduction of malaria, especially in parts of northern Australia where there is a large number of mosquitoes, many of the dwellings are open, and there is an influx of immigrants, tourists and students from high-risk endemic areas. Control of the mosquito vector is the most effective way to prevent reintroduction of the disease.

Australian travellers to endemic areas are at risk of contracting malaria. Between 700 and 1000 cases of malaria contracted outside Australia are notified each year. This is a huge increase in the number of cases compared with 20 years ago and reflects an increase in travel by Australians to endemic areas, as well as an increase in immigration. Strains of *Plasmodium* resistant to the antimalarial drug chloroquine

have appeared in many areas. Although several trials are in progress, there is currently no vaccine against malaria and travellers should consult their health department for up-to-date information on appropriate medication for the areas they intend to visit.

It is important to use preventive strategies to avoid mosquito bites: antimicrobial prophylaxis, insect repellent, long-sleeved clothing, mosquito nets and screens. Travellers should be aware that they may contract malaria, despite taking medication, if the particular strain of the organism is drug-resistant, or if the medication is not appropriate or is not taken for long enough.

Malaria should be suspected in any person who has recently returned from a malarious area and is suffering intermittent bouts of fever, headaches and nausea. Microscopic examination of blood films can give a correct diagnosis. Most cases of malaria can be treated successfully if diagnosed early in the course of the disease, but delay can have fatal consequences.

Schistosomiasis

Schistosomiasis is a chronic parasitic infection caused by trematodes, or flukes. Their complex life cycle involves a larval form, the free-swimming **cercariae**, which are released from the intermediate host, a freshwater snail. Infection of humans occurs when the cercariae penetrate the skin, usually when the person is working, swimming or wading in contaminated water. A number of cases of schistosomiasis have been recorded in travellers returning to Australia; for example, in a rugby team that had visited Zimbabwe and been white-water rafting on the Zambezi river. Other travellers had been swimming in Lake Malawi.

When correctly diagnosed, schistosome infections can be treated with praziquantel. Travellers should be aware of areas where schistosomiasis is endemic. As long as the species of freshwater snail that is needed as the intermediate host is not imported into Australia, people who are infected will not pose a health risk to other Australians.

Mosquito borne-viruses

Japanese encephalitis

Japanese encephalitis is a mosquito-borne viral zoonosis, which is widespread on the Indian subcontinent, and in South-East Asia and Papua New Guinea. Cases have occurred on Badu Island in the Torres Strait during the wet season, and there is evidence of activity on the far north tip of Cape York peninsula. A vaccine is recommended for residents in the Torres Strait Islands and for travellers to endemic areas.

Chikungunya virus

Eight cases of infection with this virus were notified in 2007 in returning travellers. *Aedes aegypti* is one of the vectors for this virus, so there is the potential for it to become established in Australia (see Chapter 1). Other viruses that are presently endemic in tropical Asian areas also have the potential to spread to Australia.

Dengue fever

Dengue fever is a mosquito-borne viral disease carried by the vector *Aedes aegypti*, which is found in some areas of Queensland. Outbreaks of dengue occur from time to time, following the importation of the virus and usually associated with good mosquito-breeding conditions (see Figure 14.1, page 327). There are four known serotypes of the virus, all of which have now been isolated in Australia.

The symptoms of dengue fever include acute headache, joint pain, rash and fever, but occasionally haemorrhagic fever and dengue shock syndrome may occur, especially in young children. Haemorrhagic dengue fever appears to occur in people who have a prior history of dengue disease and a second infection with a different serotype of the virus. Dengue fever has been known for many years but has only become a major health problem with extensive global travel, which has allowed circulation of the four serotypes.

Most cases of dengue are acquired outside Australia, but returning travellers can pose a threat. This occurred in 2000 when troops returning from East Timor to Townsville imported seven cases of dengue of two different serotypes. None of the cases was haemorrhagic and mosquito control measures ensured the disease did not spread.

In recent years the incidence of dengue has grown dramatically around the world. The WHO currently estimates there may be 50–100 million dengue infections worldwide every year. The disease is now endemic in more than 100 countries. Africa, the Americas, the Eastern Mediterranean, South-East Asia and the Western Pacific regions are the most seriously affected.

Yellow fever

Yellow fever is an acute viral disease, transmitted by mosquitoes. It is found in Africa and parts of South America, but does not occur in Australia. A vaccine is available, and travellers entering Australia from endemic areas are required to have been vaccinated or to undergo quarantine.

PUBLIC HEALTH ISSUES IN NEW ZEALAND

The distribution of infectious diseases throughout the world varies from one region to another. Each country produces its own statistics for the incidence of diseases that occur, and the policies and regulations they develop for their control may differ from those of their neighbours. Although New Zealand is geographically close to Australia, its climate and population are quite different. In this section, we examine some of the public health issues that are unique to New Zealand.

The population of New Zealand is about 3.5 million, of whom 13 per cent are Maori, 5 per cent are from the Pacific Islands and the remaining 82 per cent are of mainly European origin with an increasing number of people from Asia. The Pacific Islands population living in New Zealand is heterogeneous, consisting of people of the Samoan, Tongan, Cook Islands, Tokelauan, Niuean, Fijian and Tuvaluan ethnic groups, as well as people from Papua New Guinea, Vanuatu and the Solomon Islands. The lifestyles and health problems of the three major groups are different and contribute to the

variations in incidence statistics for the various diseases. The New Zealand government health reports and policies are available at: <www.health.govt.nz>.

Surveillance

The *New Zealand Public Health Surveillance Report* (NZPHSR) is prepared by the Institute of Environmental Science and Research (ESR) and published jointly by the Ministry of Health and the ESR; it is available online at <www.surv.esr.cri.nz>. In 2009 the list contained about 45 notifiable diseases, including vaccine-preventable infections that should be notified to the Medical Officer of Health.

Most sexually transmitted infections are not notifiable in New Zealand, and their surveillance has traditionally been based on data from specialist sexual health clinics. STI cases reported through the clinic-based surveillance system probably underestimate the true burden of disease because a substantial percentage of STIs are diagnosed by other health providers, particularly general practitioners.

Separate surveillance reports are available for notifiable diseases, influenza, sexually transmitted infections and chemical injuries. The Antibiotic Reference Laboratory at ESR is responsible for the national surveillance of antimicrobial resistance among human pathogens. The ESR public health virology laboratory collates, analyses and reports all-year-round laboratory-based virological surveillance, and the Enteric Reference Laboratory is responsible for the confirmation of isolates of the notifiable diseases *Salmonellae*, *Shigellae*, *Vibrio cholera* and VTEC.

Comparison of notification rates of infectious diseases in Australia and New Zealand

Several important infectious diseases have a higher *rate* of notification in New Zealand than in Australia. They include tuberculosis, meningococcal meningitis and rheumatic fever.

Meningococcal disease

Meningococcal disease is a serious illness caused by *Neisseria meningitidis*. There are three major serogroups—A, B and C—and two minor groups—Y and W-135. A tetravalent vaccine is available for serogroups A, C, W-135 and Y and is used when outbreaks occur. There have been several epidemics of meningococcal disease in New Zealand. When the cases are due to a vaccine-preventable serogroup, a vaccination program for children is instigated. Meningococcal disease presents as meningitis, septicaemia and other diseases. Children under 5 years of age are most at risk, especially Maori and Pacific Island children. There were 96 cases in 2010, down from a peak of 650 in 2001. There were 25 cases of the New Zealand epidemic strain and a 6.3 per cent fatality rate.

Gastrointestinal disease

Gastrointestinal disease continues to be a significant cause of morbidity. *Campylobacter* is the most common pathogen and the rate is almost four times that in Australia. Norovirus and *Salmonella* are the other main pathogens reported. About 50 per cent of infections are associated with retail food outlets. An additional 20 per cent of *Campylobacter* cases reported contact with farm animals. Most gastrointestinal infections show seasonal variations. In recent years, there has been an increase in incidence of cryptosporidiosis and giardiasis.

Blood-borne infections

AIDS, but not HIV, is notifiable in New Zealand. Data are collected by the AIDS epidemiological group in Otago University <http://dnmeds.otago.ac.nz>. In 2010 there were 39 new AIDS cases, 65 per cent in the homosexual community and 28 per cent heterosexual, whereas in Australia more than 85 per cent of new infections involve homosexual transmission.

Streptococcal infections

Compared with other developed countries, New Zealand has high rates of post-streptococcal disease such as **rheumatic fever**. The disease presents as arthritis and carditis, affecting the aortic and mitral valves of the heart, and sometimes causing residual damage to the valves of the heart. The person is susceptible to further streptococcal infections and damage to the heart valves. Rheumatic fever is a notifiable disease in New Zealand, and a register of patients was set up in 1986. The incidence is highest among Maori and Pacific Island children. In 2010 there were 155 new cases reported, 95 in Maoris (16.8 per 100 000) and 56 in Pacific Islanders (23.9 per 100 000). In Australia the incidence of rheumatic heart disease is highest among Aborigines (see page 350).

Health status of New Zealand's ethnic groups

The health status of different groups within the community is affected by socioeconomic conditions as well as ethnicity. Various measurements are used to determine the relative health status of the ethnic groups that make up the New Zealand population. These include general health, prevalence of disease or disability, the infant mortality rate, incidence of infectious diseases, and death rates.

At the beginning of the 20th century, a series of epidemics of infectious diseases (influenza, whooping cough, measles), a high mortality rate and land wars drastically reduced the Maori population. The situation was addressed by public health measures such as improvements in hygiene, sanitation, water supplies and vaccination. However, the 1994 report by the Public Health Commission of New Zealand on the health of the Pacific Islands people in New Zealand found that differences still existed in health, incidence of infections and outcome of diseases for the country's major ethnic groups.

Despite an overall decline in infant mortality in New Zealand in the last 20 years, the rate for Maoris is still almost twice that for Europeans. The rate for Pacific Islanders is similar to that for Europeans. Sudden infant death syndrome (SIDS) is five times higher in Maori infants.

The rate of notification of some infectious diseases varies for people of different ethnic origin. Of the gastrointestinal infections, Pacific Island peoples and Asians had higher rates of **hepatitis A**, while **campylobacteriosis** was higher among

Europeans. Of concern is the high incidence of meningococcal disease in Maoris and Pacific Islanders, compared with Europeans. The incidence of hepatitis B in New Zealand is quite high, mainly in young adults and injecting drug users. Maoris have a high rate of infection and a greater chance of subsequent **hepatitis B** carriage, leading to chronic hepatic disease.

The incidence of **rheumatic fever** in Pacific Islanders is 20 times that for Europeans and higher than the incidence for Maoris. Hearing loss resulting from **otitis media** affects 6–10 per cent of New Zealand school children. It is more likely to occur in children under the age of 7 and is more common among males and Maori children (15 per cent). Pacific Island children have a similar rate to Maori children.

Tuberculosis has declined in most developed countries but there is concern that the incidence will increase as the number of immunosuppressed people rises and there is spread of multi-resistant strains. In New Zealand there is a marked difference in the incidence of TB between geographical regions and ethnic groups. The incidence of new TB notifications in 2010 was highest among Asians, with 176 cases (51 per 100 000), whereas the numbers for other groups were: Europeans 26, Maoris 33, Pacific Islanders 45, and 12 for other ethnic origins. Many of the cases had been born, or had contact with people who were born, outside New Zealand.

Immunisation

The New Zealand Department of Health's recommended vaccination schedule (Table 14.7) is contained in the *Immunisation Handbook* and is available online at: <www.immune.org.nz>. The schedule is revised continually and health professionals should ensure they are aware of the most recent version. The target is 95 per cent vaccine coverage at 24 months of age, but at present there is only about 88 per cent cover. Higher coverage is needed to prevent serious outbreaks of measles and whooping cough.

A lower percentage of Maori children were immunised compared with children of European origin. A National Immunisation Register has been set up to facilitate follow up of children for vaccination. Various strategies are being introduced to improve coverage and reach families who do not participate in vaccination programs. A major challenge is to overcome the mistrust shown by caregivers about the safety of vaccines.

TABLE 14.7 **New Zealand immunisation schedule commencing July 2011***

Antigen	DTaP-IPV-Hep B/Hib	PCV10	MMR	Hib	DTaP-IPV	Tdap	HPV	Td	Influenza
Brand	Infanrix-hexa	Synflorix	M-M-R II	Act-HIB	Infanrix-IPV	Boostrix	Gardasil	ADT Booster	
Manufacturer	GSK	GSK	MSD	Sanofi-aventis	GSK	GSK	CSL/MSD	CSL	
6 weeks	•	•							
3 months	•	•							
5 months	•	•							
15 months		•	•	•					
4 years			•		•				
11 years						•			
12 years (females only)							• 3 doses		
45 years								•	
65 years								•	• (annually)

Note: For ease of reading, vaccine trade names have been written in the standard font, and as proper nouns.

Key: D = diphtheria; T = tetanus; aP = acellular pertussis; IPV = inactivated polio vaccine; Hib = *Haemophilus influenzae* type b; Hep B = hepatitis B; PCV10 = 10-valent pneumococcal conjugate; MMR = measles, mumps and rubella; Td = adult tetanus and diphtheria vaccine; d = adult diphtheria; ap = adult acellular pertussis; HPV = human papillomavirus.

* The date the new vaccines are released for use may be later than 1 July 2011 while existing stocks are used up.

Source: <www.health.govt.nz/system/files/documents/pages/natl-immunisation-sched-21mar-11.pdf>. Ministry of Health, New Zealand Government.

SUMMARY

THE AUSTRALIAN HEALTH SCENE

- The decline in mortality from infectious diseases in the 20th century was due to an improved standard of hygiene, the discovery of antibiotics and the development of vaccines.
- Public health refers to the promotion of health in the community.
- Responsibility for the delivery of healthcare in Australia rests with various state, territory and federal authorities.

NOTIFIABLE DISEASES

- A core group of communicable diseases is required by law to be notified to the health authorities when diagnosed in a patient.
- The NNDSS coordinates the adoption of the NHMRC list of diseases for notification and the reporting of notifiable diseases at a Commonwealth level.

INFECTIOUS DISEASES IN AUSTRALIA

- The annual report of the NNDSS is published in the *CDI* bulletin and online.
- The report describes the incidence and pattern of infectious diseases in all states and territories.

PRIMARY HEALTHCARE

- Primary healthcare involves the provision of services such as health education, health promotion, disease prevention and treatment of illness.
- In Australia the major factors that influence health status are socioeconomic conditions.
- Aborigines and Torres Strait Islanders have a much lower general standard of health than other Australians.

SCREENING PROCEDURES

- Screening of women during pregnancy is useful to detect the presence of, or susceptibility to, pathogens that may cause congenital defects.
- The Tuberculin skin test (TT), or Mantoux test (Mx), is a skin test that can determine whether a person has been exposed to and infected by the tubercle bacillus.
- In Australia all blood and blood products supplied by the blood bank are screened for the presence of infectious agents such as the human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV), *Treponema pallidum* (syphilis) and human T cell leukaemia virus I (HTLVI).
- Pap smears can detect changes in the cells of the cervix that may lead to invasive carcinoma.

IMMUNISATION

- Major reasons for improvement in health in the 20th century were the development of vaccines and the implementation of immunisation programs.
- Active immunisation is the administration of a vaccine to stimulate the body's immune system to produce both specific antibodies and cellular immunity.
- The Australian Health Department provides free vaccination for most childhood diseases.
- The NHMRC publishes information on immunisation procedures and recommendations for administration.
- The most effective type of vaccine consists of live, attenuated (weakened) forms of the pathogen. Some vaccines consist of toxoids; some contain killed, whole cells of the pathogen; others consist of fragments of the pathogen.

COMPLIANCE WITH IMMUNISATION

- The principle of herd immunity means that, for a population to be protected from a disease, a certain percentage must be immunised against that disease.
- In Australia, compliance with immunisation schedules is now over 90 per cent at 24 months of age and 82 per cent at 5 years.
- The risks from immunisation are much less than the risks from having the disease.
- Children who are not vaccinated against a particular disease will be excluded from school when an outbreak of the disease occurs.
- Vaccines are available for travellers and health workers.
- The NHMRC warns that homoeopathic 'immunisation' has not been shown to give protection against infectious diseases.

INFECTIOUS DISEASES IN CHILDCARE CENTRES

- Australian children aged 0–5 years attending childcare centres have a high risk of acquiring infections.
- Infections are transmitted in aerosol droplets, by exposure to faecal material and by handling contaminated toys.
- Skin infections are easily transmitted and can develop into impetigo.
- Cross-infection can be prevented by good hygiene, practising regular handwashing, teaching children to wash hands and by separation of tasks.
- Staff should be immunised and children should be up to date with the immunisation schedule.
- Occupational risks include exposure to childhood infections, and pregnant workers should be aware of the risk of congenital infections.

HEALTHCARE IN RURAL AND REMOTE AREAS

- Factors such as climate, socioeconomic conditions and access to health services affect the incidence of infectious diseases in remote areas.
- The Northern Territory Department of Health and Community Services collects and coordinates the publication of reports of infectious diseases in the monthly *Northern Territory Communicable Diseases Bulletin*.

Aboriginal health

- There is a disparity in general health and incidence of infectious diseases between Aboriginal and non-Aboriginal people.
- The overall notification rate for communicable diseases in the Northern Territory is six times the national average.

- The infant mortality rate is two to three times higher than in the rest of Australia.
- Aborigines have a much higher incidence of sexually transmitted infections and tuberculosis than other Australians.

Infectious diseases in the Aboriginal population

- Overcrowded living conditions and poor hygiene in Aboriginal communities lead to a high rate of disease and cross-infection.
- Complications (sequelae) of bacterial infections include chronic bronchitis, blindness, infertility, deafness, rheumatic heart disease, and renal failure in adult life.
- Skin infections with Group A *Streptococcus pyogenes* (GAS) may have outcomes such as post-streptococcal glomerulonephritis and rheumatic fever.
- Chronic otitis media (OM) and associated hearing loss are prevalent among Australian Aborigines.
- Trachoma is still a serious disease in some remote areas. It can be treated with azithromycin.
- The introduction of the Hib vaccine has reduced the incidence of Hib disease in the Northern Territory as well as in the rest of Australia.
- The incidence of gastrointestinal infections is significantly higher in the Northern Territory than in other parts of Australia.

Unusual diseases of rural and remote areas

- Melioidosis is the most common cause of fatal, community-acquired bacterial pneumonia in the Northern Territory.
- *Leptospira interrogans* is the cause of an acute renal failure with a 5–10 per cent fatality rate. Other unusual diseases include Q fever and dengue fever.
- Arboviral infections occur during the wet season.

Implications for healthcare

- There are special healthcare procedures to be implemented in remote areas—specific immunisation schedules, additional notifiable diseases, antibiotic guidelines for Central and Northern Australia, and special treatment protocols.

INFECTIOUS DISEASES FROM OUTSIDE AUSTRALIA

- Diseases that are not endemic in Australia may be imported by travellers.
- Eighty per cent of all notifications of active tuberculosis in Australia are in foreign-born people, mainly from Indochina.
- Multi-drug-resistant strains of *Mycobacterium tuberculosis* (MDR-TB) occur in many countries. At present, these strains are not common in Australia.
- Australian travellers to endemic areas are at risk of contracting malaria.
- Dengue fever is an imported viral disease, occurring usually in northern parts of Australia where the vector *Aedes aegypti* is found.
- Yellow fever is an acute viral disease, transmitted by mosquitoes. It is found in Africa and parts of South America, but does not occur in Australia. A vaccine is available.
- Cases of schistosomiasis have been recorded in travellers returning to Australia from Africa.

PUBLIC HEALTH ISSUES IN NEW ZEALAND

- Some public health issues are unique to New Zealand, because of its different climate and population.
- The *New Zealand Public Health Surveillance Report* (NZPHSR) is prepared by the Institute of Environmental Science and Research (ESR) and published jointly by the Ministry of Health and the ESR.
- The health status of the different ethnic groups within the community is affected by socioeconomic conditions as well as ethnicity.
- The infant mortality rate for Maoris is almost twice that for Europeans, while the rate for Pacific Islanders is similar to that for Europeans.
- The incidence of several infectious diseases varies among the different ethnic groups.
- Vaccination rates are below the target of 95 per cent.

STUDY QUESTIONS

1. What is meant by a 'notifiable' disease?
2. Who is responsible for the notification of infectious diseases?
3. Where can you find information about the incidence of infectious diseases in Australia?
4. What are the functions of the NHMRC?
5. What are some of the public health strategies for the prevention and control of infectious diseases?
6. How does immunisation protect a person from a particular disease?
7. What are the current recommended schedules for childhood vaccination?
8. Why do some vaccines require more than one dose?
9. Why is vaccination for rubella particularly important for pregnant women?
10. What is homoeopathic immunisation? Does it work?
11. Why are antenatal screening tests carried out for some infectious diseases?
12. What information can be gained from a Mantoux test?
13. What is a Pap smear?
14. Which infectious diseases occur most frequently in childcare centres?
15. Name three ways to minimise the spread of infection in childcare centres.
16. What are some of the major differences between the health of Aboriginal Australians and other Australians?
17. What are some of the infectious diseases that particularly affect Aboriginal people in the Northern Territory?
18. Describe some of the unusual infectious diseases that are seen mainly in remote or rural areas.
19. Name two diseases that are not presently endemic in Australia, and describe how they could become established.
20. How does the incidence of infectious diseases in New Zealand differ from that in Australia?

TEST YOUR UNDERSTANDING

1. How do health departments use data on the incidence and prevalence of infectious diseases?
2. What is meant by 'herd immunity'?
3. What reasons do some people give for non-compliance with vaccination schedules?
4. What are some of the advantages of screening programs?
5. Why should all sexually mature women have regular Pap smears?
6. Why do children in childcare have more infections than children cared for at home?
7. What are some of the challenges for the provision of healthcare in remote areas?
8. Why is Australia free of some serious diseases? How could this change?

FURTHER READING

Australian Bureau of Statistics 2008, *The Health and Welfare of Australia's Aboriginal and Torres Strait Islander Peoples, 2008*, Cat. No. 4704.0. Available at: <www.abs.gov.au/ausstats/ abs@.nsf/mf/4704.0>.

Australian Commission on Safety and Quality in Health Care 2008 (draft), *Reducing Harm to Patients from Health Care Associated Infection: The Role of Surveillance*. Available at: <www.safetyandquality.gov.au>.

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Currie, B. et al. 2004, Melioidosis epidemiology and risk factors from a prospective whole population study in Northern Australia. *Tropical Medicine and International Health* 9(11): 1167–74.

Mathews, J., T. Weeramanthri and P. DíAbbas 1995, Aboriginal health. *Today's Life Science* 7(8).

National Health and Medical Research Council (NHMRC) 2005, *Staying Healthy in Child Care: Preventing Infectious Diseases in Children* (Canberra: Australian Government Publishing Service). Available at: <www.nhmrc.gov.au/guidelines>.

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Paterson, B.J., J.S. Mackenzie, D.N. Durrheim and D. Smith 2011, A review of the epidemiology and surveillance of viral zoonotic encephalitis and the impact on human health in Australia. *NSW Public Health Bulletin* 22(6): 99–104. Available at: <http://dx.doi.org/10.1071/NB10076>.

World Health Organization 2011, *Global Tuberculosis Control*. Available at: <www.who.int/tb/publications/global_report/2011>.

Websites

Government reports and guidelines, and most of the information regarding the incidence of notifiable diseases, are available from internet sites.

- Australian Department of Health and Ageing: this is the starting point for all information relating to health in Australia, <www.health.gov.au>.
- This is the introduction to the public health site, <www.health.gov.au/pubhlth>.
- This is the *Communicable Diseases Intelligence (CDI)* website with links to reports of notifiable diseases, immunisation schedules, etc, <www.health.gov.au.pubhlth/cdi>.
- This site provides access to state and territory government department sites; other university departments and health laboratories; World Health Organization; international government organisations; New Zealand Ministry of Health; US Centers for Disease Control and Prevention (CDC); and international travel information, <http://health.gov.au/pubhlth/cdi/cdilinks.htm>.
- This site contains surveillance reports on HIV, hepatitis B and C, as well as trachoma: Kirby Institute (formerly the National Centre in HIV Epidemiology and Clinical Research), <www.unsw.edu.au/nchecr>.
- New Zealand public health reports and notifiable diseases data, <www.health.govt.nz/nzphr.html>.
- New Zealand surveillance data, <www.surv.esr.cri.nz>.
- This site provides access to the CDC publication *Emerging Infectious Diseases*, a series of current review articles on world infectious diseases, <www.cdc.gov.ncidod/EID/index.htm>.
- A monthly review of notifications of outbreaks of infectious diseases in the European community, <www.eurosurv.org>.

UNIT
4

Infections of body systems

CHAPTER

15

Microbial techniques for diagnosis of infection

CHAPTER FOCUS

- What methods are used in the microbiology laboratory to diagnose infections?
- What types of specimens are collected for microbiological analysis?
- What are the important considerations in the proper collection and transport of specimens for microbiological analysis?

INTRODUCTION

Relatively few infectious diseases can be accurately diagnosed solely on clinical grounds, so for most infections laboratory diagnosis, in addition to a clinical diagnosis, is required. Speed and accuracy are the primary imperatives in the laboratory diagnosis of infectious diseases. Some infections, such as meningitis or bloodstream infections, can have serious outcomes for the patient within hours of the onset of symptoms. So, the earlier results become available, the sooner specific steps can be taken for appropriate management of the patient, lowering the risk of morbidity and mortality. Faster diagnosis increases the likelihood of appropriate infection control precautions and antibiotic treatment being used, reducing the risk of spread of infection to other people as well as reducing the need for broad spectrum antibiotics.

The accuracy of a laboratory diagnosis is an important issue because there are many potential errors that can lead to misdiagnosis by the laboratory. If the specimen is collected from the wrong body site or is poorly collected, the causative agent may not be identified. It is also possible for the infection to be attributed to the wrong microorganism. This can occur if the specimen is contaminated with normal flora organisms that are capable of causing the infection. For example, *Staphylococcus epidermidis* can cause septicaemia, but is also a normal inhabitant of the skin. If a blood sample taken from a patient is contaminated with this organism as the needle is passed through the skin, the organism could be mistakenly identified as the cause of the patient's septicaemia. The issues of speed and accuracy are paramount and will be discussed further throughout this chapter.

TYPES OF MICROBIOLOGY LABORATORY TESTS

The clinical microbiology laboratory performs tests on a patient's specimen(s) according to the presumptive diagnosis and requests of the clinical team. For an accurate laboratory diagnosis, it is essential that an appropriate specimen is properly collected. To achieve this, one must have a good understanding of the pathogenesis and possible cause of infection. Laboratory tests are requested based on the nature of the symptoms and the possible causative agent(s). One or more specimens may have to be collected, and the doctor, nurse or other qualified healthcare worker must perform the collection procedure correctly. The specimen must then be transported promptly to the laboratory for processing. Laboratory findings are made available to the clinical team as soon as possible to facilitate the prompt diagnosis and appropriate treatment of the disease.

A variety of methods are used in the laboratory for the diagnosis of infections. The methods appropriate for the situation are determined by the possible causative agents and the site(s) of infection. Generally, laboratory tests are designed to detect (1) the microorganism itself in a specimen from the patient, or (2) a component of the organism in the specimen, or (3) evidence of an immune response to the causative organism in the serum (blood) of the patient. Five main methods are employed (see Figure 15.1).

1. *Direct microscopic examination of a specimen*. A direct microscopic examination of a specimen can provide a very rapid presumptive diagnosis in certain situations. It may also provide information about the host cells present in the specimen, which can indicate the probable type of infection (e.g. viral versus bacterial) as well as the quality of the specimen (e.g. a mucoid versus a purulent sputum).
2. *Culture and isolation of microorganisms from patient material*. A specimen from the patient is used to inoculate appropriate microbiological media (see Figure 15.2) and, after incubation, the cultures are examined for the presence of pathogens. In some cases (e.g. urinary tract infections), the number of microorganisms in the specimen is estimated. When the causative agent is bacterial, the organism's sensitivity to antibiotics can also be determined. Unfortunately, not all pathogens can be cultured (especially some viruses and protozoa). Another major disadvantage is that culture results may not be available for 18 hours or more for many bacteria, and much longer for viruses. Thus, faster diagnostic methods are continually being sought.
3. *Identification of antigens of the microorganism in patient material*. This involves the detection of a particular microorganism or a component (antigen) of it in a specimen from the patient. Antigen detection methods are also useful when culture of the pathogen is not possible, or is too difficult or too time-consuming.
4. *Identification of microbial nucleic acids in a specimen from the patient*. A gene sequence that is specific for a particular microorganism can be detected in patient material by the use of a DNA probe. This method is usually used in conjunction with DNA amplification techniques such as the polymerase chain reaction (PCR). There have been numerous significant developments in these DNA techniques in the last decade. They are increasingly used to provide faster diagnoses, and for the diagnosis of infections that are difficult or impossible to confirm by other methods.
5. *Identification of infection using serologic (or immunologic) reactions*. This involves the detection of specific antibodies to a microorganism in the serum or other body fluid of a patient. A serologic method is generally used when culture of the pathogen is not possible or too difficult, or when culture takes too long (e.g. viruses, protozoa, some bacteria).

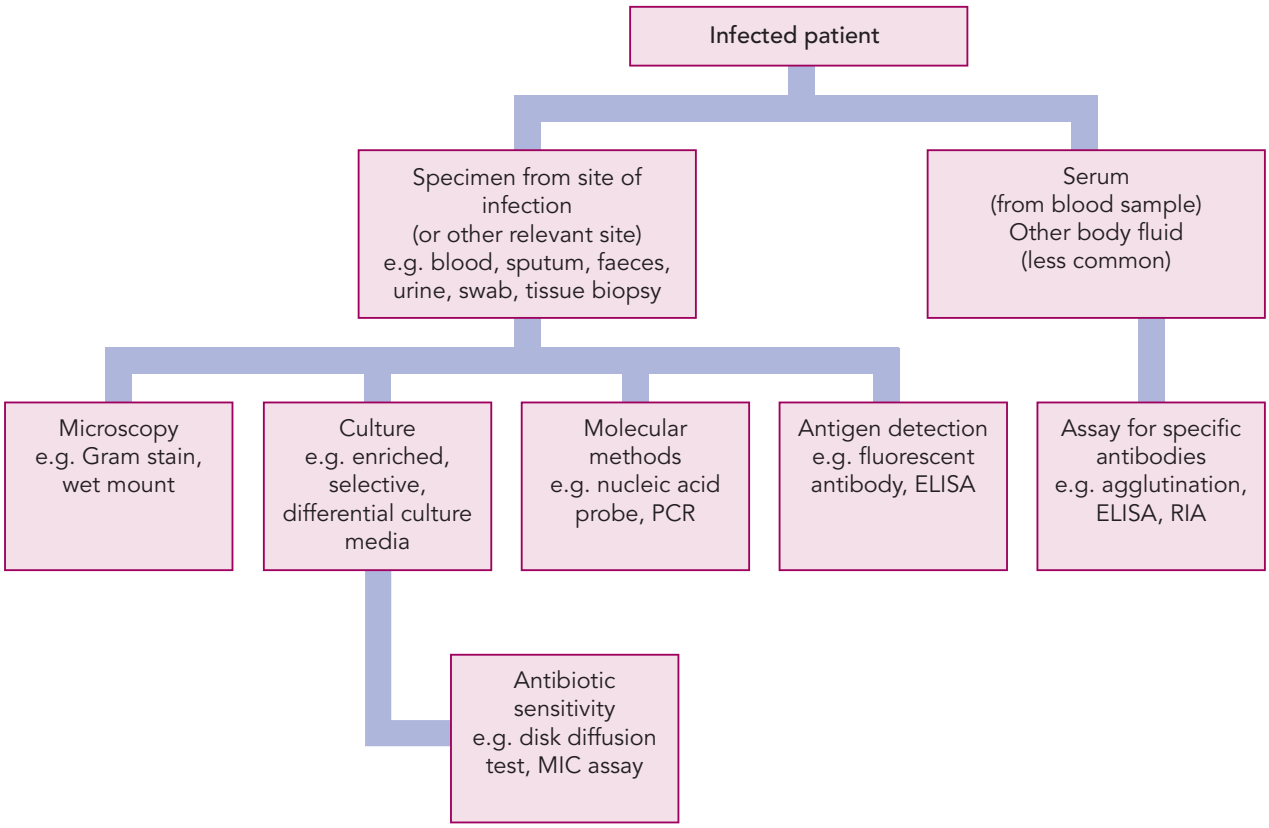


FIGURE 15.1

Outline of methods for the laboratory diagnosis of infectious diseases

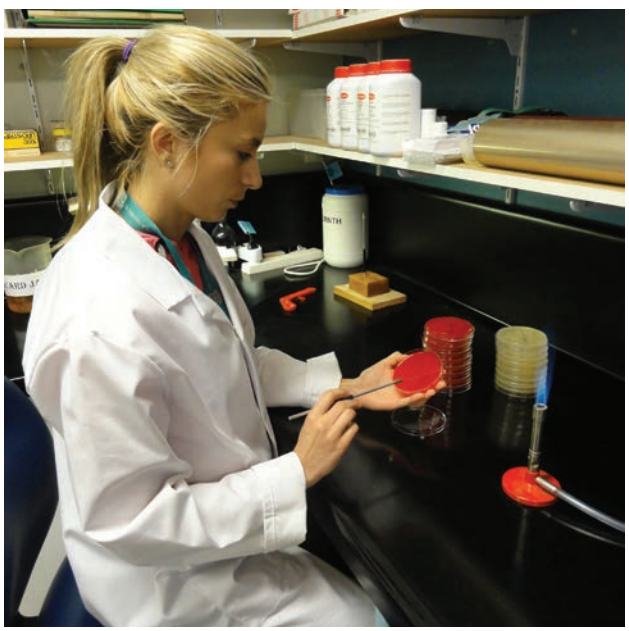


FIGURE 15.2

Culture of specimens in the microbiology laboratory

All diagnostic techniques have their advantages and shortcomings (see Table 15.1). Not all the methods outlined above are suitable or available for all infections; and the fastest methods are not always the most accurate. Despite relentless efforts to develop more rapid methods, culture of the causative agent remains the 'gold standard' for many infections, especially for those caused by bacteria and fungi. The standard time frames for microscopic and cultural methods are summarised in Figure 15.3. It should be noted that the times given are for typical organisms, and that there are many exceptions to those shown in the figure. There are also rapid methods now in use that can reduce culture time for some organisms to several hours.

In relation to the accuracy of a test or method, two important attributes are its sensitivity and specificity. The *sensitivity* of a test refers to the proportion of people with a particular disease that are identified as having the disease by the test. The more people with the disease that are not identified by the test, the less sensitive is the test. *Specificity* refers to the proportion of people without the disease who yield a negative test result. The more people without the disease who are indicated by the test as having the disease, the less specific is the test. A positive test result in a person who does not have the disease is called a **false positive**. A **false negative** is a negative test result in a person who does have the disease.

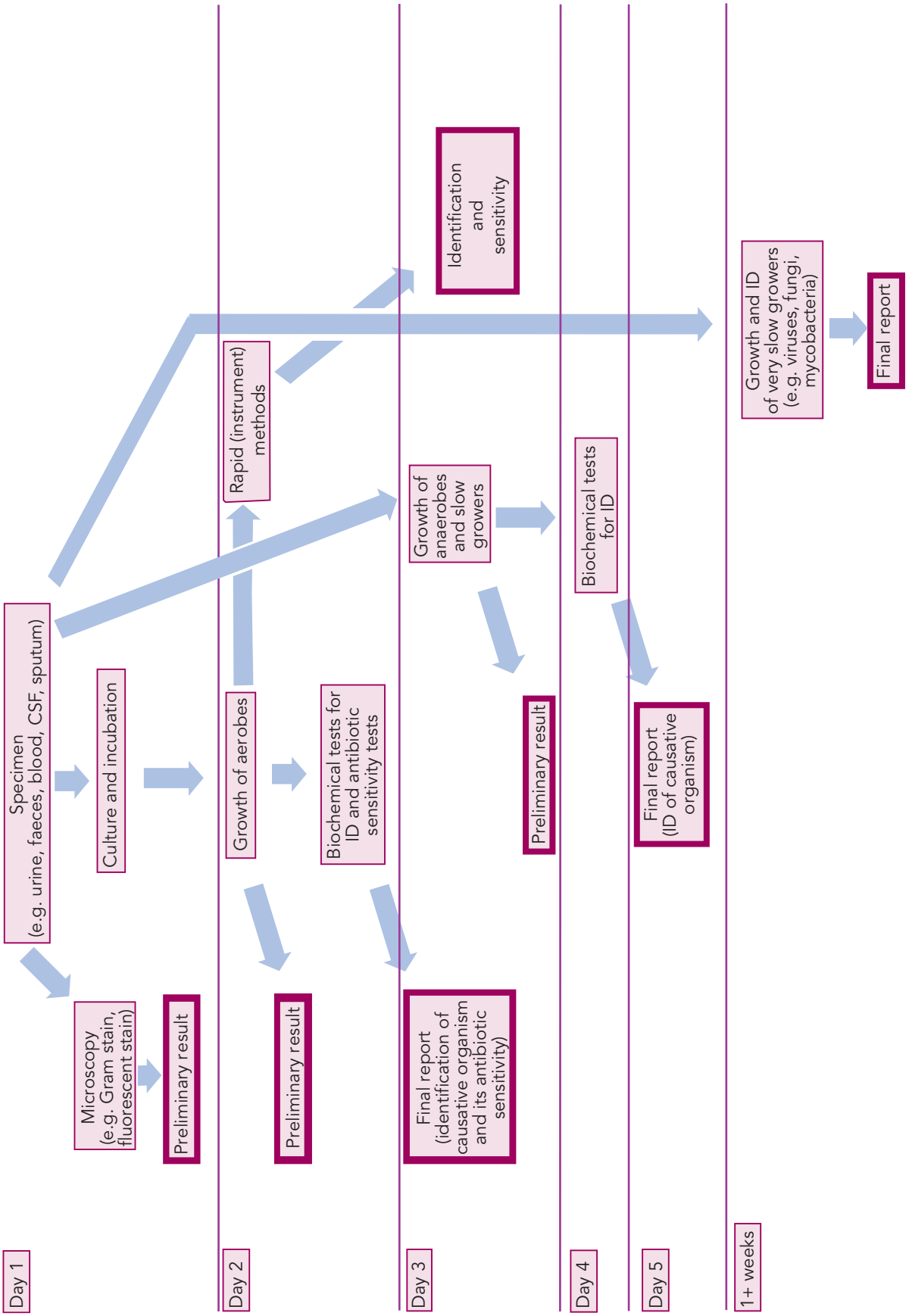


FIGURE 15.3 **Laboratory diagnosis of infectious diseases by culture methods**

TABLE 15.1 Advantages and limitations of diagnostic techniques

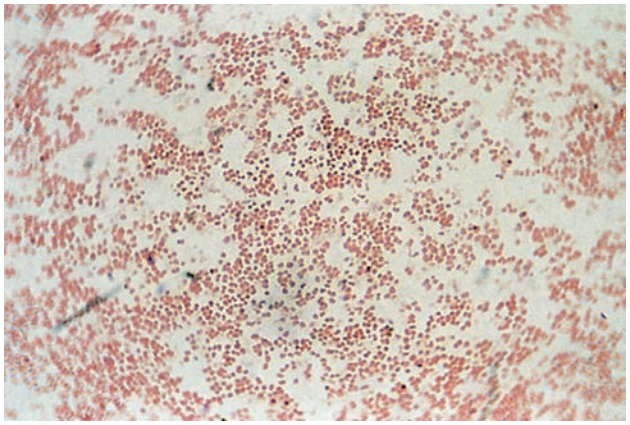
TECHNIQUE	ADVANTAGES	SHORTCOMINGS
Microscopy	Very fast	Poor sensitivity Cannot test for antibiotic sensitivity
Culture	Definitive, if performed correctly Antibiotic sensitivity can be obtained Fungi can be cultured	Time consuming (18–72 hours) Not very useful for slow-growing organisms Not appropriate/available for most viruses Problem distinguishing between infection and colonisation Biohazard
Serology	Good for non-culturable organisms (e.g. viruses, fastidious organisms) Can be quick, depending on method	False positives possible False negatives possible Difficult interpretation May need two tests (slow) Cannot test for antibiotic sensitivity
Antigen detection	Can be quick Good for non-culturable organisms Can get positive diagnosis early in the disease (compared to serology)	False positives possible May have narrow detection period Cannot test for antibiotic sensitivity
Nucleic acid detection	Fast Good for non-culturable and slow-growing organisms Can detect very small numbers of organisms Can be quantitative (real-time PCR)	False positives possible False negatives possible Specialised equipment required Mostly cannot test for antibiotic sensitivity

MICROSCOPIC TECHNIQUES

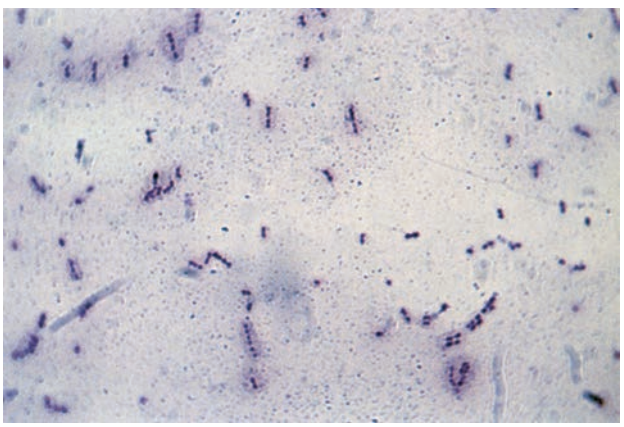
Direct microscopic examination of certain specimens can sometimes provide a presumptive diagnosis within an hour, although several hours may be required for more complex staining and microscopic methods. Direct microscopy using a light microscope is most useful in the following circumstances.

- When the site of infection is a normally sterile part of the body. For example, in a case of meningitis, a Gram stain of cerebrospinal fluid (CSF) can provide rapid and vital information about the likely cause. Possible causes of acute bacterial meningitis, such as *Neisseria meningitidis* (a Gram-negative coccus) and *Streptococcus pneumoniae* (a Gram-positive coccus), can be distinguished in a Gram stain (see Figure 15.4).
- When the pathogen is readily distinguishable by its morphology (size, shape and other physical attributes) under the microscope. Certain intestinal parasites can be identified in a wet mount of faeces or other specimen—for example, *Giardia* (a protozoan) and *Ascaris lumbricoides* (a roundworm), as shown in Figure 15.5. Malaria is usually diagnosed by microscopic examination of a blood smear. Microscopy can be crucial for parasite infestations, since many cannot be or are difficult to culture.
- When the pathogen can be differentially stained so that it can be specifically identified under the microscope. For example, mycobacteria (causing tuberculosis) can be distinguished from other microorganisms in a Ziehl-Neelsen stained (acid-fast stain) smear of sputum. *Cryptococcus neoformans* can be identified in CSF using an India ink stain. Another particularly useful staining technique involves the use of fluorescent dyes attached to antibody molecules specific for a particular microorganism. The organisms are identified if they fluoresce under a microscope using an ultraviolet light source. This technique can be used in many situations, such as for the identification of *Legionella*, *Pneumocystis jiroveci*, and for *Treponema pallidum* in fluid from a genital lesion (see Figure 15.6). It is also useful as a rapid diagnostic tool for some acute viral infections.

A further important advantage of direct microscopic examination of a specimen is that the host cells present in the specimen can be identified. This can provide information about whether or not infection exists, the type of infection and the quality of the specimen. For example, the presence of pus cells (polymorphonuclear leukocytes) in a sample of urine is suggestive of infection (see Figure 15.7), whereas large numbers of epithelial cells in urine suggest that the specimen was improperly collected (i.e. not a mid-stream sample). The presence of pus cells in CSF suggests an



(a)



(b)

FIGURE 15.4

Gram stains of the common causes of bacterial meningitis

(a) Neisseria meningitidis; *(b)* Streptococcus pneumoniae.

Source: G. Jayachandran, Sydney Medical School, University of Sydney.

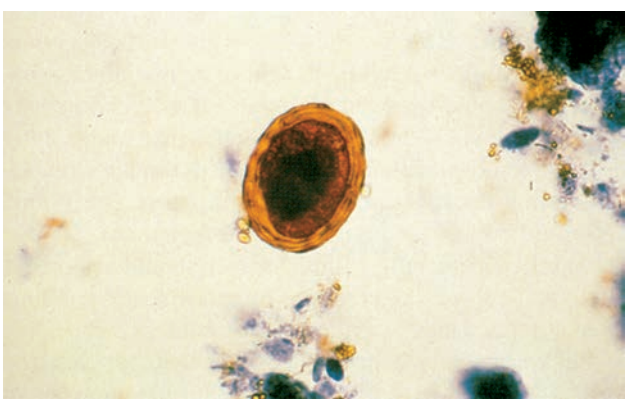
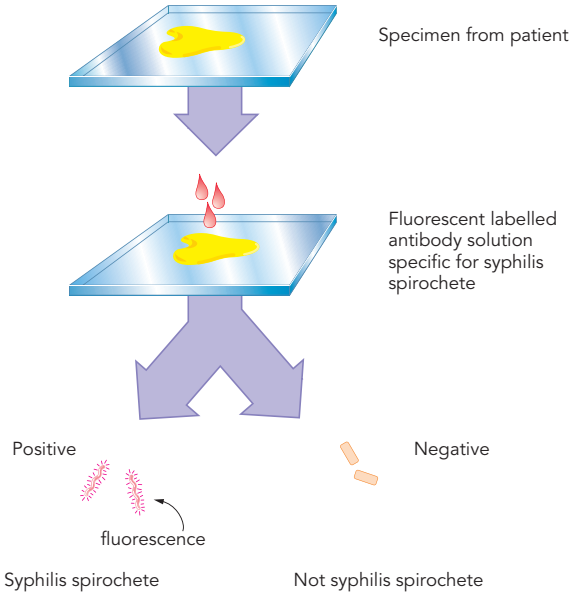


FIGURE 15.5

Egg of *Ascaris lumbricoides* as seen in a light microscope

Source: Stephen Neville, Department of Microbiology, South Western Area Pathology Service.



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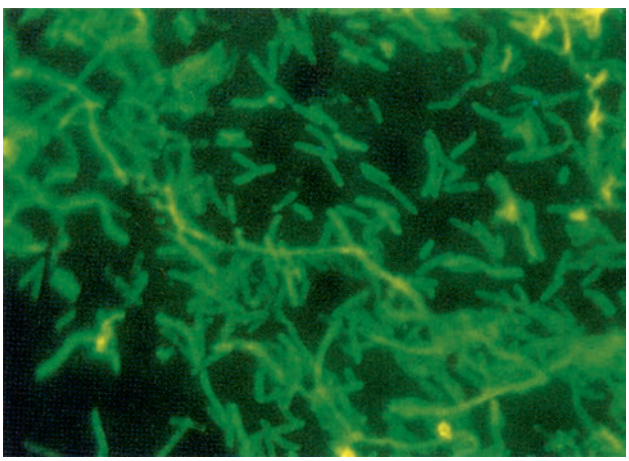


(b)

FIGURE 15.6

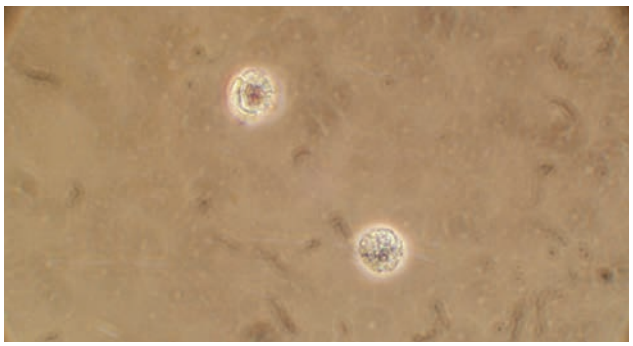
The fluorescent antibody technique

(a) Fluorescent antibody stain results of Treponema *and an unrelated microorganism in a specimen; (b) photomicrograph of treponemes fluorescing.*

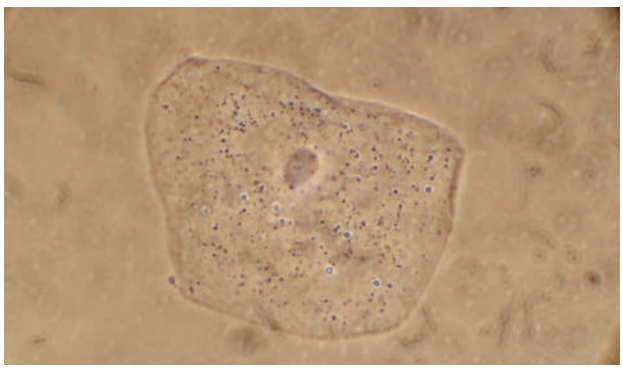
Source: Dr Penny Bishop.

acute bacterial infection, whereas lymphocytes suggest that a non-bacterial cause (e.g. virus, fungus) or a tuberculous meningitis is more likely.

Electron microscopy is a complex procedure that generally takes much longer than light microscopy, mainly because of the more complicated specimen preparation that is required. However, it can be a valuable tool for the identification of very small microbes, such as viruses. Virus culture takes days or even weeks, and some viruses cannot be cultured at all. The major limitation of electron microscopy is that the microscope is a highly specialised piece of equipment, usually found only in specialist virology laboratories or research institutes.



(a)



(b)

FIGURE 15.7

Direct microscopic examination of urine

(a) Pus cells (polymorphonuclear leukocytes) in urine are indicative of infection; (b) epithelial cells suggest poor specimen collection.

Source: Dr Gary Lee.

SPECIMEN COLLECTION FOR CULTURE

The most direct method for the diagnosis of an infection is by culture and identification of the cause from a patient specimen. The specimen is cultured on appropriate microbiological media and an identification of the causative organism is then made, based on one or more of colony morphology, biochemical properties and antigenic properties (see later section). The microbiological analysis in the laboratory is only as reliable as the quality of the specimen allows. Thus, the correct selection, timing, collection and transport of patient specimens are of the utmost importance.

When specimens are not properly collected and handled:

- the causative organisms may not be present in the specimen at all; or
- the organisms may die if subjected to adverse conditions; or
- the causative organisms may be overgrown by normal microbial flora also present in the specimen.

As stated earlier, a major disadvantage of cultural methods is the length of time required for a result. Culture of most bacteria takes 18–48 hours, but some slow growers (e.g. fungi and mycobacteria) can take several days to weeks to grow. Blood cultures (see later section) may require up to 5–7 days of incubation. Then another 5–24 hours may be required for antibiotic sensitivity results; and much longer for slow growers.

Specimen selection

Many different types of specimens are sent to the laboratory for culture, including blood, urine, faeces, sputum, pus and swabs from sites of infection. These specimens are generally readily obtained. However, for some infections, tissues or body fluids from less accessible sites may have to be collected, such as fluid from a deep abscess, bone marrow, cerebrospinal fluid or peritoneal exudate. The selection of the most appropriate specimen type(s) to be collected should be based on the nature of the infection, and they should be collected from a site where the organism is most likely to be found. For example, a patient with respiratory symptoms and a productive cough should have sputum collected for examination. However, it is not always so straightforward. For instance, *Salmonella typhi*, the cause of typhoid fever, is most likely to be isolated from blood in the first two weeks of infection, but then from faeces or urine in the next two weeks. A good knowledge of the course of the infection is obviously necessary.

Timing of specimen collection

It is important that, wherever possible, specimens for laboratory culture are collected before antibiotic therapy is initiated. Once antibiotic therapy has begun, the likelihood of culturing the causative organism from the specimen is lessened. In certain situations, however, it may not be possible to delay antibiotic therapy until a specimen is collected. For example, in a case of meningitis, which can be life-threatening, antibiotic therapy may have to be initiated before a lumbar puncture for cerebrospinal fluid (CSF) collection can be performed.

Knowledge of the usual clinical course of a given infection, combined with a careful observation of the patient's signs and symptoms, should indicate the appropriate time for specimen collection. For most infections, the collection of the specimen should simply be done as soon as possible but, for some, more precise timing is required. For example, early morning sputum specimens should be collected for the diagnosis of tuberculosis to maximise the chance of finding organisms.

Correct timing of the collection of blood samples for culture is critical because organisms may only be intermittently released from the infection source. In septicaemia, chills followed by fever represent the showering of microorganisms into the bloodstream. Cultures are most likely to be positive when they are collected during such episodes.

Proper collection of specimens

The proper collection of specimens is vital for four main reasons: (1) to obtain an appropriate and useful specimen; (2) to prevent contamination of the specimen during collection; (3) to prevent harming the patient during specimen collection; and (4) to ensure the healthcare worker is not

contaminated during the collection. The following general procedures should be observed.

- Hospital Standard Precautions guidelines should be followed at all times. All specimens should be treated as potentially hazardous.
- Sterile, leak-proof containers should be used. Care should be taken not to contaminate the external surface of the collection container and its accompanying paperwork. If contamination does occur, appropriate steps for their disinfection or disposal must be taken.
- Specimen collection should be performed with care and tact to avoid harming the patient, or causing discomfort or undue embarrassment. If the specimen is to be collected by the patient (e.g. sputum or urine), he or she should be given clear and detailed instructions.
- All specimens should be collected in a manner that will keep contamination by commensal microflora and external microorganisms to a minimum.
- A sufficient quantity of the specimen should be obtained to enable all necessary tests to be performed. The volume of specimen may be critical.
- The specimen container must be properly labelled and accompanied by the appropriate laboratory requests. The label and request form should identify the patient and the source of the specimen, and the date and time of collection. Any information that will aid the laboratory in performing the appropriate analyses should be included in the clinical history.
- Specimen containers should always be sterile. Faecal specimen containers are an exception since faeces has a very high microbial load and is cultured on highly selective media.
- Certain specimen types, especially swabs, may require special transport systems to protect the pathogens from drying or being overgrown by commensals.

Swabs are frequently used to collect specimens for microbiological analysis. There is a tendency to take a swab of an infected site, rather than some other type of specimen (e.g. a volume of pus or a tissue biopsy) because the former is easier to collect. In some situations (e.g. pharyngitis) a swab may be the only possible type of specimen, but in other situations it is less than ideal. The main problems with swab specimens are that only a limited amount of material can be collected and specimens are highly susceptible to desiccation, leading to reduced viability of microorganisms. After collection, swabs should usually be placed into a transport medium for transport to the laboratory.

Specimen transport

During transport, specimens may deteriorate or change from the state they were in when collected. Specimens should be delivered promptly to the laboratory so that microbiological examination can begin as soon as possible. Also, it is important that the results of the analysis accurately represent the microbiological population present in the specimen at the time of collection. If delivery is delayed, some fragile pathogens may die; and if the specimen is from a site in the body with a normal microflora, the more hardy or faster-growing members of the flora may overgrow the pathogens.

While it is most desirable to submit specimens to the laboratory quickly and in an unaltered state, this is not always possible. Therefore, certain specimens should be sent to the laboratory in a transport device, containing a **transport medium**. This is a liquid or semi-liquid, designed to prevent the specimen from drying and to maintain microorganism viability, while retarding their growth prior to culture. It is designed to maintain the microbial population in the specimen as close as possible to its composition at the time of collection.

Not all specimens need to be transported in a transport medium, but it is strongly recommended for specimens that could easily dry out, such as swabs and small amounts of fluid, tissue or biopsy material. For some specimens (e.g. a small amount of biopsy material) the medium may simply be 0.85 per cent saline solution. There is also a variety of specialised devices, such as swab transport systems, anaerobe transport systems and viral transport media.

It should always be remembered that clinical specimens may contain pathogenic microorganisms. Careless handling of the specimen during collection or transport could result in contamination of the outside of the container and the accompanying paperwork. Anyone who handles these may then be exposed to any pathogens present.

COMMON SPECIMEN TYPES FOR CULTURE

Blood cultures

Despite the successful development of rapid molecular methods for diagnosis of many infectious diseases (see later sections), culture of blood is still considered the gold standard for the diagnosis of bloodstream infections. A blood culture is usually performed in the investigation of a patient with fever or other manifestations of systemic infection. The blood is inoculated into one or two bottles of culture medium, depending on the type(s) of organisms expected. One bottle is for growth of aerobes and a second bottle is inoculated if anaerobes are suspected. Less than one bacterial cell may be present in a millilitre of blood, even in serious illness. Thus, the volume of blood collected for culture is critical. The recommended volume of blood for the blood culture system in use should be followed.

Because the number of microorganisms is often low, and because they may be shed into the bloodstream only intermittently, it is recommended that three sets of separately collected blood cultures are obtained over a 24–48-hour period. A single blood culture may miss intermittent bacteraemia. Also, a differentiation between pathogens and contaminants is possible if more than one sample is collected. Timing of the collections is important because intermittent bacteraemia usually coincides with the onset of fever or chills, although these symptoms may occur as much as an hour after the organisms are shed into the bloodstream.

It is essential that blood for culture is collected aseptically. This is done by first disinfecting the venipuncture site with an alcohol pad (70 per cent isopropyl or ethyl alcohol). The intended venipuncture site should not then be touched except with similarly disinfected, gloved fingers. The stoppers of the culture bottles or collection tubes should be disinfected with alcohol prior to their inoculation.

Optimally, the blood should be inoculated directly into the culture bottles with the same syringe and needle at the bedside of the patient. Figure 15.8 shows blood culture bottles inoculated with blood.

In the laboratory, cultures are incubated for up to seven days in an automated blood culture system (see Figure 15.9). Automated systems regularly monitor the blood cultures for microbial growth as they incubate, usually by measuring carbon dioxide production or pressure changes in the bottle. Such systems are designed to provide as early a detection of positive cultures as possible. Once a culture becomes positive it is then subcultured on to agar plates for ultimate identification and, if necessary, sensitivity testing of the organism.

Intravascular catheters

Intravascular catheters may become colonised and serve as a source of microorganisms in septicaemia. Culturing of a catheter tip may help to determine if there is a relationship between the catheter and infection. A distal segment of the catheter (approximately 5 cm) should be obtained by aseptically clipping off the end of the catheter directly into a screw-cap sterile container at the time the catheter is removed. This specimen should be sent immediately to the laboratory before excessive drying occurs. The number and type of viable bacteria in the catheter specimen may then be determined.

FIGURE 15.8

Blood culture bottles inoculated with blood from a patient

Blood culture bottles, showing different-coloured tops for aerobic (green) and anaerobic (red) cultures.

Source: Dr Gary Lee.

FIGURE 15.9

Automated blood culture system

The incubator and detection unit are connected to a computer that signals when a culture becomes positive.

Source: Dr Gary Lee.

Specimens for urinary tract infections

During urination, bladder urine passes through the urethra and generally becomes contaminated with bacteria that colonise the urethra. Irrespective of whether or not the bladder urine is infected, the urethral colonisers can be mistaken for infecting organisms. Contamination of the bladder urine with urethral organisms can be reduced by collecting a 'clean catch' or midstream urine specimen. If this method is properly performed, the urethra is flushed out with the first portion of urine and the middle portion is collected for urinalysis.

Proper instruction of patients on how to collect a midstream specimen of urine (MSU) is critical to ensure the collection of a good specimen and hence the accuracy of laboratory results. An understanding by healthcare personnel of the proper methods for obtaining urine specimens from bedridden patients is also very important. If the patient is collecting the specimen, he or she should be given detailed instructions, including diagrams.

To collect an MSU specimen from a female:

1. The urethral opening and vaginal vestibule are cleansed with soapy water or clean gauze pads soaked with liquid soap.
2. The area is rinsed well with sterile water or wet gauze wipes.
3. The labia are held apart during voiding.
4. A few millilitres of urine are allowed to pass. The flow of urine should not be stopped.
5. The midstream portion of urine is collected into a sterile container.

To collect an MSU specimen from a male:

1. The foreskin is retracted (if not circumcised) and the penis is washed with soapy water.
2. The area is then well rinsed with sterile water.
3. With the foreskin still retracted, a few millilitres of urine are allowed to pass. The flow of urine should not be stopped.
4. The midstream portion of urine is collected into a sterile container.

Soap rather than disinfectants should be used for cleaning the urethral area, because disinfectants introduced into the urine during collection may inhibit the growth of microorganisms.

Urine is an excellent growth medium for microorganisms. If a urine sample is left standing at room temperature, contaminating bacteria may increase from low to high numbers. In large numbers they may inhibit the growth of the pathogen or be mistaken for the pathogen. A urine specimen should be sent to the laboratory within one hour of collection. If this is not possible, it should be refrigerated.

Urine is cultured quantitatively for bacteria, since the number of viable bacteria or colony-forming units (CFU) per litre is critical in the interpretation of urine cultures. A urinary tract infection is indicated if the number of bacteria in a clean-catch MSU exceeds 10^8 organisms per litre, if a single type is cultured, and if increased numbers of white cells are present. Lower numbers of organisms may be considered significant in certain situations (e.g. if antibiotic therapy has been commenced). Chapter 21 gives a more detailed description of how urinalysis is interpreted.

In patients with indwelling urethral catheters attached to a closed drainage system, urine is collected by disinfecting the wall of the catheter at its juncture with the drainage tube and puncturing it with a needle attached to a syringe (see Figure 15.10). The connection between the catheter and the drainage tube should not be broken for specimen collection and the material in the drainage bag, which may have been standing for several hours, must not be used. Urine may also be collected for culture during the course of cystoscopy or retrograde pyelography.

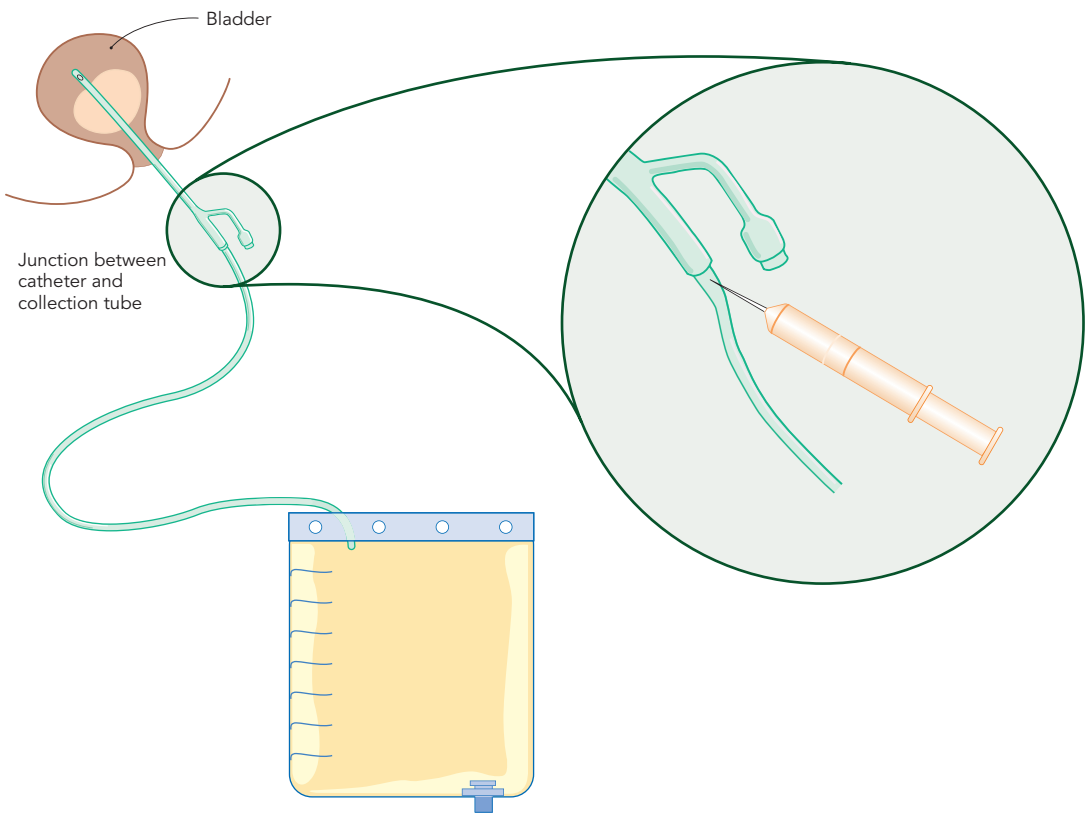


FIGURE 15.10

Collection of a catheter specimen of urine

A needle is inserted just below the junction between the catheter and collection tube.

Specimens for central nervous system infections

In a case of suspected meningitis, cerebrospinal fluid (CSF), obtained by lumbar puncture, is investigated (see Case History 15.1). A lumbar puncture must be performed under conditions of strict asepsis to protect the patient. Also, if contamination of the specimen occurs, the isolation and identification of the true causative agent may be impaired.

The skin at the puncture site should be disinfected with an antiseptic such as povidone-iodine. Specimens should be collected in sterile containers that are sealed with a screw cap to prevent leakage and loss or contamination of the contents. Usually, three portions of 1.5–2 ml each are placed in separate containers for microbiology, cytology and biochemistry testing. If only one tube of CSF is obtained, it should be submitted to the microbiology laboratory first so that it can be opened aseptically.

Meningitis is a life-threatening infection, warranting rapid transport of the CSF specimen to the laboratory, rapid diagnosis and treatment. Blood cultures should also be collected from patients suspected of having meningitis.

CASE HISTORY 15.1

Meningococcal meningitis

A 32-year-old woman went to the emergency department of her local hospital complaining of a sore throat that had persisted for three days, muscle and joint pain, and a mild fever. She told the doctor that the previous day she had had a severe headache with neck stiffness, nausea and vomiting. She did not have a skin rash and was not taking any medications.

On physical examination, her temperature was 37.8°C; pulse, 100; and blood pressure, 110/70 mm Hg. Her neck was stiff and her pharynx was slightly inflamed. A lumbar puncture was taken soon after examination and produced a cloudy cerebrospinal fluid (CSF). Within an hour of CSF collection the microbiology laboratory reported that a Gram stain of the CSF showed large numbers of white blood cells (90 per cent polymorphonuclear leukocytes) and Gram-negative diplococci, suggestive of *Neisseria meningitidis*. The patient was immediately given high-dose intravenous penicillin G. The diagnosis of meningococcal meningitis was confirmed by culture the next day. The patient recovered completely.

Questions

1. What was the value of the Gram stain result in this case?
2. Why were the Gram stain results so definitive in this case?
3. If the Gram stain was negative, would the staff have waited until the next day before commencing antibiotic therapy? Explain.

Specimens of other body fluids

Apart from blood, urine and CSF, other normally sterile body fluids that may be collected include pleural, peritoneal and synovial fluids. These are usually collected by percutaneous needle aspiration using strict aseptic technique.

The number of microorganisms in these specimens may be low, primarily because of their dilution by fluid accumulation at the site. It is therefore necessary to collect as large a specimen sample as possible.

Specimens for upper respiratory tract infections

A number of different types of specimens may be collected from the upper respiratory tract, including throat, nasal and nasopharyngeal swabs, nasopharyngeal fluid and nasal washings. The type of specimen is determined by the type of organism suspected.

Throat swabs (pharyngeal specimens) are collected primarily for the detection of infection by Group A streptococci (*Streptococcus pyogenes*), *Neisseria gonorrhoeae* (in pharyngeal gonorrhoea), *Candida albicans* (thrush) and *Haemophilus influenzae*. The procedure involves:

1. depressing the tongue gently with a tongue depressor
2. extending a sterile swab between the tonsillar pillars and behind the uvula (avoiding contact with the cheeks, tongue, uvula and lips)
3. sweeping the swab back and forth across the posterior pharynx, tonsillar areas, and any inflamed or ulcerated areas to obtain the sample.

A throat sample should not be collected if the epiglottis is inflamed, as sampling may cause serious respiratory obstruction.

Nasal swabs are collected primarily for the detection of carriers of *Staphylococcus aureus*. The procedure involves:

1. inserting a sterile swab into one nostril until resistance is met at the level of the turbinates
2. rotating the swab against the nasal mucosa
3. repeating the process on the other nostril using the same swab.

Nasopharyngeal suction is performed for the detection of carriers of Group A streptococci, *Neisseria meningitidis*, *Corynebacterium diphtheriae* and *Bordetella pertussis*. Material is suctioned from the nasopharynx and collected into a sterile container.

Nasopharyngeal swabs are collected primarily for the detection of carriers of *N. meningitidis* and to diagnose *B. pertussis* infections. In this procedure a flexible wire swab is carefully inserted through the nose into the posterior nasopharynx. The swab is rotated, keeping it near the septum and floor of the nose.

Nasal washings are collected primarily for culture of viruses. With the patient's head hyper-extended (about 70° angle), approximately 5 mL of sterile isotonic saline is instilled

into each nostril. To collect the material, the head is tilted forward and the fluid allowed to run out of the nares into a sterile container; or the fluid is aspirated using a rubber bulb syringe. The nasal washings are placed in an equal volume of viral transport medium, or transported in a sterile container.

Specimens for lower respiratory tract infections

The specimen most often collected in the investigation of lower respiratory infection is expectorated sputum (purulent mucus). This material must pass through the upper airways, which are colonised by large numbers of different bacteria. Specimen quality is of utmost importance, so healthcare personnel should be aware of the need for a fresh, clean specimen of purulent material produced from a deep cough. In some patients, sputum may need to be induced by having them inhale a warmed saline aerosol. A poor-quality specimen (predominantly saliva) is likely to be contaminated with oropharyngeal flora, some of which are potential pathogens and may therefore be incorrectly assumed to be the cause of infection. This can make it difficult to determine which of the organisms isolated is responsible for the infection. Thus, culture of sputum does not always yield clear-cut results.

Specimen quality may be assessed in the laboratory by performing a Gram stain. Sputum specimens with excessive squamous epithelial cells are judged to be unsatisfactory because such specimens are likely to contain a large amount of saliva and to be significantly contaminated with oropharyngeal flora.

A better specimen may be obtained by bronchial aspiration through a bronchoscope, or by inserting a needle into the trachea below the glottis (trans-tracheal aspiration). Needle biopsy of the lungs may be necessary for the diagnosis of infections in which there is no productive cough (e.g. fungal and viral infections).

If tuberculosis is suspected, extreme care should be exercised in handling the sputum specimen because the organism can be transmitted from the specimen to the healthcare worker.

Specimens for gastrointestinal tract infections

Faeces (stools) may be collected for the culture of bacteria causing gastroenteritis or for the detection of protozoa, helminths or viruses. The proper collection and preservation of faeces are frequently neglected, but are important requirements for the isolation of microorganisms responsible for intestinal infections.

A stool specimen can be effectively obtained by:

- passing the stool directly into a wide-mouth, leak-proof container with a tight-fitting lid; or
- passing the stool into a clean, dry bedpan and then transferring the stool into a sterile leak-proof container with a tight-fitting lid.

Toilet paper should not be used to collect the stool because it may contain chemicals that are inhibitory for some faecal pathogens. The specimen should be transported to the laboratory promptly, or refrigerated if a delay is unavoidable. If a stool specimen cannot be processed within several hours of collection, it may be mixed with a suitable transport medium. Deterioration of parasites (protozoa and helminths) in stool samples may be prevented by the use of appropriate fixatives, which preserve the morphology of the protozoa and helminths.

In selected circumstances, such as in the detection of rectal gonorrhoea, a rectal swab may be necessary. The swab should be passed beyond the anal sphincter, carefully rotated and withdrawn. The swab should be placed in a screw-cap tube containing a preservative such as Cary-Blair medium and transported to the laboratory.

Other specimens that may be required include gastric aspirates for certain parasites (e.g. *Giardia*, *Cryptosporidium*) and gastric biopsies (for detection of *Helicobacter pylori*).

A single negative stool culture or examination for eggs and parasites cannot be regarded as sufficient for ruling out a particular gastrointestinal pathogen. For many infectious diarrhoeas, up to three stool specimens may need to be collected and examined.

Specimens for genital tract infections

Genital tract specimens are submitted mainly for the detection of such pathogens as *Neisseria gonorrhoeae*, *Chlamydia trachomatis,* herpes simplex virus, genital wart virus, *Trichomonas*, *Haemophilus ducreyi*, Group B streptococci, *Candida albicans* and certain anaerobic bacteria.

In females, a number of specimens may be collected depending on the site of infection. For example:

- cervical swab
- vaginal swab
- urethral swab
- amniotic fluid
- rectal swab
- vesicle fluid (e.g. on vulva).

In males, typical specimens include:

- urethral swab
- fluid or swab from penile lesion
- rectal swab.

Specimens from infected wounds and tissue

A number of different specimens may be collected from infected wounds and tissues. As for other specimen types, specimen quality is of utmost importance. A sample of tissue, pus or fluid is generally superior to a swab specimen.

Tissue and biopsy specimens should be placed into a sterile, screw-cap container and transported immediately to the laboratory. If the specimen is small, it should be immersed in sterile isotonic saline to prevent it from drying out. From an infected wound a volume of pus should be collected if possible. There are circumstances, however, when only a swab specimen can be obtained. In such cases, the swab should be used to sample as much of the lesion as possible. Swabs should be transported to the laboratory in transport medium.

Specimens for anaerobic bacteria

A variety of anaerobic bacteria can cause infections in humans, including species of *Bacteroides*, *Clostridium* and *Peptostreptococcus*. Anaerobes may be involved in appendicitis, cholecystitis, periodontal infections, endocarditis, endometritis, brain abscess, osteomyelitis, peritonitis, empyema, salpingitis, sinusitis, and wound infections following bowel surgery or trauma. These bacteria may be overlooked or missed if the specimen is not properly collected and transported to the laboratory and then subjected to appropriate laboratory procedures.

Anaerobes vary in their sensitivity to oxygen, but a brief exposure of less than ten minutes to atmospheric oxygen is enough to kill the more sensitive organisms. If anaerobes are suspected, the laboratory request form should indicate it, and the specimen should be exposed to air for as brief a time as possible.

One of the best specimens for anaerobic culture is pus, obtained by using a needle and syringe. In a volume of pus the bacteria are somewhat protected from oxygen and drying. The material, however, should *not* be transported in the needle and syringe. Needle transport is very unsafe because there is the potential risk of a needlestick injury, and syringe transport poses a risk because the specimen may be expelled during transport, creating a threat to people and the environment. So, aspirated material should be transferred to an anaerobic transport vial. Large volumes of purulent material may be transported in a sterile, screw-cap container. The needle and syringe should be disposed of in the appropriate way (see Chapter 13).

Tissue and biopsy samples are also very good specimens for anaerobic culture. After collection they should be placed into an anaerobic transport device, or a sterile tube or petri dish, which is then placed into a sealable plastic bag that generates an anaerobic atmosphere. When a swab must be used to collect a specimen, a commercial anaerobe swab system should be used.

Extremes of heat or cold should be avoided. If delays are unavoidable, the specimens should be held at room temperature until processing.

Specimens for fungal culture

A variety of specimens may be collected for the culture of fungi, depending on the site of infection. For superficial infections, skin scrapings, hair or nail clippings from the site of infection are most appropriate. For subcutaneous infections, possible specimens include sputum, blood and pus. Specimens for the culture of fungi should be collected aseptically, placed in sterile containers and delivered to the laboratory within several hours. Swabs are the least suitable, but specimens from certain body sites, such as the ear canal, nasopharynx, throat, vagina and cervix, are not readily collected by other means.

Specimens should be transported in sterile, humidified, leak-proof containers. Only dermatological specimens (skin, hair or nail clippings) should be transported in a dry container. Transport medium should not be used unless the specimen can be easily and completely retrieved from the medium.

Specimens for viral culture

Typical specimens for viral culture are listed in Table 15.2. In addition to a specimen from the clinical site of infection, blood, throat washings and faeces are also often collected. The chance of virus recovery is best if the specimen is collected within three days of the onset of symptoms; for many viruses it is greatly reduced beyond five days. Specimens other than fluids should be placed in a sterile, leak-proof container with viral transport medium. However, with the development of rapid molecular techniques, especially PCR (see later section) virus culture is infrequently used for diagnosis of infection.

TABLE 15.2 Specimens required for viral culture

CLINICAL DISEASE	SPECIMENS REQUIRED
Measles (rubeola)	Blood, urine, nasopharyngeal secretions
German measles	Throat and nasal swabs, blood, urine
Chickenpox	Vesicle fluid
Respiratory syncytial virus	Nasal washings
Herpes simplex—skin lesions	Vesicle fluid
Herpes simplex—encephalitis	Cerebrospinal fluid, brain biopsy
Diarrhoea (e.g. rotavirus infection)	Faeces, rectal swab

CULTURING BACTERIA AND FUNGI

Culture media

A nutrient composition prepared for the growth of microorganisms is called a **culture medium**. Culture media are sterilised when they are made so that they have no microorganisms in them before they are used. When microbes are grown on a culture medium the growth is called a **culture**. It is possible to grow the majority of bacteria and fungi in artificial (chemically prepared) culture media, but there is no single medium in which all types of bacteria and fungi are able to grow. Whereas many (but not all) bacteria can be grown within 18 hours on culture media, most fungal species require several days to grow.

Some bacteria have very special nutrient requirements. For example, *Streptococcus pyogenes* can grow only if red blood cells are supplied in the medium, and *Neisseria gonorrhoeae* can grow only if the blood is heated (to release the nutrients) before being added to the medium. Microorganisms that require special growth factors, such as these, are said to be **fastidious**, requiring **enriched media**. There are some bacteria that are so fastidious they can't be grown in culture media at all—for example, *Treponema pallidum* and

Mycobacterium leprae; and others such as the chlamydiae and rickettsiae cannot be grown in artificial media but can be grown in cell cultures (cultures of living animal cells).

Some culture media are designed to allow the growth of certain microorganisms, while inhibiting the growth of others. For example, Sabouraud dextrose medium has a low pH and high sugar content, which inhibits bacteria but allows fungi to grow (see Figure 15.11). MacConkey medium contains bile salts which inhibit non-enteric bacteria. These are called **selective media**. MacConkey medium is also an example of what are termed **differential media**, because certain bacteria can be differentiated from others by the particular nature of their growth on the medium. The medium contains lactose and a pH indicator, so colonies of lactose fermenters (e.g. *E. coli*) are pink/red coloured, whereas colonies of non-lactose fermenters are cream/yellow (see Figure 15.12). In the last decade, many chromogenic media have been developed, and are very useful types of differential media on which different organisms can be distinguished by their different-coloured colonies (see Figure 15.13).

Pure cultures

Culture media may be liquid, but most of the time they are made solid by the addition of a gelling agent, called **agar**. A solid medium contained in a petri dish (a circular plastic dish) is called an **agar plate**. Solid media are often used because bacteria and fungi can grow on the surface of the media, forming colonies composed of millions of cells. All the cells in a colony are identical because they are the progeny of a single cell that was initially implanted on to the surface of the agar. If a single colony is transferred to a fresh agar plate and spread over the surface, numerous colonies will grow on the plate and all the colonies will comprise the same species of organism.

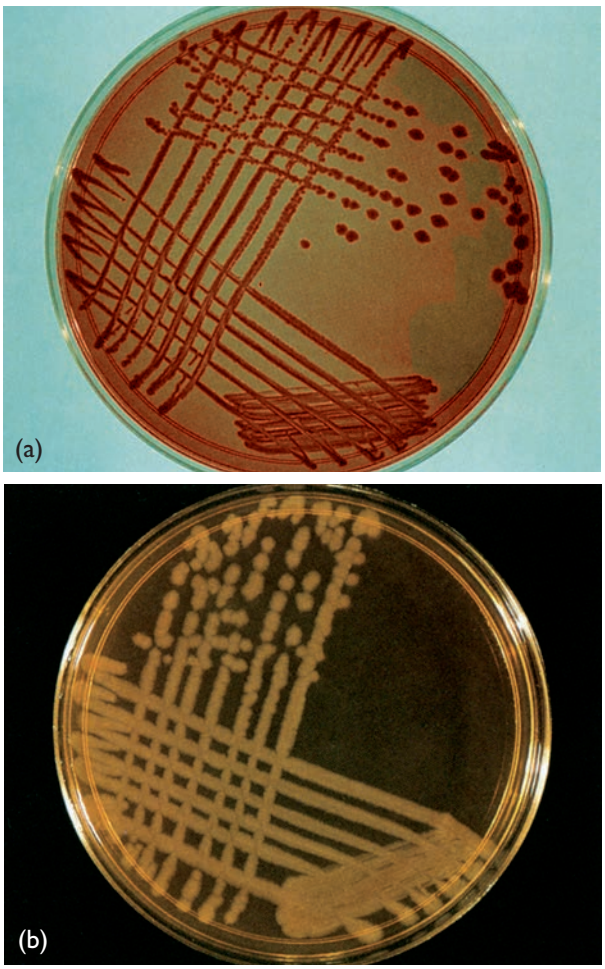


FIGURE 15.12

Differential growth medium

Differentiation of (a) Escherichia coli; *and (b)* Pseudomonas aeruginosa *on MacConkey agar.*

Source: G. Jayachandran, Sydney Medical School, University of Sydney.

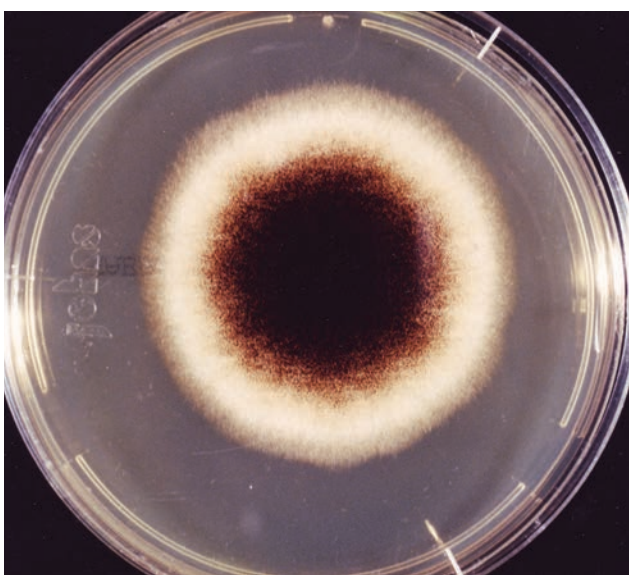


FIGURE 15.11

Culture of *Aspergillus niger*

The fungus has been grown on Sabouraud dextrose agar.

Source: G. Jayachandran, Sydney Medical School, University of Sydney.

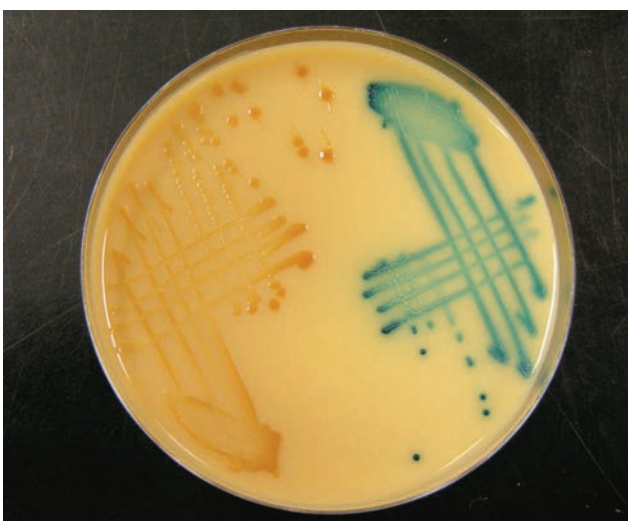


FIGURE 15.13

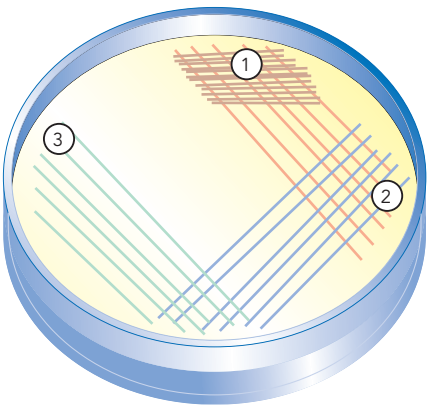
Chromogenic medium

A type of differential medium.

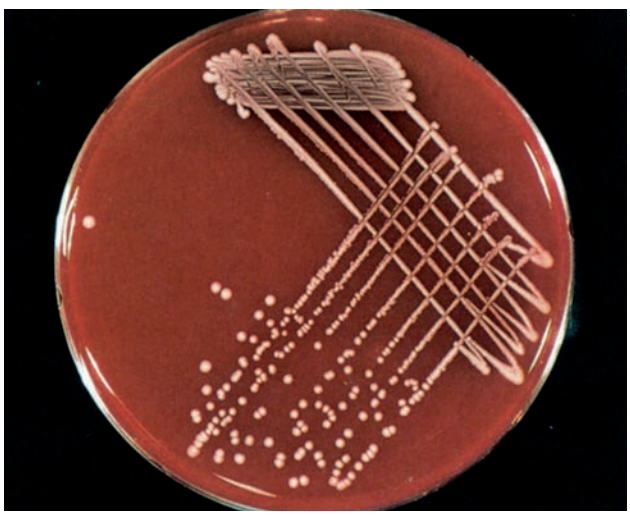
Source: Dr Gary Lee.

This is called a **pure culture**. In order to perform tests on a microorganism it is essential to have a pure culture, so that the test results reflect the attributes of that organism alone.

The way in which pure cultures are usually prepared is called the **streak plate method**. Bacteria are picked up by a sterile wire loop and, as the loop is moved lightly over the surface of the agar in lines (or streaks), they are deposited on the surface of the plate on the lines. The loop is sterilised by flaming between different areas of streaking, resulting in fewer and fewer bacteria being deposited. This has a diluting effect and results in single bacterial cells being deposited in the areas that are streaked last. When the plate is incubated, a separated, single colony will grow where each cell is deposited (see Figure 15.14).



(a)



(b)

FIGURE 15.14

The streak plate method for obtaining pure cultures

(a) Organisms are picked up on a sterile wire inoculating loop and lightly streaked across the agar in region 1; the loop is sterilised by flaming and more streak lines are made in region 2; the loop is sterilised again and the streaking is repeated in region 3; after incubation, well-separated colonies of bacteria appear on streak lines, especially in region 3; (b) a streak plate after incubation.

Source: (b) G. Jayachandran, Sydney Medical School, University of Sydney.

Streak plating to obtain pure cultures is necessary because the majority of clinical specimens contain mixtures of microorganisms, particularly if the specimen has been taken from a body site which has a normal microbial flora. Streak plating allows the separation of different organisms and their subsequent purification.

Sometimes, liquid media are used first to increase the numbers of organisms, followed by culture on solid media. Liquid media are used for culturing blood, because the numbers of bacteria in a sample of blood may be very small. Liquid media are also used for culturing of swabs if the presence of anaerobic bacteria is suspected. These are often liquid media containing particles of meat (called a cooked meat medium—see Figure 15.15), within which anaerobes can grow. A liquid medium has the added advantage of diluting any interfering substances in the specimen, such as antibiotics.

Incubation

The majority of bacteria and some fungi (yeasts) grow from single cells to macroscopic colonies on an agar plate in 18–24 hours, although anaerobes often require 48 hours or longer. Some bacteria (e.g. mycobacteria) and moulds grow much more slowly and can take several days or sometimes weeks to form visible colonies. Cultures for anaerobic incubation are placed in an airtight container together with a satchel that chemically removes the air.

Identification of cultured organisms

Once a culture of a pathogenic bacterium is obtained, various criteria and tests are used to establish the identity of the organism. Different criteria are needed to identify different organisms, but a presumptive identification is usually based on:

- Gram reaction (see Chapter 4)
- cell morphology (i.e. the shape and arrangement of the cells)
- colony morphology (i.e. the characteristics of the colonies on solid culture media)
- ability to grow aerobically (in the presence of oxygen) or anaerobically, or both
- specialised growth requirements.

To confirm the identity of an organism other properties usually need to be examined, such as:

- the production of special enzymes (e.g. coagulase by *Staphylococcus aureus*)
- biochemical activities (e.g. the ability to metabolise different sugars)
- its motility (ability to swim through liquid).

The biochemical profile may be performed using commercially available multi-test systems, such as that shown in Figure 15.16. These require incubation for 4–24 hours, depending on the type of kit used. These methods are gradually being replaced by automated bacterial identification systems, with which an identification and antibiotic

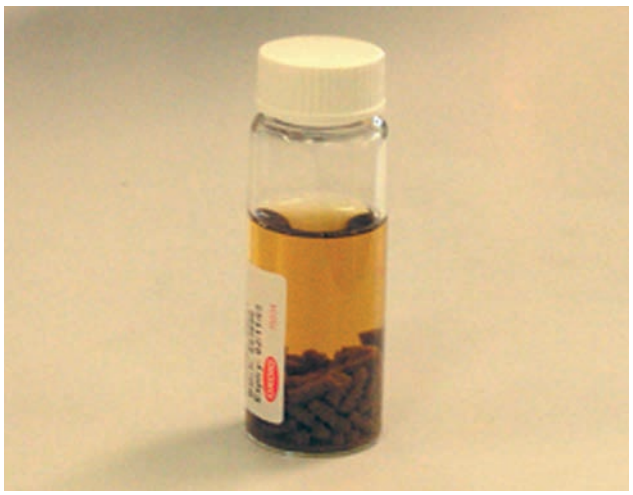


FIGURE 15.15

Cooked meat medium for the growth of aerobic and anaerobic bacteria

Source: (b) Dr Gary Lee.

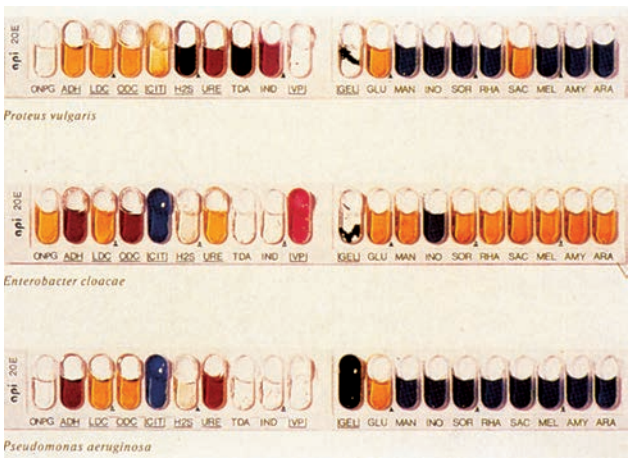


FIGURE 15.16

Multi-test systems are used to perform multiple biochemical tests on a microorganism simultaneously

Shown here is the commercially prepared API 20E system being used to distinguish between three different bacteria.

Source: Copyright 1996–2011 bioMérieux Australia Pty Ltd.

sensitivity profile can be obtained for many bacteria in 4–8 hours.

Fungi are usually identified on the basis of their colony appearance (e.g. colour) and the microscopic morphology of their cells and reproductive structures (see Chapter 6). A biochemical analysis is used for some fungi, especially yeasts. Molecular methods for identification of fungi are becoming more common.

Overall, for most bacteria and yeasts, culture and identification results are usually available within 24–48 hours from the time of arrival at the laboratory. However, slow-growing bacteria and moulds can take from several days to weeks to culture.

Antibiotic sensitivity tests

Bacteria isolated in pure culture may also have their sensitivity to different antibiotics determined. This is required for organisms whose antibiotic sensitivity pattern cannot be predicted. Many of the bacteria that cause healthcare-associated infections fall into this category.

One method, using filter paper discs impregnated with antibiotics, is described in Chapter 12. Briefly, colonies from a culture of the test bacterium are used to seed the whole surface of a plate, and antibiotic discs are then placed on the plate. During incubation, growth of the bacterium is inhibited around the discs containing the antibiotics to which it is sensitive. Thus, antibiotic sensitivity testing requires a further overnight incubation period, although a number of automated sensitivity testing instruments can now give results for some bacteria in 3–8 hours. To obtain a quantitative antibiotic sensitivity result, called a *minimal inhibitory concentration* (MIC) (see Chapter 12), a culture method with an overnight incubation may be required. Automated methods, if available, can provide an MIC in 4–8 hours.

CULTURE OF OTHER MICROORGANISMS

Viruses and the bacteria belonging to the genera *Chlamydia* and *Rickettsia* are obligate intracellular parasites and must therefore be grown in cell or tissue cultures, as described in Chapter 5. Growth in cell cultures usually requires a minimum of several days.

Cultured viruses are identified by the cytopathic (cell damaging) effect the virus has on the cells it has been grown in and/or by electron microscopic examination of the cultured viruses. Although virus isolation gives the most accurate diagnosis of infection, in most clinical settings it is not practical. The culture of viruses (as well as *Chlamydia* and *Rickettsia*) is time-consuming and involves highly specialised techniques, so alternative diagnostic methods, especially PCR (polymerase chain reaction) and serology, are generally used by clinical microbiology laboratories. Culture is done mainly by specialist virology or research laboratories.

Very few protozoa and helminths can be cultured *in vitro*. Thus, laboratory diagnosis of parasitic infections is usually done by non-cultural methods. Many parasites can, in fact, be quickly identified on morphological grounds by microscopic examination of the specimen. Infections caused by other parasites are usually diagnosed by immunological methods, antigen detection or PCR (see following sections).

SEROLOGY (IMMUNOLOGIC DIAGNOSIS)

The study of antigen-antibody reactions in the laboratory is called **serology**. A serologic diagnosis of infectious disease is based on the principle that when a person has an infection they ultimately produce antibodies that are specific for the microorganism causing the infection (see Chapter 9). These antibodies are detectable in serum (or blood) and other body fluids. Thus, a serologic diagnosis is performed by testing a person's serum (or sometimes other body fluid) for

antibodies against antigens of the microorganism thought to be causing the infection. The antigens may be whole microorganisms, or parts of them, such as viral coats, or bacterial walls, flagella or toxins. Serological tests are useful if the microorganism:

- is impossible or difficult to grow (e.g. *Treponema pallidum*, viruses and parasites); or
- it grows slowly (e.g. *Cryptococcus*, *Legionella* and viruses).

The detection of antibodies can also be used to assess a person's immune status to a particular microorganism. For instance, immunity to rubella in pregnant women is determined by measurement of antibodies to the virus in serum. Serological techniques are also used in non-microbiological tests, such as in blood banks for typing blood, for typing tissue before transplant operations, and for typing of immunoglobulins in certain immunological disorders.

Most serologic diagnoses are based on measurements of antibody concentration (titre) in serum. These have the disadvantage that usually one to several weeks or more must elapse from the time of infection before a clearly detectable antibody response is produced. Another drawback is that a positive test only represents exposure to the organisms at some time and not necessarily active disease (see Chapter 9).

For a positive diagnosis, it is most desirable to demonstrate **seroconversion** in a patient—that is, no (or low) antibodies to the microorganism in the early stage of an illness, and then demonstration of a significant amount (or increase) of antibodies to it a week to several weeks later. An alternative criterion for a positive serological diagnosis is demonstration of a fourfold increase in antibody content in sera collected two weeks apart (usually termed *acute* and *convalescent sera*—see under 'Development of disease' in Chapter 7, page 149).

The principle of antibody titration is shown in Figure 15.17. The patient's serum antibody titre is determined by adding the microbial antigens to serial dilutions of the patient's serum; for example, tube 1 = 1/20, tube 2 = 1/40, tube 3 = 1/80, tube 4 = 1/160, tube 5 = 1/320, etc. The greatest dilution that shows a positive reaction is the antibody titre of the patient's serum. Nowadays, these titrations and reactions are usually performed in automated systems. An antibody titration can also be performed on other body fluids (e.g. urine, CSF).

Several methods are available to determine the amount of antibody in an individual's serum. The method used depends mostly on whether the antigen is a whole cell, a toxin, a cell component or a virus. Because all antibodies have two or more antigen-binding sites (see Chapter 9), a reaction between antibody and antigen forms a latticework (or aggregate) of many antigen-antibody molecules that becomes quite large in appropriate conditions. If the antigen is on or fixed to particulate material, such as a bacterial cell or latex particles, the end result is a clumping of the particles, or **agglutination**. If the antigens are fixed to red cells, the positive reaction is called a **haemagglutination**.

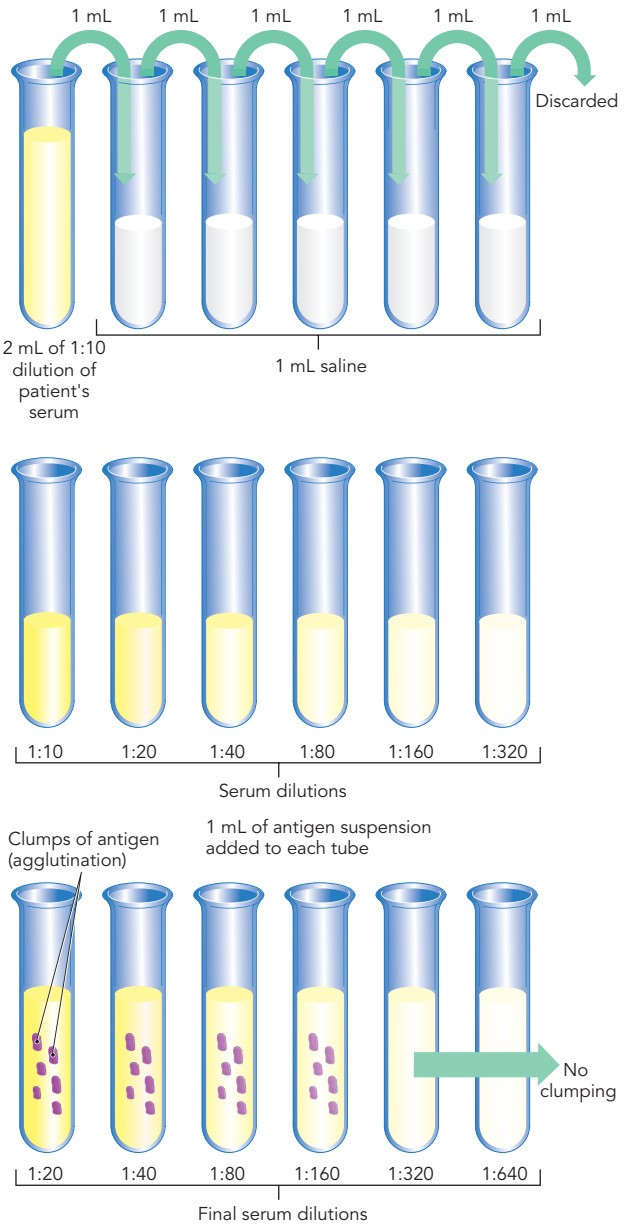


FIGURE 15.17

The principle of serial dilution for antibody titration

The titre is the last tube that shows a positive reaction (clumping), which in this example is 160.

Other commonly used serological assays are the fluorescent antibody test, the enzyme-linked immunosorbent assay (ELISA), immunoblotting and radioimmunoassay. Different methods are required for different types of antigens.

IgM testing

A major drawback of serological diagnosis of infection is the need to demonstrate an increase in antibody titre in paired sera (acute and convalescent). For this reason, a serological diagnosis is usually retrospective. This can be overcome if specific IgM antibodies can be detected. As we saw in Chapter 9, IgM is present early in infection and usually

disappears after several weeks. It is therefore indicative of active, rather than past infection, permitting a diagnosis to be made from a single specimen.

ANTIGEN DETECTION

Detection of antigens of a microorganism in an appropriate specimen is a method used for diagnosis of some infections. Like serological methods, antigen detection methods are most commonly used for diseases caused by organisms for which culture is not possible, or is too difficult or too time-consuming. Antigen detection methods are also useful in providing a rapid, presumptive diagnosis in emergency situations, such as bacterial meningitis or acute pneumonia. Commercial antigen detection kits are available for a range of viruses (e.g. norovirus, respiratory syncytial virus, herpes simplex viruses, cytomegalovirus), parasites (e.g. *Giardia*), fungi (e.g. *Cryptococcus*) and bacteria (e.g. *Legionella*, *Clostridium difficile* toxin, *Neisseria meningitidis*, pneumococcus).

In some diseases, antigens of the microorganism can be detected before the patient produces specific antibodies. For example, cryptococcal antigen can be detected in the CSF of patients with cryptococcal meningitis early in the infection. Antigens of the human immunodeficiency virus (HIV) can be detected in blood during the antibody negative (window) period, which can exceed three months in some people (see Chapter 19).

Antigen detection methods utilise specific antibodies to identify antigens of the organism in patient material. The methods used include a variety of enzyme immunoassays, immunofluorescence and latex agglutination tests.

DETECTION OF MICROORGANISMS USING MOLECULAR TECHNIQUES

Molecular-based techniques have been proclaimed by many people to be the most significant development in diagnostic microbiology in decades. They are becoming more and more widely used because of their superior sensitivity, relative speed, and ability to identify pathogens that are slow growing or difficult or impossible to culture. These methods identify the cause of an infection by detecting fragments of nucleic acids of the microorganism in a specimen from the patient or from a culture of the isolated organism. A variety of molecular-based techniques have so far been developed (see Table 15.3), allowing a faster and more sensitive detection of microorganisms than conventional methods.

Molecular techniques are particularly useful for:

- reducing the time for a result from days (by culture) to hours—especially when speed of diagnosis is vital (e.g. with pneumonia)
- diagnosis of infections caused by organisms that are difficult or impossible to identify by other methods (e.g. viruses, some bacteria and fungi)
- quantifying the amount of nucleic acid, and hence the numbers of microbes, in a clinical specimen (e.g. to monitor the course of human immunodeficiency virus or hepatitis C infection)
- the detection of specific virulence or antibiotic resistance genes in bacteria (e.g. mecA gene for methicillin resistance in *Staphylococcus aureus*)
- the subtyping of viruses (e.g. hepatitis C subtypes)
- epidemiological studies.

TABLE 15.3 Molecular techniques used in clinical microbiology laboratories

Hybridisation with nucleic acid probes
Polymerase chain reaction (PCR)
Ligase chain reaction (LCR)
Nucleic acid sequence-based amplification (NASBA)
Trans-mediated amplification (TMA)
Branched chain DNA signal amplification (bDNA)
Strand displacement amplification (SDA)
Pulse field gel electrophoresis
165 ribosomal RNA sequencing

With technological improvements, the development and introduction of these methods are proceeding rapidly and they are becoming common procedures in the clinical microbiology laboratory. They also play vital roles in cancer diagnosis, human genetics, forensic science and archaeology.

Nucleic acid probes

Virtually all microorganisms contain some unique nucleotide sequence in their genome which, in theory, can be utilised as a fingerprint for rapid identification. A nucleic acid probe is a single-stranded segment of DNA or RNA that is complementary to the nucleic acid that is to be detected. The probe binds to a complementary nucleic acid sequence, if present, to form a novel duplex molecule (see Figure 15.18). Probes are labelled with radioisotopes (such as ^{32}P), enzymes or substances that give colour or fluorescent reactions, so that the formation of duplex molecules can be readily detected. Probes can be made that are specific to the genus, species or even strain, by varying the nucleotide sequence that is used.

A probe can be used to identify unknown organisms growing in culture. For example, gene probes for *Mycobacterium tuberculosis* and *M. avium intracellulare* have reduced the time required to identify cultures of these organisms from weeks to hours. A number of commercially available probes have been produced for culture confirmation.

Gene probes can be used to detect genetic sequences that code for antibiotic resistance. One of the best characterised genes that can be detected with gene probes is the *mecA* gene of *Staphylococcus aureus*, which codes for methicillin resistance.

Another use for probes is in the detection of pathogens in a specimen. The direct detection of microbial nucleic acid in a clinical sample is the most exciting potential for probes because it has the advantage of speed and specificity. Probes for enterotoxins of *Escherichia coli* or cholera toxin have

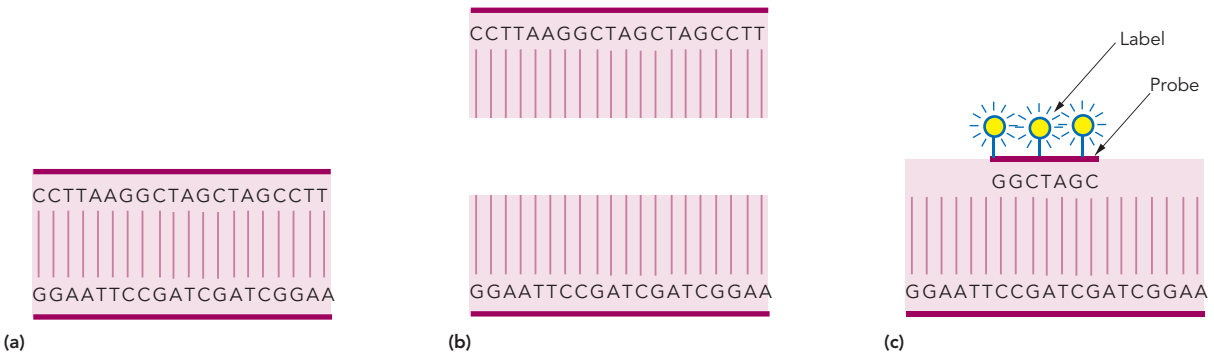


FIGURE 15.18

Use of a DNA probe to identify a particular DNA sequence

(a) A sample of double-stranded DNA for testing; (b) separation of the DNA segment; (c) binding of the labelled probe DNA to the sample DNA, thereby identifying it.

been applied directly to faeces. A commercially available ^{125}I-labelled DNA probe directed against sequences specific for *Mycoplasma pneumoniae* has been used successfully to detect the organism in sputum specimens.

A major limitation of this technology is that gene probes are generally unable to detect low numbers of organisms (few copies of the gene) in clinical specimens. Nucleic acid amplification methods (e.g. the polymerase chain reaction) are a solution to this problem.

Polymerase chain reaction

Nucleic acid amplification tests for the rapid detection of infectious microorganisms have evolved dramatically over the last two decades. The polymerase chain reaction is the most commonly used of these. The **polymerase chain reaction** (**PCR**) is a technique that can increase the quantity of a specific nucleotide sequence contained within a sample by a process of directed DNA synthesis. The PCR is a powerful amplification and detection technique that can theoretically generate billions of copies of DNA from a single molecule of the nucleic acid in a few hours.

PCR technology was first described in 1985. In this process, DNA is first extracted from the clinical specimen and then passed through a temperature-controlled cycle that amplifies a specific segment of the target DNA (see Figure 15.19). The target DNA is mixed with sequence-specific primers (that are complementary to, and select, the segment of DNA of interest), free nucleotides and the enzyme DNA polymerase.

The mixture is first heated to 90–95°C to separate the two strands of the target DNA. Each strand of the DNA acts as a template for DNA synthesis. The mixture is then cooled to 45–60°C to allow annealing (joining) of the primers to a specific part of the target DNA. The primers are extended by the addition of nucleotides complementary to the target DNA by DNA polymerase, ultimately yielding two identical replicas of the original target DNA. Thus, the numbers of copies of the target DNA is doubled during every cycle. The cycle of heating and cooling is repeated (by an automated thermocycler) to exponentially increase the number of copies of the target DNA.

PCR has been used to great effect in the diagnosis and management of some diseases and offers enormous potential in diagnostic microbiology in general. For example, it is now commonly used for the diagnosis of some infections caused by organisms that are slow-growing or difficult to grow, such as viruses, *Mycobacterium tuberculosis* and *Chlamydia trachomatis*. These microbes typically take two or more weeks to grow, making culture of limited clinical usefulness.

PCR also plays a vital role in assessing disease progression, patient infectivity and prognosis, and the effectiveness of therapy in some very important viral infections (e.g. human immunodeficiency virus (HIV), hepatitis B and hepatitis C). A further application of PCR is in the subtyping of viral infections (e.g. herpes simplex viruses 1 and 2 and subtypes of hepatitis C).

PCR shows great promise in other medical areas such as the amplification of genes associated with genetic diseases including cystic fibrosis and haemophilia.

The basic process of PCR has been modified in a variety of ways to significantly expand its versatility and applications:

- Multiplex PCR is a modification in which multiple sets of primers are used to detect several target DNA sequences simultaneously. That is, a single process can test for multiple potential causes, provided they have been included in the test system.
- RNA can be detected by converting RNA into complementary DNA and then amplifying it in a process called reverse transcriptase PCR (RT-PCR). This modification is useful for the identification of RNA viruses and for distinguishing between active (viable) and inactive (latent) infection.
- If broad-based primers are used (called broad-range PCR), DNA in clinical specimens can be randomly amplified and unknown microbes can be detected and possibly characterised. This methodology was responsible for the relatively rapid identification of the

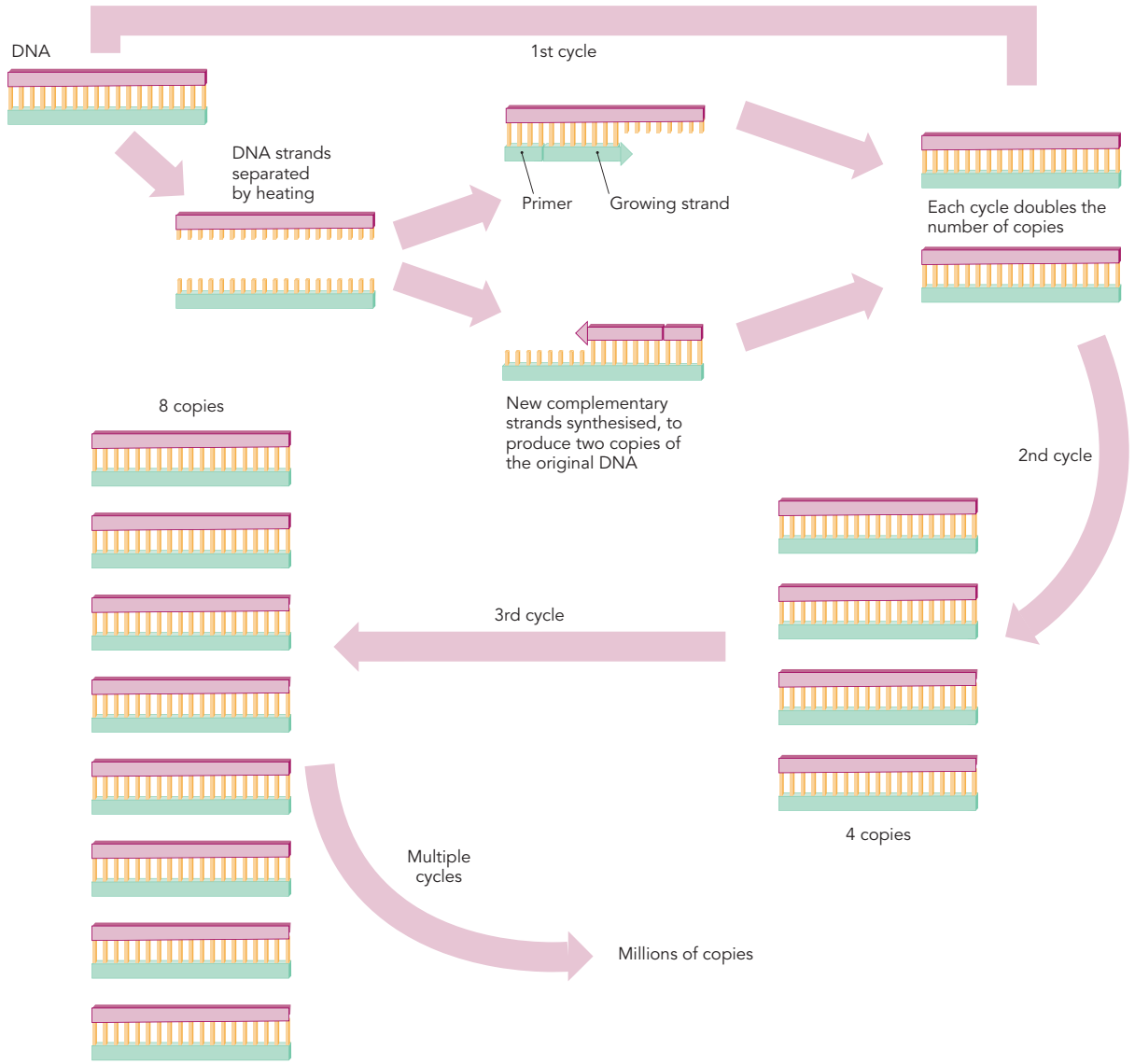


FIGURE 15.19

Mechanism of the polymerase chain reaction

The polymerase chain reaction is a nucleic acid amplification technique allowing a small number of molecules of DNA to be copied many times over.

cause of SARS (severe acute respiratory syndrome) as a coronavirus within weeks of the start of the outbreak in late 2002. This was a major factor that led to the early control of the outbreak.

- A significant development of the PCR technology is real-time PCR. In this method, amplification and detection of amplified products occur in the same reaction vessel. This major breakthrough enables detection and quantitation of the product to occur simultaneously with the amplification step, making the whole process even faster. By comparison with controls, the starting amount of DNA in the sample can be quantified, which may be useful in some circumstances in determining the severity of infection. Furthermore, this has become an important means of monitoring viral load and the effectiveness of therapy in some viral infections (e.g. hepatitis C). Real-time PCR is also referred to as quantitative PCR (qPCR).
- PCR tests are being developed to detect the presence of genetic determinants of antimicrobial resistance. This will partially address the inability of non-culture methods of assessing antibiotic sensitivities.

The use of PCR and other nucleic acid amplification methods provides significant advantages in the diagnosis of some infectious diseases. The advantages include faster turnaround time, better sensitivity and specificity in some cases, strain typing, greater use of automated processes, and their suitability for specimens collected after the commencement of antibiotic therapy. However, PCR has some important limitations. It requires scrupulous technique and considerable technical skill to perform. Even then, false positive and false negative results are a constant concern. Also, if antibiotic

CASE HISTORY 15.2

Herpes simplex encephalitis

A 10-year-old girl, who was previously well, presented with a nine-day illness, characterised by fevers and headache. On the day before her admission her temperature was noted to be 'very high'. She had rigors, seemed delirious with abnormal speech and was becoming increasingly drowsy. By the day of admission, she was agitated, abusive and incomprehensible. She was admitted to a base hospital where investigations were all normal, except for a CT scan that showed a left uncal haemorrhage.

The CSF taken on the day of admission contained 100×10^6/L red blood cells, 400×10^6/L lymphocytes, 100×10^6/L polymorphs, 3.7 mmol/L glucose, 0.88g/L protein, and intracranial pressure 13–15 cm.

Polymerase chain reaction (PCR) for herpes simplex virus (HSV) DNA was positive. Serum taken on day 2 of admission was negative for HSV IgM and low positive for IgG.

A second CSF was taken on day 3 of admission, containing 420×10^6/L red blood cells, 550×10^6/L lymphocytes, 24×10^6/L polymorphs, 2.8 mmol/L glucose, and 0.8 g/L protein. The CSF was sterile for both bacteria and viruses, negative for HSV IgM and IgG, and again positive by PCR. A third CSF taken eight days after admission had borderline HSV IgM and positive HSV IgG, but was negative by PCR.

When the PCR results were known, ceftriaxone therapy was ceased and aciclovir treatment was continued for a total of 14 days.

Source: Adapted from J. Montanaro 1992, Early detection of herpes simplex encephalitis by polymerase chain reaction. *Communicable Diseases Intelligence* 16(4), 24 February: 76–77.

Questions

1. What is the advantage of PCR in this case?
2. Why were antibody tests initially negative?
3. How is PCR able to detect infection so early?

sensitivity is required, culture of the causative agent must still be performed. Other limitations are that most methods do not distinguish between viable and non-viable organisms, and they are less effective in identifying an infection caused by multiple organisms (except multiplex PCR).

The cost and availability are also limiting factors. Perhaps the greatest limitation of PCR is that it requires a fairly accurate preliminary diagnosis to be effective. PCR cannot detect an organism for which primers have not been provided in the reaction, although multiplex and broad-range methods have reduced this problem to some extent. Despite these limitations, PCR technology has now replaced conventional diagnostic methods (e.g. culture) in many situations.

OTHER MODERN DIAGNOSTIC TECHNOLOGIES

As stated throughout this chapter, there are constant efforts to develop diagnostic techniques that are faster and that have better specificity and sensitivity than the methods in current use. A relatively new technology that is on the verge of being widely adopted in clinical microbiology laboratories involves the use of mass spectrometry.

Mass spectrometry

A major contribution to the revolution in diagnostic microbiology is a technology referred to as 'matrix-assisted laser desorption ionisation time-of-flight mass spectrometry' (MALDI-TOF MS). This method analyses microbial proteins using mass spectrometry, and is able to accurately identify, within minutes, bacteria, yeasts or fungi from a single colony grown on a culture medium. The spectral fingerprints (patterns) vary between microorganisms, and are specific to the genus, species and sometimes sub-species level. The fingerprint of the test organism is compared to a database to identify the organism by matching fingerprints. Figure 15.20 shows the spectral fingerprints of five different bacteria. There are also methodologies currently being developed to enable MALDI-TOF MS to be used directly on positive blood culture broths, allowing identification of the causative agent within one hour. MALDI-TOF MS is likely to become a standard technique in routine microbiology laboratories in the near future.

DNA sequencing

DNA sequencing involves an analysis of the order of nucleotides in a DNA molecule. Determination of the DNA sequences of microorganisms is an emerging technology that could provide, in the near future, major breakthroughs in the rapid diagnosis of infectious diseases, especially those that are currently difficult to diagnose, and in the monitoring of outbreaks. Substantial research effort is currently being undertaken to develop cost-effective, high-throughput DNA sequencing technology, and to determine the whole genome sequences of pathogenic microorganisms.

POINT OF CARE TESTING

Point of care testing is an analytical test performed outside of a diagnostic laboratory, using a device that can easily be transported to the vicinity of the patient. That is, it is a rapid test that can be performed at the bedside, in a doctor's office or in the field. Point of care testing has received enormous attention in recent years, because it can potentially provide almost immediate test results that facilitate rapid clinical decision-making and intervention. Rapid tests, suitable for point of care use, have been developed for a number of microorganisms, including influenza viruses, HIV, MRSA, *Chlamydia trachomatis*, *Treponema pallidum*, *Streptococcus pneumoniae*, respiratory syncytial virus, rotavirus, *Clostridium difficile*, *Entamoeba histolytica*, *Plasmodium* sp., *Mycobacterium tuberculosis* and *Helicobacter pylori*. These tests are

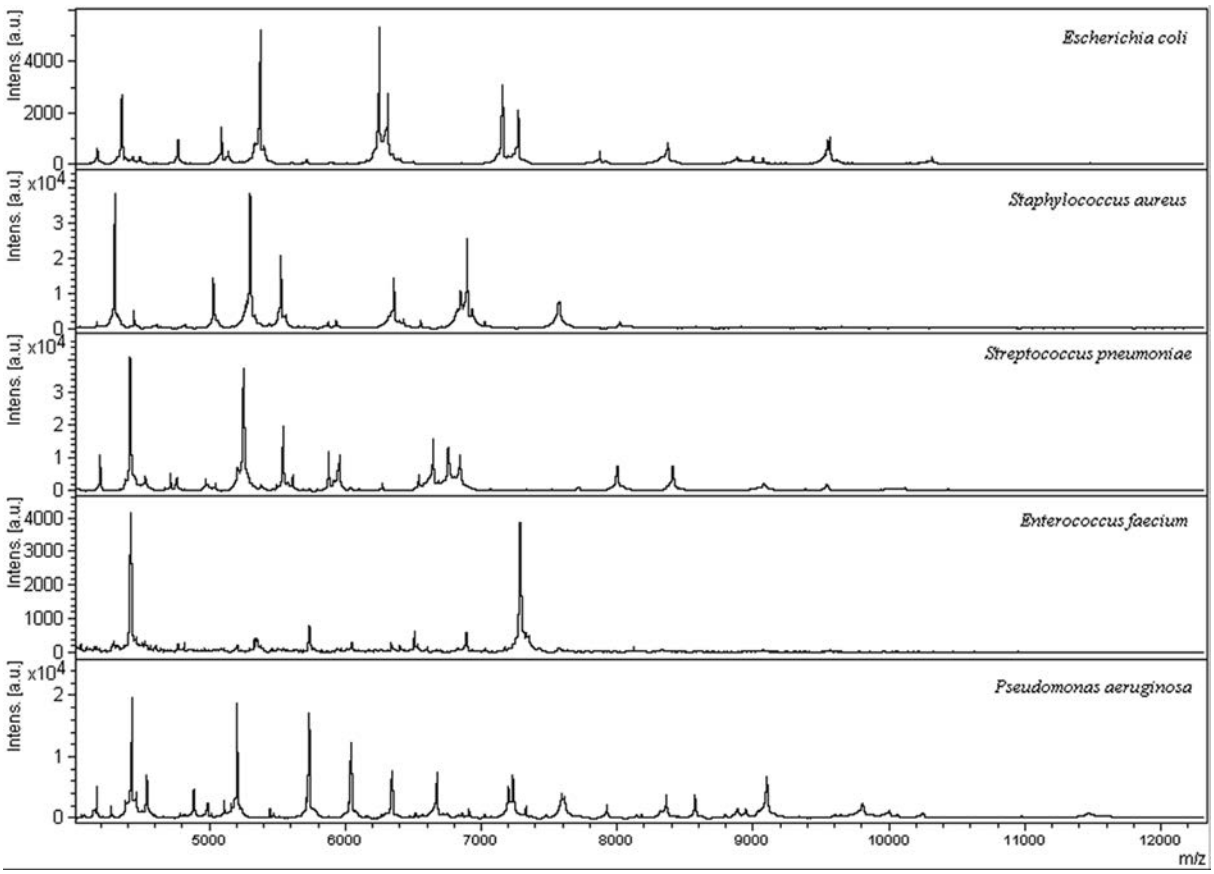


FIGURE 15.20

Spectral fingerprints of five different bacteria using MALDI-TOF MS technology

Source: Carbonnelle, E. et al. 2011, MALDI-TOF mass spectrometry tools for bacterial identification in clinical microbiology laboratory. *Clinical Biochemistry* 44: 106, Figure 1. (Summarises the principles and value of the new MALDI-TOF MS technology in microbial diagnostics.)

usually based on antigen or antibody detection methods, such as agglutination and ELISA, or PCR. The World Health Organization recommends point of care testing for HIV and syphilis in high-incidence, undeveloped countries as an important approach to reduce the spread of infection in those areas.

Despite the enormous promise of rapid point of care tests for diagnosis of infectious diseases, there is much debate regarding their general value and appropriateness. There are significant concerns regarding test sensitivity as well as the accuracy of results if testing of specimens is undertaken by untrained staff and outside accredited laboratories. Other concerns include equipment accuracy and calibration, the current high cost of rapid tests, the inability of current tests to detect multiple organisms, and ultimately whether the standard laboratory tests can be replaced by point of care testing. Nevertheless, rapid, point of care testing is considered to be of value in situations where laboratory support is limited (e.g. in rural areas and underdeveloped countries), as a surveillance tool for outbreaks of infection, in community screening programs in at-risk groups, in situations where patients may not return for treatment (e.g. sexually transmissible infections), and for life-threatening conditions where immediate treatment is warranted.

SUMMARY

- Few infectious diseases can be accurately diagnosed on clinical grounds, so for most infections laboratory diagnosis is needed.
- Speed and accuracy are the primary imperatives in the laboratory diagnosis of infectious diseases.
- For an accurate laboratory diagnosis, it is essential that an appropriate specimen is properly collected and transported promptly to the laboratory for processing.

MICROSCOPIC TECHNIQUES

- Direct microscopic examination of certain specimens can sometimes provide a presumptive diagnosis within an hour.

SPECIMEN COLLECTION FOR CULTURE

- Microbiological culture of the causative agent remains the 'gold standard' for many infections, especially for bacteria and fungi.
- A major disadvantage of cultural methods is the length of time required for a result; usually at least 18 hours and sometimes as long as several weeks.
- Wherever possible, specimens for laboratory culture are collected before antibiotic therapy is initiated.

COMMON SPECIMEN TYPES FOR CULTURE

- A blood culture is usually performed when investigating a patient with fever or other manifestations of systemic infection.
- Intravascular catheters may become colonised and serve as a source of microorganisms in septicaemia.
- Contamination of urine with microflora of the urethra can be reduced by collecting a 'clean-catch' or midstream specimen of urine (MSU).
- In suspected meningitis, cerebrospinal fluid (CSF), obtained by lumbar puncture, is investigated.
- Several types of specimens may be collected from the upper respiratory tract—throat, nasal and nasopharyngeal swabs; nasopharyngeal fluid; nasal washings.
- The specimen most often collected in the investigation of lower respiratory infection is sputum.
- Faeces (stools) may be collected for the culture of bacteria causing gastroenteritis, for the detection of specific toxins, or for the detection of protozoa, helminths or viruses.
- In females, specimens that may be collected for the diagnosis of genital tract infections include a cervical swab, vaginal swab, urethral swab, amniotic fluid, rectal swab and vesicle fluid. In males, typical specimens include a urethral swab, fluid or a swab from penile lesions, and a rectal swab.
- When collecting a specimen from an infected wound or sample of tissue, pus or fluid is preferable to a swab specimen.
- If anaerobes are suspected in an infected wound, the specimen should be exposed to air for as brief a time as possible.
- Pus, tissue samples and biopsy samples are very good specimens for anaerobic culture.
- For superficial fungal infections, skin scrapings, hair or nail clippings from the site of infection are collected.
- For culture of viruses, a specimen from the clinical site of infection plus blood, throat washings and faeces are usually collected.

CULTURING BACTERIA AND FUNGI

- A nutrient composition prepared for the growth of microorganisms is called a culture medium.
- When microbes are grown on a culture medium, the growth is called a culture.
- Culture media may be liquid, but most are made solid by the addition of a gelling agent called agar.
- A solid medium contained in a petri dish is called an agar plate.
- Solid media are used because bacteria and fungi can grow on the surface of the media, forming colonies.
- A pure culture is one in which all the colonies comprise the same species of organism.
- Most bacteria and yeasts grow from single cells to macroscopic colonies on an agar plate in 18–24 hours.
- Bacteria isolated in pure culture may have their identification and sensitivity to different antibiotics determined.

CULTURE OF OTHER MICROORGANISMS

- Viruses and some bacteria are obligate intracellular parasites and must therefore be grown in cell or tissue cultures.

SEROLOGY (IMMUNOLOGIC DIAGNOSIS)

- A serologic diagnosis of infectious disease is performed by detection of antibodies against antigens of an infectious agent in serum or other body fluids.
- Serological tests are useful if the microorganism is impossible or difficult to grow, or if it grows slowly.
- The detection of antibodies may be used to assess a person's immune status to a particular microorganism.
- For a positive serological diagnosis of infection, a demonstration of seroconversion may be necessary.
- IgM-specific antibody is present early in infection and is indicative of active infection.

ANTIGEN DETECTION

- Detection of antigens of a microorganism in an appropriate specimen is used to diagnose some infections.

DETECTION OF MICROORGANISMS USING MOLECULAR TECHNIQUES

- Molecular techniques identify the cause of an infection by detecting fragments of nucleic acids of the microorganism in a specimen or from culture of a specimen.
- Molecular techniques allow a faster and more sensitive detection of microorganisms and are useful for infections that cannot be diagnosed by other methods.
- A nucleic acid probe is a single-stranded segment of DNA or RNA that is complementary to the microbial nucleic acid that is to be detected.

- The direct detection of microbial nucleic acid in a clinical sample has the advantage of speed and specificity.
- The polymerase chain reaction (PCR) is a commonly used DNA amplification and detection technique.
- PCR plays a vital role in assessing disease progression, patient infectivity and prognosis, and effectiveness of therapy in some important viral infections

OTHER MODERN DIAGNOSTIC TECHNOLOGIES

- Matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS) analyses microbial proteins using mass spectrometry, and is able to accurately identify, within minutes, microorganisms from a single colony.
- DNA sequencing of microorganisms could provide major breakthroughs in the rapid diagnosis of infectious diseases.

POINT OF CARE TESTING

- Point of care testing is a rapid analytical test that can be performed at the patient's bedside, in a doctor's office or in the field.

STUDY QUESTIONS

1. What are the major advantages of a direct microscopic examination of patient material where possible?
2. How does one determine what type of specimen should be collected for the diagnosis of a particular infection?
3. Why should specimens be collected before starting antibiotic therapy where possible?
4. List the important procedures that should be followed when collecting a specimen for microbiological examination.
5. Although swabs are commonly collected for microbiological analysis, they are not always the most appropriate specimen to collect. Explain.
6. What is a 'transport medium', and why is it used?
7. Why are three samples of blood often collected over a 24–48-hour period from a patient suspected of having a septicaemia?
8. What is a 'midstream specimen of urine', and why is it the preferred specimen for the diagnosis of urinary tract infections?
9. What is the main problem associated with the delayed transport (several hours) of specimens such as urine and sputum to the microbiology laboratory?
10. What important principles should be considered when collecting a specimen from an infected wound?
11. In general, what are the best specimens to collect for the diagnosis of anaerobic infections?
12. What, in microbiological terms, is a 'culture'?
13. Explain the purpose of the streak plate method in microbiology.
14. What type of organisms cannot be grown on agar plates?
15. What is serology?
16. What is meant by the term 'seroconversion'?
17. What are nucleic acid probes? What are their potential uses in microbiology?
18. What is PCR, and what are its potential clinical applications?

FURTHER READING

Barken, K.B., J.A.J. Haagensen and T. Tolker-Nielsen 2007, Advances in nucleic acid-based diagnostics of bacterial infections. *Clinica Chimica Acta* 384: 1–11. (Reviews the commonly used nucleic acid-based diagnostic methods.)

Bissonnette, L. and M.G. Bergeron 2010, Diagnosing infections—current and anticipated technologies for point-of-care diagnostics and home-based testing. *Clinical Microbiology and Infection* 16: 1044–53. (Discusses the advantages and disadvantages of point of care diagnostic systems.)

Brooks, G.F., J.S. Butel and S.A. Morse 2004, *Jawetz, Melnick & Adelberg's Medical Microbiology*, 23rd ed. (New York: McGraw-Hill). (A general text of medical microbiology that covers specimen collection handling and diagnostic methods in a brief but comprehensive fashion.)

Carbonnelle, E. et al. 2011, MALDI-TOF mass spectrometry tools for bacterial identification in clinical microbiology laboratory. *Clinical Biochemistry* 44: 104–09. (Summarises the principles and value of the new MALDI-TOF MS technology in microbial diagnostics.)

Dong, J., J.P. Olano, J.W. McBride and D.H. Walker 2008, Emerging pathogens: Challenges and successes of molecular diagnostics. *Journal of Molecular Diagnostics* 10(3): 185–97. (Reviews the methods for the laboratory diagnosis of emerging infections.)

Ince, J. and A. McNally 2009, Development of rapid, automated diagnostics for infectious disease: Advances and challenges. *Expert Reviews of Medical Devices* 6(6): 641–51. (An excellent review of the advantages and limitations of conventional and modern diagnostic techniques for infectious diseases.)

CHAPTER

16

Skin, wound and eye infections

CHAPTER FOCUS

- What microorganisms cause infections of the skin?
- What are the clinical features of and important infection control considerations for skin infections?
- What are the common causes and clinical features of surgical wound infections and burns wound infections?
- What infection control procedures prevent infections of surgical and burns wounds?
- What are the common causes and clinical features of eye infections?

INTRODUCTION

The skin is composed of two distinct layers—a thin outer layer called the **epidermis**, and a thicker underlying layer called the **dermis** (see Figure 16.1). The subcutaneous layer (also known as the superficial fascia) lies under the dermis, and is generally considered as separate from the skin. The epidermis is composed of four to five layers of epithelial cells which contain large amounts of the tough protein keratin. The basal epithelial cells, which are strongly bound to the dermis, continuously divide, pushing the older cells closer towards the surface. The outer layers of epidermal cells are dead and are shed as new cells are produced below. The dermis is a strong, elastic layer due to the presence of collagen and elastic fibres. It also contains capillaries, lymphatics and sensory neurons, and therefore provides structural and functional support for the epidermis. Hair follicles, sebaceous glands and sweat glands are primarily in the dermis, and protrude through the epidermis to the skin surface.

Intact skin is an excellent frontline defence against invasion by most microorganisms. It acts as a structural barrier and has various chemical attributes that inhibit microorganisms, such as a low pH, high salt concentration, lysozyme and fatty acids (see Chapter 9). Although the skin is a relatively inhospitable place for microorganisms, it does have a normal resident flora that helps to defend against potential pathogens. The flora includes a variety of aerobic and anaerobic organisms such as staphylococci (especially *Staphylococcus epidermidis*), micrococci, diphtheroids and propionibacteria. Many other organisms, such as streptococci and Gram-negative enteric bacteria (e.g. *Escherichia coli*, *Proteus* spp.), may transiently colonise the skin. The moister areas of skin such as the axilla (armpit) and groin support relatively large numbers of organisms.

INFECTIONS OF THE SKIN

Tissue damage is the most common factor leading to infection of the skin, although in some situations apparently normal skin is infected. Minor trauma, such as abrasions, small cuts or cracks, can lead to infection of the skin. More severe trauma, such as that due to surgery, puncture wounds or burns, can lead to serious skin infection, often with involvement of underlying soft tissue. Once the skin has been damaged, infection can be caused by a variety of organisms from different sources. Endogenous infection may be caused by skin flora, such as *Staphylococcus epidermidis*, by temporary skin residents, such as *Staphylococcus aureus*, or by upper respiratory tract flora, such as *S. aureus* (nose) and *Streptococcus pyogenes* (throat). A relatively newly identified type of *S. aureus*, referred to as community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA), is now recognised as a major cause of skin and soft tissue infections in the community. Exogenous infection may be caused by organisms in the natural environment, such as *Clostridia* in soil, or by organisms contaminating the hospital

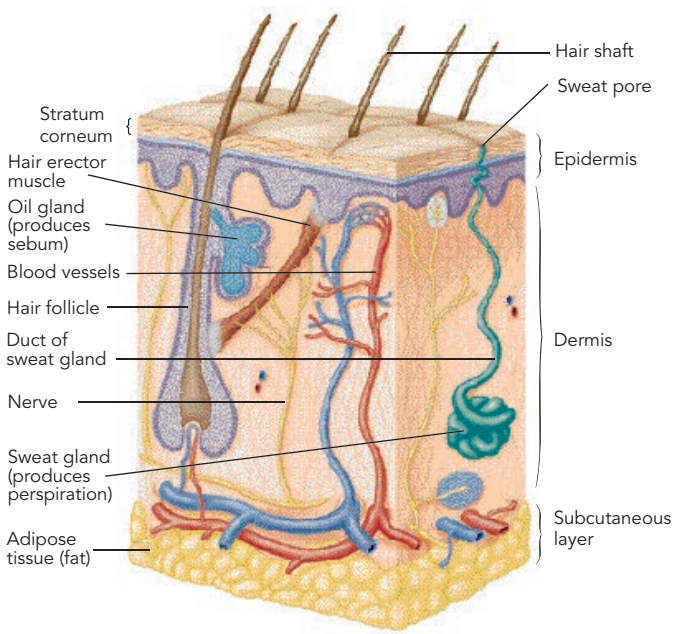


FIGURE 16.1

A section through skin

The top layers of the cells contain the tough protein called keratin.

environment. In addition, there are some systemic infections in which organisms are carried in the bloodstream and localise in skin tissue. A skin rash may be the major manifestation of these infections, such as occurs in measles, rubella and chickenpox. The terms used in dermatology to describe different types of lesions on skin are listed and explained in Table 16.1.

Bacterial skin infections

Folliculitis, boils and carbuncles

Folliculitis, an infection of the hair follicles, can be caused by bacteria, viruses or fungi. The most common cause is *Staphylococcus aureus*, which often comes from the person's own flora (skin or nose) or from another person's skin flora. Folliculitis occurs mostly on the scalp, face or limbs and is often precipitated by some irritation (e.g. following shaving, use of a loofah sponge or dermatitis). *Pseudomonas aeruginosa* may also cause folliculitis, often associated with use of a spa (hot tub) or wetsuit. When the deeper areas of a hair follicle are affected, a larger, deeper, pus-filled nodule develops, called a **furuncle** or **boil**. Usually, a boil continues to expand slowly, eventually coming to a head on the surface of the skin. Nasal carriers of virulent *Staphylococcus aureus* may suffer recurrent boils anywhere on the skin. A disturbing trend is the increasing incidence of skin infections, such as furunculosis, caused by community-associated methicillin-resistant *S. aureus* (CA-MRSA).

Boils may discharge to adjacent areas of skin, resulting in multiple abscesses, called a **carbuncle** (see Figure 16.2). Carbuncles tend to develop at particular sites, such as on the neck, upper back and buttocks. In addition to the abscesses, there are often systemic symptoms such as fever, malaise and lymphadenopathy. Septicaemia may complicate even simple lesions, especially in the immunocompromised.

Mild cases of folliculitis usually resolve on their own, but recurrent infections and boils and carbuncles may need treatment (see later section). Diagnosis is usually made on clinical grounds, but a laboratory confirmation of the causative organism is readily achieved by culture of pus.

TABLE 16.1 Dermatological terms for skin lesions

Bulla	A large blister containing clear fluid, more than 0.5 cm in diameter
Crust ('scab')	The dried exudate from an erosion or ulcer
Erosion	A superficial, circumscribed loss of epidermis, which heals without scarring
Erythema	Area of redness due to vasodilation
Exanthem	A widespread skin rash
Excoriation	An area of skin denuded of epidermis by scratching
Macule	A circumscribed, flat area of altered skin colour
Nodule	A circumscribed, elevated area of skin, larger than 1 cm in diameter
Papule	A circumscribed, elevated area of skin, less than 1 cm in diameter
Petechia	A small flat haemorrhage
Purpura	Numerous petechiae
Pustule	A vesicle or bulla containing cloudy fluid
Scale	An abnormal accumulation of keratin; scaling may occur on normal skin, but more often forms on abnormal skin
Ulcer	An area of tissue loss, varying in depth; an ulcer may involve skin only, or may extend more deeply
Vesicle	A small blister containing clear fluid, less than 0.5 cm in diameter
Wheal/ Urticaria	A raised, often itchy erythematous lesion

Impetigo

Impetigo is a highly contagious, pyogenic (pus-forming) infection caused by staphylococci, streptococci, or both (Figure 16.3). It occurs predominantly in young children, where minor skin damage due to insect bites, eczema or scabies is the usual predisposing factor, although it can develop on apparently healthy skin. Lesions often start on the face and may then spread to other sites of the body. Scratching and other abrasions can result in new lesions, as well as transmission of the organism to others in close contact. It is readily spread on hands, toys and furniture, and can rapidly spread through schools and daycare centres.

There are two main forms of impetigo—the bullous form, caused mainly by *Staphylococcus aureus*, and the non-bullous form, caused by staphylococci and/or Group A streptococci

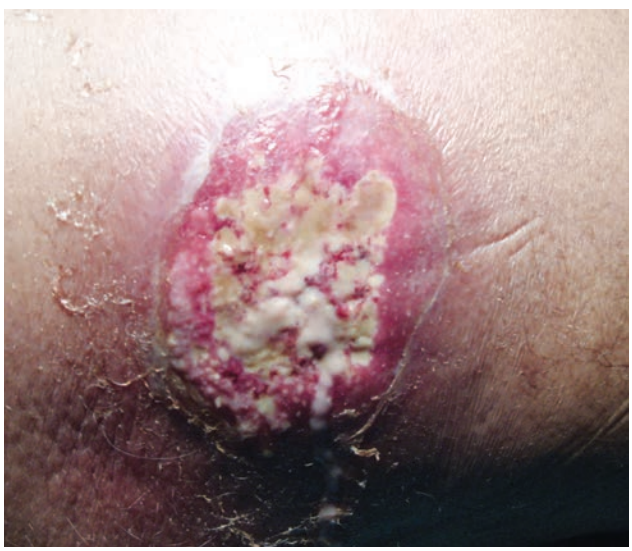


FIGURE 16.2

Carbuncle on skin caused by *Staphylococcus aureus*

Source: Drvgaikwad on Wikimedia.

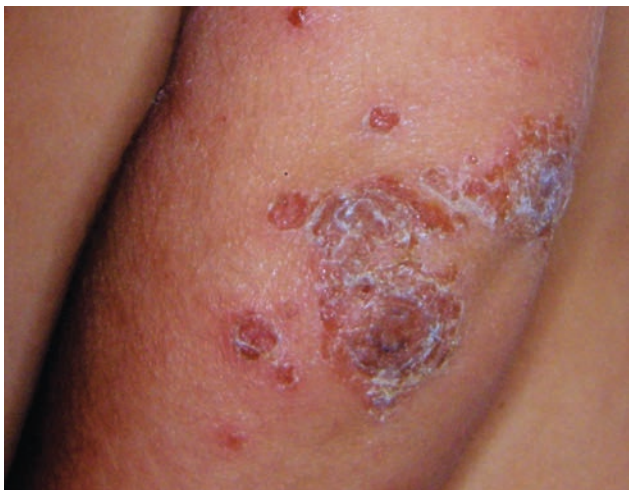


FIGURE 16.3

Impetigo

A highly contagious disease characterised by vesicles on the skin which break down to a crust form.

Source: Reproduced with permission of New Zealand Dermatological Society Incorporated at DermNetNZ.org.

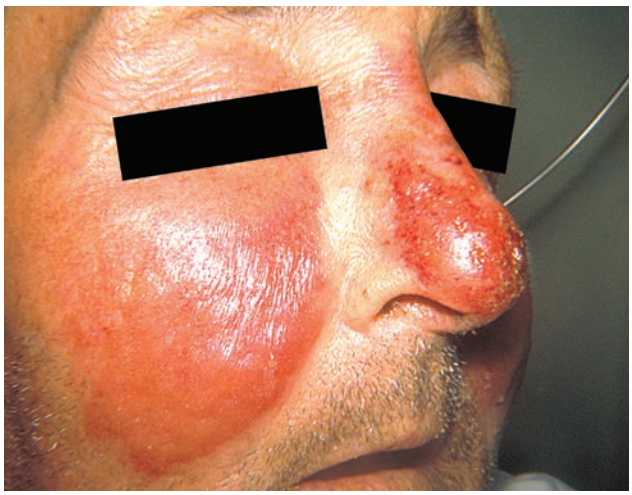


FIGURE 16.4

Erysipelas

Source: CDC/Dr Thomas F. Sellers/Emory University.

(especially *Streptococcus pyogenes*). The non-bullous form is characterised by small vesicles which rupture easily, resulting in weeping, honey-coloured crusts that dry and self-resolve. Bullous lesions are larger, with clear fluid that later becomes cloudy and yellow. After rupture, brownish crusts form. There is generally no systemic illness unless deeper tissues are involved. Impetigo usually heals spontaneously within two weeks. Laboratory diagnosis is based on culture of a swab of the lesions under the crusts.

Scalded skin syndrome is caused by strains of *Staphylococcus aureus* that produce a toxin called **exfoliatin** (or **epidermolysin**). It can occur as a complication of minor skin lesions, impetigo or cellulitis. Exfoliatin causes layers of cells in the epidermis to separate, and the outer sheets of skin are shed, exposing layers of inner skin that have the appearance of being scalded. A septicaemia may develop, often with a fatal outcome. The neonatal form is sometimes referred to as *Ritter's disease*.

Erysipelas

Erysipelas is a superficial, bacterial skin infection that has been recognised as far back as the Middle Ages, when it was known as St Anthony's Fire. It begins as a small, raised, rubbery lesion, often on the face or lower limb, and spreads rapidly. Typically, it develops into a bright red, swollen lesion with a sharply demarcated edge (Figure 16.4), followed by bullae that rupture and weep. Systemic symptoms of fever, headache and vomiting often precede the cutaneous signs. Erysipelas is usually caused by streptococci (Group A and non-Group A), although other bacteria such as *Haemophilus influenzae* are occasionally responsible. It occurs most often in adults. The streptococci usually enter through a break in the skin, but sometimes there is no apparent predisposing lesion. Without appropriate antibiotic treatment the organisms may spread through the lymphatics and cause septicaemia, pneumonia, nephritis and gangrene.

The diagnosis is usually based on clinical appearance. Laboratory diagnosis of erysipelas is difficult, but may be attempted in atypical cases by culture of blood or tissue.

Cellulitis

Cellulitis is an acute bacterial infection of the skin and subcutaneous tissues. The infected tissue becomes hot, painful, red and swollen. Fever, chills, bacteraemia and local lymph node enlargement may be present. It may occur following a superficial skin infection or after skin trauma. A number of factors predispose a person to cellulitis, including diabetes mellitus, immunodeficiency and peripheral arterial disease. The majority of infections are caused by *Streptococcus pyogenes* or *Staphylococcus aureus* (including CA-MRSA). Other possible causes include *Pseudomonas aeruginosa*, *Hemophilus influenzae* and *Streptococcus pneumoniae*. *Clostridium perfringens* and other anaerobes can cause cellulitis in poorly oxygenated tissue, especially after deep wounds and fractures.

Culture of aspirates of the leading edge of the lesion, or skin biopsy, are the usual means of laboratory diagnosis. Blood cultures should also be taken.

Bairnsdale ulcer

Bairnsdale ulcer is a chronic, relatively painless cutaneous ulcer caused by *Mycobacterium ulcerans* (see Figure 16.5). This infection was first reported in 1948 in a group of six patients in Australia, five of whom came from the Bairnsdale district of Victoria. Outbreaks have occurred in Victoria, and it has been made notifiable in that state. It is also endemic in Far North Queensland, but rare elsewhere in Australia. It occurs in many other parts of the world including South America, Papua New Guinea and Africa, although it has an unusually uneven and unexplained distribution. It is also referred to as *Buruli ulcer* because it has been seen so frequently in the Buruli region of Uganda.

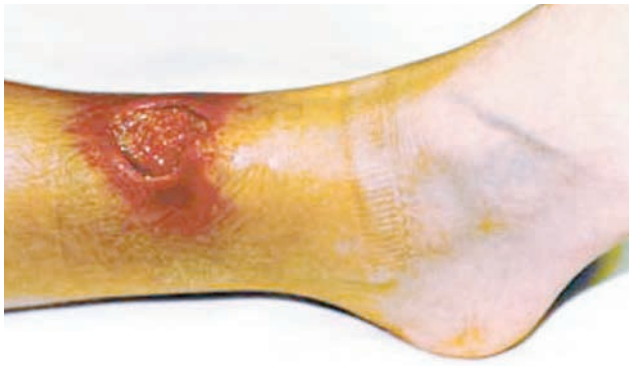


FIGURE 16.5

Bairnsdale ulcer

Source: Dr Paul Johnson.

Infection is often associated with exposure to water (e.g. swamps, slow-flowing rivers) but the exact mode of acquisition is not known. Infection of skin lesions or transmission by mosquitoes has been proposed. The major virulence factor of the organism is a toxin called mycolactone, which is a cytotoxin that causes necrosis of subcutaneous tissue. It also appears to have analgesic and immunosuppressive activities. After a mean incubation period of three months, a slowly progressive, generally painless ulcer develops in the skin and subcutaneous tissue. The ulcer often leads to scarring and permanent skin disfigurement. Bone destruction and scarring around joints can result in functional limitations. The disease is usually diagnosed by PCR, histopathology or culture.

Treatment/prevention of bacterial skin infections

In general, antibiotics enter abscesses poorly and, when they do, they are inactivated by enzymes in pus. For minor skin infections, local measures, particularly wound drainage and gentle debridement of crusted lesions, are often all that is necessary. Sometimes, treatment with topical antibiotics and bathing with antiseptics are required as an adjunct to drainage. In general, systemic antibiotic therapy is given when there are systemic symptoms such as fever, or when the infection is severe or widespread, or involves deeper tissue. Underlying skin disease, scabies infection or eczema should also be treated.

Impetigo and other streptococcal infections are highly contagious and are usually treated with an oral antibiotic such as penicillin, flucloxacillin or erythromycin, or intravenous flucloxacillin or cephalothin in severe infections. Patients with severe infections (especially children) should be isolated until the infection is under control.

Eradication of nasal and skin carriage of *Staphylococcus aureus* in patients with recurrent staphylococcal skin infections may be attempted with a nasal spray of the antibiotic mupirocin and a skin antiseptic such as triclosan.

Treatment of Bairnsdale ulcer involves surgical debridement and combination antimicrobial therapy of rifampicin plus streptomycin.

CASE HISTORY 16.1

Bairnsdale ulcer

A 42-year-old man presented in Melbourne in January 2006 with a skin ulcer over the left ankle which had been present for five months. Buruli ulcer had been diagnosed six weeks earlier when he was in the Netherlands, but the antibiotics prescribed were having no apparent effect.

The lesion was excised, a skin graft applied and antibiotics given for a further six weeks, resulting in complete resolution.

PCR testing on the excised tissue was positive for *Mycobacterium ulcerans*. Typing and epidemiological assessment indicated that it was an Australian strain, and that he probably acquired the infection seven months before the appearance of the lesion while kayaking near Eden on the southern NSW coast.

Source: Adapted from C.J. Lavender et al. 2007, First case of *Mycobacterium ulcerans* disease (Bairnsdale or Buruli ulcer) acquired in New South Wales. *Medical Journal of Australia* 186(2): 62–63.

Questions

1. What does this case demonstrate about the normal progression of Bairnsdale ulcer infection?
2. How does *M. ulcerans* produce the typical symptom of a painless skin ulcer?
3. What are the possible reasons for initial failure of therapy in this case?

Acne

Acne is a skin disorder that affects around 80 per cent of teenagers and many adults. *Propionibacterium acnes*, an anaerobic Gram-positive rod that is a normal inhabitant of skin, has been strongly implicated in the pathogenesis of acne. The organism colonises the pilosebaceous follicles (hair follicle and associated sebaceous gland) where it grows on the oily sebum produced by the sebaceous glands. Excessive sebum production, especially around the time of puberty, favours the growth of this organism, and hence increases the likelihood of acne. However, the presence of this organism on the skin of some individuals is totally innocuous.

The lesions of acne have classically been divided into closed lesions (whiteheads), open lesions (blackheads) and inflammatory lesions (papules, pustules or nodules). Whiteheads and blackheads are non-inflammatory lesions that are usually referred to collectively as comedones. These lesions develop when hair follicles and sebaceous glands become blocked with sebum. The inflammatory lesions are postulated to develop when *P. acnes* grows in the blocked follicles and activates the inflammatory response.

Acne is treatable in some people, but some cases do not respond. The mainstay of treatment is frequent cleansing of the skin, antiseptics and topical keratolytics (benzoyl

peroxide or tretinoin). Topical or oral antibiotics (e.g. tetracycline or clindamycin) may be prescribed for persistent cases, but some antibiotic resistance in *P. acnes* has been demonstrated.

Viral skin infections

Some viruses, such as wart viruses, cause skin lesions by localising in and infecting cutaneous tissue. Other viruses, such as chickenpox, measles and rubella, cause systemic infection, but produce skin lesions as the major clinical manifestation.

Rubella

Rubella, or **German measles**, is one of several systemic human viral diseases that cause a skin rash. It is caused by a togavirus that is spread mainly by the airborne route from respiratory secretions of infected individuals. It is highly contagious for about a week before and a week after the rash appears. Due to the availability of an effective vaccine, the incidence of rubella in Australia is low—less than 1 case per 100 000 population. Health authorities consider that Australia is close to elimination of endemic rubella.

Except for the congenital form (described below), rubella is generally a mild, often subclinical, disease. The incubation period is 2–3 weeks. In adults, a short period of mild fever, malaise and upper respiratory symptoms may occur first. The rash is the main symptom of rubella; it appears first on the face or trunk but quickly spreads to the rest of the body. The lesions are macular with a light rose-pink colour (Figure 16.6). Lymph nodes are usually enlarged, and in severe cases conjunctival infection may occur. The rash usually disappears after three days. Many infected individuals do not have a rash but can still transmit the virus to others.

Congenital rubella syndrome results from infection of a developing foetus when the virus crosses the placenta from the mother's circulation. The effect on the foetus varies with the gestational age at the time of maternal infection. The virus slows cell replication and differentiation, so the greatest risk of congenital defects occurs if the woman is infected in the first trimester, when foetal organ systems are developing. The most severe effects of congenital rubella include stillbirth, mental handicap, deafness, eye defects, cardiac abnormalities, hepatomegaly, splenomegaly, pneumonitis and low birth weight. Major abnormalities are rare if infection occurs after the 16th week of pregnancy. The link between rubella infection in early pregnancy and congenital defects was first suggested in 1941 by Norman Gregg, an ophthalmologist working at the Royal Alexandra Hospital for Children in Sydney.

Rubella cannot be reliably diagnosed by clinical means, so serological tests are required. Serology is also used to assess the immunity of pregnant women in prenatal screening tests.

A live attenuated rubella vaccine was first licensed in Australia in 1970. The principal aim of rubella vaccination is to prevent congenital rubella syndrome. To achieve this, the circulation of rubella virus in the whole community should ideally be stopped, so both males and females should be immunised. Rubella vaccine is now a component of the MMR (measles-mumps-rubella) vaccine recommended for all children at 12 months of age, with a second dose at 4 years of age. Vaccination is also recommended for non-pregnant, seronegative women of child-bearing age. Since it is a live vaccine, it is not recommended for women in early pregnancy, and vaccinated women should be advised to avoid becoming pregnant for 28 days after vaccination. Vaccination has resulted in a significant reduction in congenital rubella.

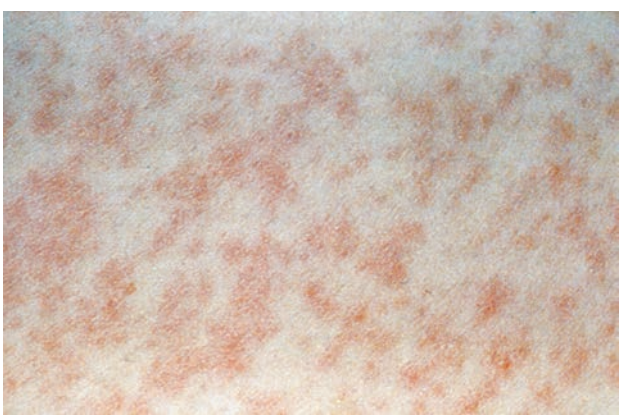


FIGURE 16.6

Rubella

The patient shows the macular rose-pink spots typical of this disease.

Source: Royal Prince Alfred Hospital, Medical Photography.

CASE HISTORY 16.2

Congenital rubella

In April 2003 an 18-year-old woman delivered her second child at 38 weeks gestation. The male infant was small for gestational age (birth weight 2220 grams) and had a head circumference on the 10th percentile (32.2 cm). His rubella IgM was positive and he had thrombocytopenia requiring platelet transfusion. Later, he was found to have severe bilateral deafness. The mother had had a rubella contact at nine weeks gestation, and was subsequently confirmed to have contracted rubella. She had missed the rubella schoolgirl vaccination program because of illness.

Source: Adapted from J.M. Forrest, M. Burgess and T. Donovan 2003, A resurgence of congenital rubella in Australia? *Communicable Diseases Intelligence* 27: 533–35.

Questions

1. How is rubella transmitted from person to person?
2. Why was the infant so severely affected?
3. Why wasn't the woman vaccinated after contact with the adolescent female with rubella?
4. Would rubella be a concern for a pregnant occupational therapist working in a hospital?

Measles

Measles, or **rubeola**, is a highly contagious, acute illness caused by a paramyxovirus. Due to the availability of an effective vaccine since the 1970s, global cases and mortality due to measles has decreased substantially. In 1980, the number of reported cases throughout the world was over 4 million. By 2009 this number had dropped to under 250 000. Nevertheless, it is estimated that more than 20 million people in the world are still affected by measles each year, and it remains a leading cause of death among young children in low-income countries. In 2008, an estimated 164 000 deaths globally were attributable to measles. Measles epidemics still occur in developed countries, such as have occurred in a number of European countries, including Britain, Switzerland, France, Spain, Italy, between 2006 and 2011, due to reduced vaccine uptake. In Australia, the incidence of measles has declined over the last decade, especially since 1998 when the Measles Control Campaign (which involved the mass vaccination of primary school children) took place. Fewer than 100 cases per year are usually now reported. The highest rate of notification tends to be in the 0–4 years age group, although outbreaks can also occur in non-vaccinated young adults, who are then a reservoir of infection for the younger age group.

The measles virus is mainly spread from infected people by respiratory secretions via the airborne route. An acute case of measles is infectious 3–5 days before the onset of symptoms and for as long as fever is present. After an incubation period of 9–14 days, symptoms of fever, runny nose, dry cough and watery eyes typically develop. **Koplik's spots**, which are areas of white necrosis on a reddened mucosa, may appear in the mouth 3–4 days later. They may also appear on other mucosa such as the conjunctiva.

Usually, within another two days, a dusky red, maculopapular rash develops, often starting behind the ears, then spreading to the face and down the body, eventually reaching the lower extremities (Figure 16.7). The Koplik's spots disappear shortly after the rash appears, and the rash fades in 5–6 days. There is usually a generalised, but minor, lymph node enlargement. The virus may invade the lungs, kidneys or brain, particularly in poorly nourished, young children (especially with vitamin A deficiency) or the immunocompromised, often with a fatal outcome.

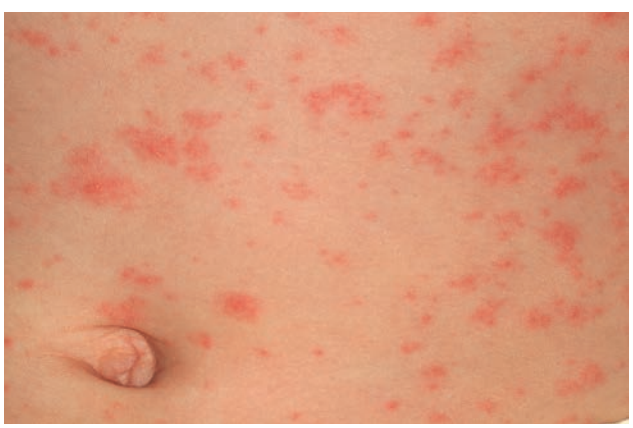


FIGURE 16.7

Measles

The disease is characterised by small, raised maculopapular lesions.

Source: Royal Prince Alfred Hospital, Sydney, Medical Photography.

Even in immunocompetent people, measles is potentially a very serious disease. **Measles encephalitis** is a severe complication, occurring in approximately 1 in 1000 patients. Many of these patients recover without problems, but 15–40 per cent suffer permanent neurological injury and 10–15 per cent may become comatose and die. In approximately 1 in 25 000 cases, the virus is not completely cleared from the body but persists in the brain. Six to eight years later, **subacute sclerosing panencephalitis** (**SSPE**) develops and is always fatal, due to viral destruction of nerve cells with progressive mental deterioration, muscle rigidity and coma. In some areas of the world, such as Africa, mortality rates due to measles or its complications can be as high as 10 per cent. Other potential complications of measles are secondary bacterial bronchopneumonia and middle ear infections. Pneumonia is the most common cause of death associated with measles.

Measles is usually diagnosed clinically by the initial catarrhal symptoms and dry cough, fever and the rash. It can be diagnosed serologically by detection of specific IgM antibodies in serum. Treatment is limited to alleviating symptoms and dealing with complications. Secondary, bacterial infections are treated with antibiotics.

Immunisation for measles is usually in the form of the MMR vaccine (containing attenuated viruses of measles, mumps and rubella). It is recommended that measles vaccination should occur at around 12 months of age, followed by a second dose at 4 years of age. Malaise, a moderate to high fever or a rash may occur 7–10 days after vaccination, and last 2–3 days. Encephalitis is a very rare complication of measles vaccination (1 in 2 million doses), much rarer than in natural infection. Anaphylaxis following the administration of MMR is also very rare (less than 1 in 1 million doses). Unfortunately, immunisation coverage in some developing parts of the world is still chronically low (see Figure 16.8), resulting in persistently high infection rates. And in developed countries vaccine uptake rates may fluctuate, leading to outbreaks as described above and in Case History 16.3.

In 1998 the *Lancet* published an article which suggested that the MMR vaccination might be linked with inflammatory bowel disease and autism. In February 2010 the journal retracted the paper, after the General Medical Council in the UK had ruled that the lead author of the article, Andrew Wakefield, had acted dishonestly in the presentation of the data. Despite the overwhelming scientific evidence that fails to support a link between childhood vaccination and autism, many people are still concerned about the safety of vaccines.

Since measles vaccine comprises living viruses, it should not be used in immunocompromised people or pregnant women. Normal immunoglobulin is used for people who are non-immune and exposed to a confirmed case.

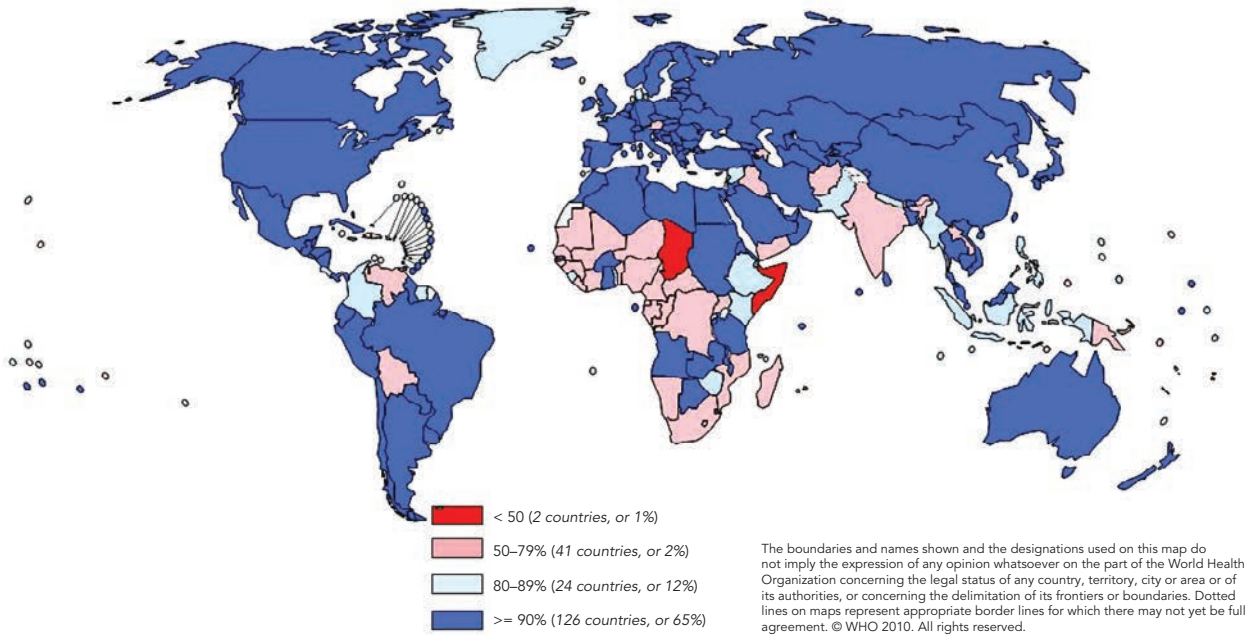


FIGURE 16.8

Worldwide measles immunisation coverage in infants, 2010

Source: World Health Organization, <www.who.int/immunization_monitoring/diseases/big_measles_map_coverage.jpg>.

CASE HISTORY 16.3

Measles

An outbreak of measles in Auckland, New Zealand, began on 1 June 2011 and was still ongoing nearly four months later on 21 September. During that period there were 164 confirmed cases, with 24 cases requiring hospitalisation. Most cases were thought to have been acquired in schools, early childhood centres or households, although transmission in tertiary institutes and workplaces was also suspected. The majority of cases occurred in West Auckland, with some spread to Central Auckland, North Shore and Manukau. There were concerns that passengers on some domestic flights may also have been exposed to the virus by fellow passengers in the early, infectious stage of measles. The Auckland Regional Public Health Service began contact tracing and issuing public health alerts from 1 June, advising people about the dangers of the disease and the importance of immunisation.

Questions

1. What does this case indicate about the transmissibility of measles?
2. What is the difference between immunoglobulin and the MMR vaccine used to control an outbreak?
3. It is thought that a vaccination rate of at least 90 per cent is required to provide herd immunity. What does this mean?
4. As a healthcare worker, what advice would you give to a pregnant client about the risks associated with the measles vaccine?

Chickenpox and shingles

The varicella zoster virus (VZV), a member of the herpes family, causes both chickenpox and shingles. **Chickenpox** (**varicella**) is a highly contagious disease, transmitted by the airborne route from the respiratory secretions or saliva of infected people, or by direct contact with skin lesions. Like other herpes viruses, VZV enters sensory nerve endings during primary infection and becomes latent in sensory ganglia. After remaining latent there for decades, it can be reactivated to cause shingles.

Varicella infections were made notifiable Australia-wide in 2006. In that year there were 1558 notifications of chickenpox, and the annual notification rate has remained at a similar level since then.

Chickenpox is usually a mild disease, but is often more severe in adults. After an incubation period of around two weeks there may be a short period of fever, malaise, runny nose and a skin rash (see Figure 16.9). The rash comprises different types of lesions at different stages of development, from macules to papules to vesicles and pustules. The lesions dry and crust over in a few days. They start on the scalp and trunk and spread to the face and limbs, sometimes to the mouth, throat and vagina. More severe infection and complications can occur, particularly in adults. Complications include interstitial pneumonia, secondary bacterial pneumonia and meningitis. Infection can be severe in women who are pregnant and in neonates infected after birth. People are infectious 1–2 days before the rash appears and up to the time when the lesions form crusts. The majority of chickenpox cases occur in children under 10 years of age.

Stress, ageing or immunosuppression can cause a reactivation of the chickenpox virus later in life to cause **shingles** (**zoster**). When reactivated, the virus spreads from the ganglion in which it has been dormant and causes clusters of painful, vesicular lesions like those of chickenpox. Lesions often appear on the trunk, but may also occur on the face and eye, usually following the location of affected sensory nerves (Figure 16.10). The rash can be accompanied by headache, fever and malaise. Prior to the development of the rash there may be a burning sensation or severe pain in the area of the nerves. These symptoms usually last 2–5 weeks. The pain can persist for up to a year (post-herpetic neuralgia) and can be severely debilitating. As in chickenpox, the lesions are highly infectious.

Shingles is most common in the elderly who have a weakened immune system (see Figure 16.11). Immunocompromised people can suffer widespread infections, sometimes involving internal organs, which can be fatal. Some people experience recurrent attacks marked by pain but with no skin lesions.

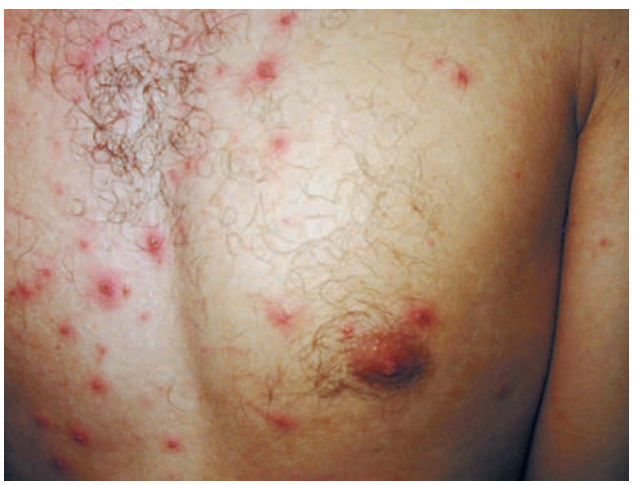


FIGURE 16.9

Chickenpox skin rash

Source: Dr Norma Scott.

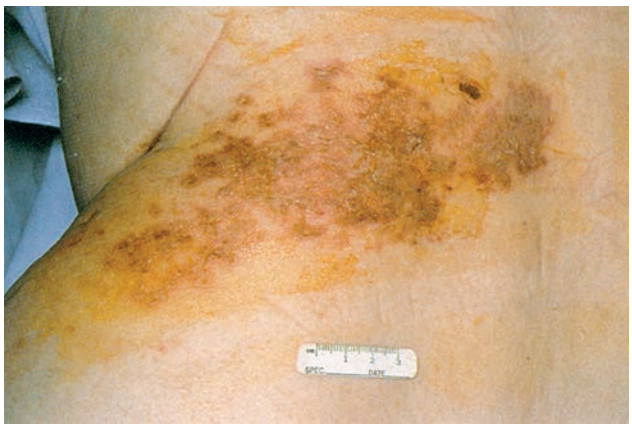


FIGURE 16.10

Shingles rash

Source: Royal Prince Alfred Hospital, Sydney, Medical Photography.

Diagnosis of chickenpox and shingles is generally based on clinical signs and symptoms. Laboratory confirmation is rarely required but, in uncertain cases, direct immunofluorescence, serology, culture, or PCR on a scraping from lesions are options. Therapy consists of supportive care, such as calamine lotion and soothing baths to relieve itchiness and scratching in chickenpox, and analgesics to relieve the pain in shingles. Aciclovir may be considered for persons at increased risk of severe infection, most notably patients older than 12 years. Aciclovir, famciclovir or valaciclovir may be used for shingles, especially in immunocompromised patients, to limit the spread of infection. These drugs are most effective if given within a few days of diagnosis and may prevent recurrent attacks. Zoster immune globulin should be given within 96 hours to those in whom varicella may be life-threatening and who have been exposed to the virus—for example, immunosuppressed people, pregnant women, neonates and premature babies.

Vaccination for varicella-zoster is now part of the Australian standard vaccination schedule. It is recommended for children at age 18 months. There is also a catch-up program for children between 10 and 13 years of age, if they have not received the vaccine and have not had the disease. Varicella vaccine is recommended for non-immune adolescents and adults, especially those in high-risk groups such as healthcare workers, teachers, childcare-centre staff and household contacts. Adolescents and adults are less responsive to the vaccine and thus require two doses, at least 4 weeks apart, to develop strong immunity. The vaccine is protective for non-immune individuals exposed to an infected person if given within 3–5 days of exposure. It is also recommended for non-immune women prior to pregnancy. Since the vaccine contains live attenuated viruses, it is not recommended for pregnant women or immunocompromised people.

Warts

Warts are caused by human papillomaviruses (HPV), of which there are over 100 different types. HPV specifically attack skin and mucous membranes, but different types tend

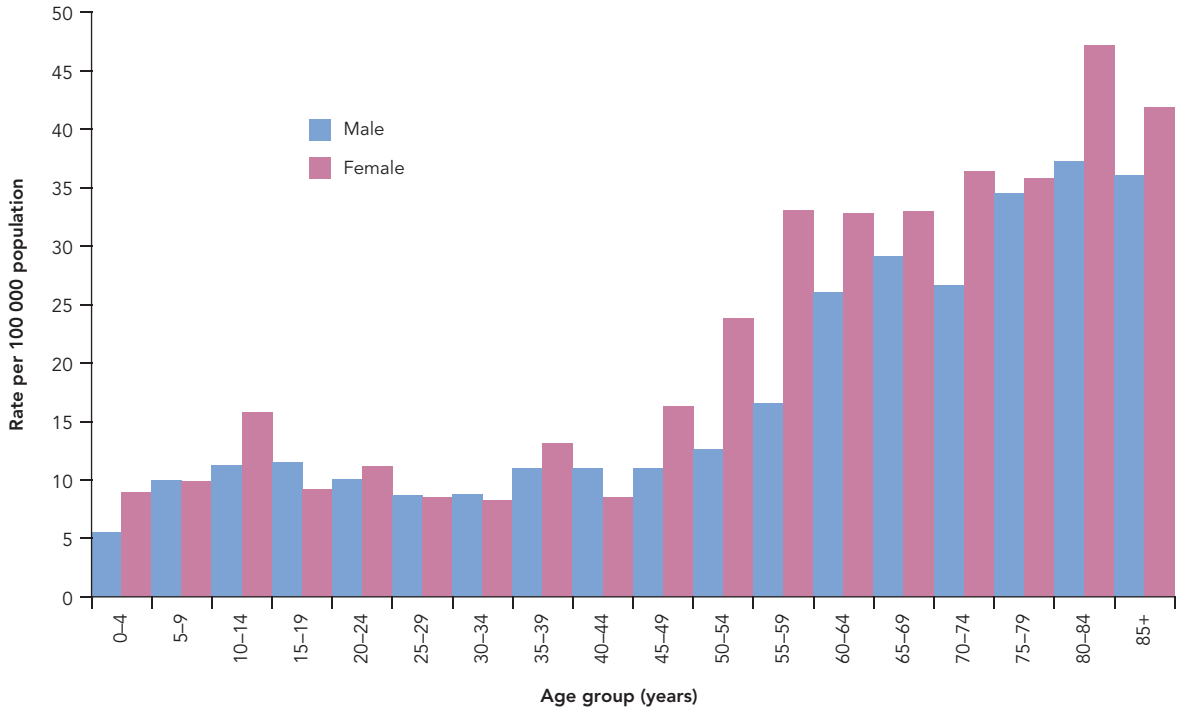


FIGURE 16.11

Notifications of shingles in Australia in 2008, by age group and sex

Source: *Communicable Diseases Intelligence* 2010, 34(3): 204.

to infect different body sites. For example, HPV 2, 3 and 4 typically cause the common warts seen on fingers and knees, whereas HPV 6, 11, 16, 18, 31, 33 and 35 cause genital warts. It is thought that infection lasts a lifetime and, when warts disappear or are removed, the virus still remains dormant in the cells of surrounding tissue.

HPV are transmitted by direct contact between people, or via fomites. Dermal warts occur when the virus enters the skin through abrasions. The virus replicates slowly, stimulating the cells to divide in an uncontrolled way to form a benign mass which protrudes above the skin surface. The incubation period varies from one week to 12 months for dermal warts. Genital warts, which are sexually transmitted, are discussed in Chapter 21.

Warts vary in appearance in different areas of the body. On normal skin, warts develop as small, painless nodules. Some may be hardly visible. Plantar warts occur in the thick skin on the soles of the feet, level with the skin surface, but with deeper underlying growth. Pressure on them when walking can cause severe discomfort.

Dermal warts usually regress spontaneously, presumably when the immune system gains control, although this may take several years. There is a clear association of some HPV with cancer, particularly serotypes 16 and 18 with cervical cancer.

Diagnosis of warts is based on clinical observation. Warts can be removed by local freezing of the tissue with liquid nitrogen or by curetting, cautery or laser. Topical treatments include podophyllin and salicylic acid. Recurrences after such treatments are common.

Herpes simplex

Primary infection with herpes simplex virus type 1 (HSV-1) usually involves the skin around the mouth or nose. Occasionally it affects other sites such as the genitalia, cheeks or forehead. Herpes simplex virus type 2 (HSV-2) involves mainly the genitalia but sometimes other sites, including the face. Genital herpes infections are dealt with in Chapter 21.

Most primary herpes skin infections are minor or subclinical in immunocompetent people, but may be severe in the immunocompromised. Primary HSV-1 infection occurs when a susceptible individual comes into close contact with a person who is symptomatic or shedding the virus asymptomatically in saliva. Most primary infections occur in childhood and are asymptomatic.

Herpes facial lesions (cold sores) usually begin as areas of tender erythema, followed by the development of closely grouped vesicles (Figure 16.12) which ultimately progress through pustular and crusting stages. The lesions contain large amounts of virus. In patients with impaired immunity (e.g. due to corticosteroids, cytotoxic drugs or HIV infection) the lesions may be more extensive and spread into the mouth, and resolve more slowly.

Primary infection can sometimes occur at other sites such as the finger (called herpetic whitlow), the conjunctiva (keratoconjunctivitis) or areas of the face subject to frequent rubbing (e.g. 'scrum pox' in rugby footballers).

During primary infection, herpes viruses enter sensory nerve endings and travel along the axons to the sensory ganglion, where they remain latent (see Figure 16.13).

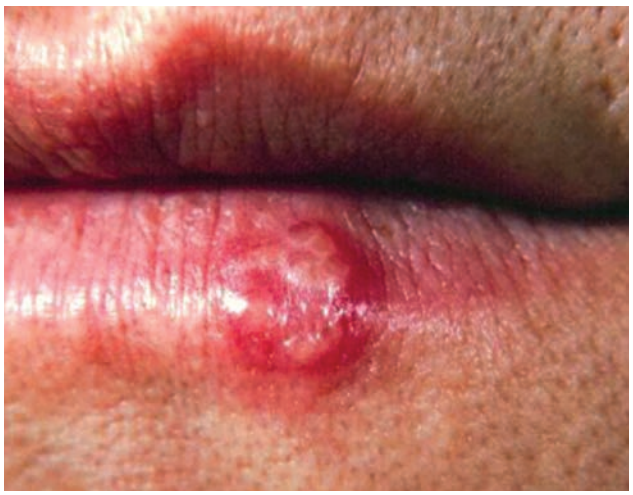


FIGURE 16.12

Cold sores caused by herpes simplex virus

Source: CDC/Dr Herrmann.

The virus remains in the sensory ganglion for life without expressing any viral proteins, which is how it remains hidden from the immune system in periods of latency. The virus can be reactivated by many stimuli, such as sunlight, trauma, viral respiratory infection, stress or immunosuppression, and it then travels back down the same sensory nerves to cause lesions at the same site. The interval between recurrences of symptoms can vary from several months to a year, or there may be no recurrence at all.

The infection is readily recognised clinically, so a laboratory diagnosis is usually not necessary. Isolation of the virus from lesion fluid, scraping or biopsy is the definitive diagnostic method, but a diagnosis can also be made by direct antigen detection or PCR on lesion material.

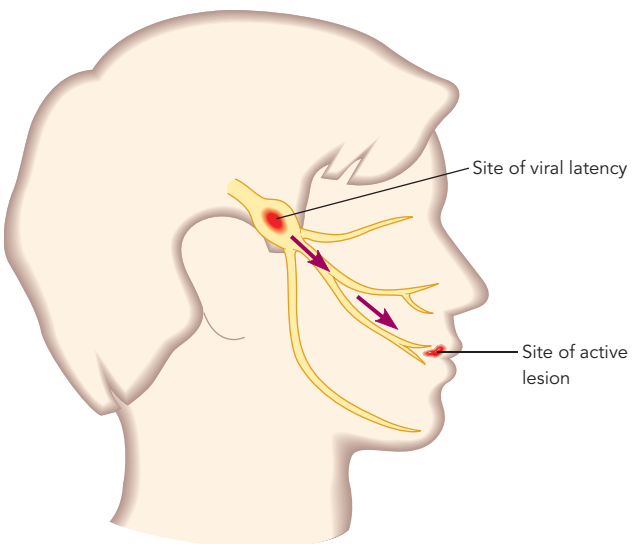


FIGURE 16.13

Site of latency of herpes virus type 1 in the trigeminal nerve ganglia

In the immunocompetent, lesions usually resolve spontaneously in 4–5 days. Treatment with saline bathing, peroxide or other astringents may help. In severe infections in the immunocompromised, treatment is usually with aciclovir, which suppresses symptoms and recurrences. Secondary infection of herpes lesions by *Staphylococcus aureus* or streptococci may occur.

Molluscum contagiosum

Molluscum contagiosum is a common viral infection, with worldwide distribution, caused by viruses of the pox group. It is most commonly seen in school-age children or young adults. It is transmitted from person to person by direct contact, or indirectly, via such things as clothing, gym equipment or swimming pools. A genital form of the disease is spread by sexual contact. The virus infects epidermal cells, causing epidermal hyperplasia. The infection is characterised by clusters of small (2–5 mm), flesh-coloured papular lesions scattered over the skin. The lesions are disseminated to other areas by scratching. The infection is generally mild and self-limited, but in the immunocompromised it can result in a severe disfiguring disease.

Diagnosis is usually made by the clinical appearance. PCR or microscopic examination of biopsy material may be used to confirm the diagnosis. Spontaneous resolution in the immunocompetent usually occurs within 6–9 months, but therapy is desirable to prevent spread of infection. Treatment has traditionally been achieved with physical means (e.g. superficial curettage or cryotherapy) or with chemical paints (podophyllin or trichloroacetic acid). For serious, chronic disease in the immunocompromised, treatment with antiviral agents (e.g. cidofovir) may be required.

Fungal skin infections

Tinea (ringworm)

The fungi that infect keratinised tissue, causing **tinea** (or **ringworm**), are called **dermatophytes**. Fungal skin infections are called **dermatomycoses**. These diseases are caused by organisms from three genera: *Epidermophyton*, *Microsporum* and *Trichophyton*. The common species include *E. floccosum*, *T. rubrum*, *T. verrucosum*, *T. mentagrophytes* and *M. canis*. Different species have a preference for different body sites.

Tinea is spread by contact with arthrospores of the fungi, which may be acquired directly from another person or indirectly from communal bathrooms, dressing sheds or swimming pools. Superficial abrasions or wounds increase the risk of infection. The fungi use keratin as a nutrient and therefore invade skin, nails and hair. The characteristic lesion of tinea is an annular (hence the name 'ringworm', although a worm is not involved), scaling patch of dry skin with a red, raised margin. In hairy parts of the body, the hair might be lost where the lesions develop. The lesions are often itchy. Dermatophytes occasionally penetrate subcutaneous tissues after invading follicles, and may then cause severe pustular lesions.

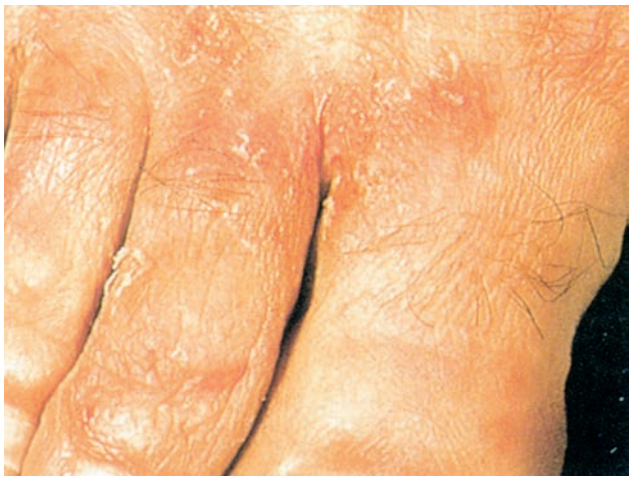


FIGURE 16.14

Tinea pedis, or athlete's foot, caused by fungi called dermatophytes

In **tinea pedis**, or **athlete's foot**, infection usually starts between the toes. It produces dry, scaly lesions between and under the toes, and subsequently causes the skin to crack and peel (Figure 16.14). The toenails are often infected (**tinea unguium**, tinea of the nails) and may become discoloured, thickened and brittle. Secondary infection by bacteria or *Candida* leads to itchy, soggy, white areas between the toes.

Tinea corporis (tinea of glabrous skin) is characterised by one or more annular, scaly lesions, often on the trunk or limbs. **Tinea cruris** (tinea of the groin), sometimes called 'jock itch', occurs in skin folds in the pubic region, and sometimes extends down the thighs. **Tinea capitis** (tinea of the scalp) is most common in children and young adolescents. Some hair loss and scaling of the skin are typical.

A clinical diagnosis of tinea is usually adequate. If necessary, a laboratory diagnosis is made by direct microscopic examination of skin scrapings or nail or hair clippings. Culture of these specimens may be performed, but this can take up to two weeks. Some species of dermatophytes fluoresce under ultraviolet light and can be seen when the light is shone on to the infected skin. Treatment generally involves the use of a topical antifungal drug (an azole or allylamine) and/or a keratolytic agent such as Whitfield's ointment (benzoic acid compound). If lesions are widespread or involve nails or hair, oral griseofulvin or terbinafine is usually used.

Prevention of athlete's foot depends on maintaining healthy, clean, dry skin and avoidance of contact in communal facilities.

Candidiasis

Candida albicans, an oval, budding yeast, is present among the normal flora of the digestive, respiratory and urogenital tracts of humans. It can cause infection (termed **candidiasis**) in a number of body sites. Superficial infection of mucous membranes causes **thrush**, which appears as milky white patches of inflammation on the tongue, inner cheeks or throat. *Candida albicans* can also cause **vaginitis**.

Candida skin infections generally occur in areas of warmth, moisture and maceration, such as in skin folds and under tight clothing and in association with nappy rash. *Candida albicans* is an opportunistic pathogen (see Chapter 7), usually requiring some predisposing factor such as diabetes mellitus or antibiotic therapy to cause infection. Obesity also predisposes to *Candida* skin infection, the moist skin folds providing an ideal site for the yeast to grow (see Figure 16.15).

Skin infection is characterised by areas of moist erythema with an irregular, soft, white edge. Satellite areas of infection in the form of red papules or pustules are usually present.

In immunocompromised individuals, *Candida* can invade the lungs, kidneys and heart (see Chapter 19). Candidiasis is a commonly seen fungal infection in hospitalised patients with serious disease such as leukaemia.

Diagnosis is usually based on clinical examination. A laboratory diagnosis is made by microscopy and culture of a swab of the lesion. Treatment usually involves the use of topical creams or ointments containing nystatin or an imidazole. Systemic therapy may be necessary in severe infections.

Tinea versicolor (pityriasis versicolor)

Tinea versicolor is a common, superficial infection caused by yeasts of the genus *Malassezia*—mostly *M. furfur* and *M. globosa*—which can also be found on normal skin. The trunk is most often affected, but there may be spread to other areas including the upper arms, neck and abdomen. The lesions are small, sharply demarcated, scaling macules. The organism interferes with pigment production—on white skin the lesions are dark and on dark skin they are lighter, hence the name 'versicolor'. The infection is basically cosmetic, causing little irritation, but it can persist for years if untreated.

The organism has recently been implicated as a cause of, or contributor to, seborrhoeic dermatitis (dandruff or 'cradle cap' in babies).

Treatment consists of topical applications of selenium sulfide or an antifungal agent, such as econazole. Recurrence is common.

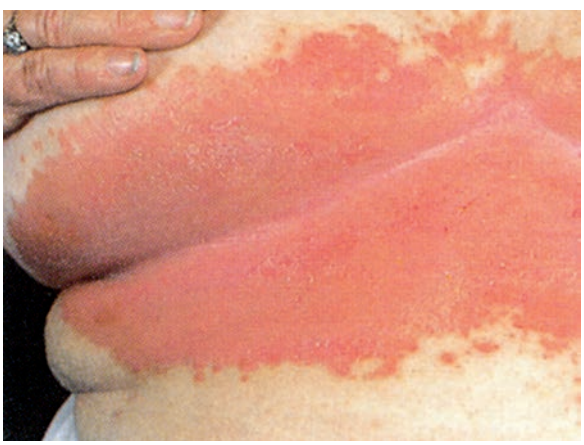


FIGURE 16.15

***Candida albicans* infection of the skin**

The infection is characterised by areas of moist erythema.

Source: Bayer AG.

Arthropod infestations

Scabies

Scabies is a highly contagious infestation caused by the mite *Sarcoptes scabiei* var. *hominis*. It has a worldwide distribution, and affects hundreds of millions of people annually. It is endemic in remote Indigenous communities in Northern and Central Australia.

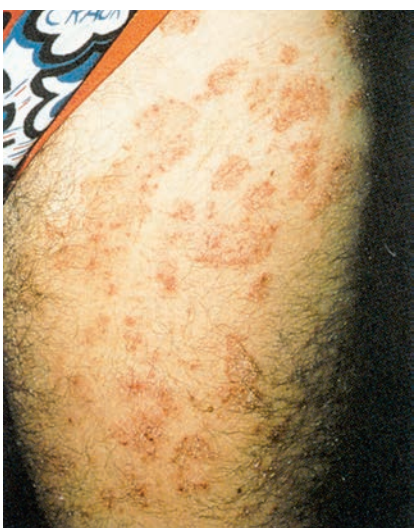
The adult female mite is about 0.4 mm in length, just visible to the naked eye. It burrows in the epidermis, laying eggs as it goes (Figure 16.16a). The typical scabies lesion is the burrow, a slightly raised, red-brown line, a few millimetres in length. Papules, pustules and nodules are also usually seen (Figure 16.16b). Itchiness, representing a sensitisation to mites, eggs and faeces in the skin, is severe and is often worse at night. Secondary infection of the lesions by streptococci or staphylococci is a common complication when they are scratched.

The severe form, called crusted (Norwegian) scabies, is characterised by formation of thick crusts on the face, scalp, hands, feet and pressure-bearing areas. It is typically found in patients who are either immunologically or neurologically impaired, such as transplant patients receiving immunosuppressive agents, patients with leukaemia and individuals with trisomy 21. In this form, the host may be harbouring thousands of mites.

The mites are mainly spread by close personal contact, and less frequently by indirect contact with bedding or clothing. An infested person can spread the mites even if asymptomatic. There may be a long period of up to ten weeks between the infection, which is when the patient becomes contagious, and the onset of clinical manifestations. Outbreaks can occur in hospitals and nursing homes. Healthcare workers are also at risk in such outbreaks.

The clinical presentation of scabies infestation is variable and often misdiagnosed. Nevertheless, diagnosis is usually based on clinical examination and microscopic demonstration of mites or eggs in a skin burrow, scraped with a needle point. A number of scabicides, such as permethrin, provide effective treatment. Close contacts should also be evaluated for infestation. Although scabies is less frequently transmitted by clothing, towels or bedding, dry cleaning or washing in hot water of these items is recommended.

(a)



(b)

FIGURE 16.16

(a) An adult scabies mite; (b) scabies infection

Source: Stephen Neville, Department of Microbiology, South Western Area Pathology Service, Liverpool, NSW.

Pediculosis

Pediculosis, or lice infestation, may involve the scalp, the pubic area or other parts of the body. Three species of lice infest these sites: *Pediculus humanus capitis* (head louse), *Pediculus humanus corporis* (body louse) and *Phthirus pubis* (crab louse). The major symptoms of pediculosis are itching and a maculopapular rash. Scratching can cause crusting and secondary bacterial infection. Enlarged lymph nodes are common.

Head lice infestation affects mainly young school children. The head louse (Figure 16.17) lays its eggs (called nits) at the base of hairs, to which they are firmly attached. After 8–10 days the eggs hatch, leaving translucent shells fixed to the hair. Pink or white dots can be seen near the scalp. Transmission is by hair-to-hair contact, brushes or hair apparel.

FIGURE 16.17

The head louse, *Pediculus humanus capitis*

Source: Stephen Neville Department of Microbiology, South Western Area Pathology Service, Liverpool, NSW.

Body lice infestation is generally related to poor hygiene, especially when clothes are infrequently laundered. The body louse lives on the clothing, depositing eggs there, and then travels to the body to feed.

Louse infestation of the pubic area (sometimes called 'the crabs') is usually sexually transmitted. Patients with this infestation often have another sexually transmitted disease, such as gonorrhoea. *P. pubis* prefers the pubic area, but can infest other sites such as the perianal area, thighs and trunk.

Lotions or shampoos of permethrin or other insecticides kill lice and nits, which can then be removed from hair with a fine-tooth comb. Family and other close contacts should be examined and treated if necessary. Clothes and linen should be dry cleaned or washed in hot water.

WOUND INFECTIONS

Preventing the infection of wounds has been a focus of medical practitioners for centuries. Hippocrates (c. 460–370 BC) used vinegar to irrigate open wounds and wrapped dressings around wounds to prevent further injury. In the 1860s, Joseph Lister pioneered the use of antiseptics to prevent surgical site infections. Nevertheless, wound infection, especially of wounds acquired in healthcare, remains a major cause of morbidity and mortality.

The common types of wound infection and their causative agents are listed in Table 16.2. In this section we will focus on infections of surgical and burns wounds, and on necrotising types of infections. Some people are hospitalised because of wounds (trauma or burns), and some people in hospital acquire wounds from surgery, intravascular catheters or pressure sores, all of which increase their risk of infection. Most wounds contain a serous exudate which, together with any dead tissue, provides an excellent culture medium for bacteria. Thus, it can be expected that virtually any open skin wound will contain microorganisms. However, this does not necessarily mean that the wound is infected.

If the wound contains non-replicating microorganisms only, it is said to be *contaminated*. If there are replicating microorganisms adhering to the wound surface without causing any tissue damage, the wound is said to be *colonised*. Wound infection can be defined as the invasion of replicating microorganisms into the wound with evidence of tissue injury.

It is now recognised that bacteria form biofilms when chronically colonising or infecting wounds. Biofilm formation also occurs on infected medical devices, such as endotracheal tubes, central venous catheters and orthopaedic devices. A biofilm is a community of bacteria, often different species, embedded in an extracellular matrix of polysaccharides secreted by the bacteria. Biofilm formation is important because it enables microbes to attach to tissue and/or a medical device and protects the bacteria within it from host defences and antibiotics. The role of biofilms in bacterial pathogenesis is discussed more fully in Chapter 10.

Clinical signs and symptoms of wound infection include increased pain and exudate, excessive inflammation, an unhealthy friable appearance of the wound and a failure of the wound to heal. However, certain factors (e.g. age, poor nutrition, diabetes, immunodeficiency) can mask the signs and symptoms of infection so that failure to heal may be the only sign.

Whether or not a colonised wound progresses to infection depends on a number of factors, particularly:

- the total number of microorganisms present
- the number of different species present
- the virulence of the organisms present
- the synergistic interactions between the different species
- the immune capacity of the affected person.

TABLE 16.2 Types of wound infections and the common causative organisms

TYPE OF WOUND	COMMON CAUSES
Surgical wounds (clean)	*Staphylococcus aureus* Coagulase negative staphylococci Enterococci Gram-negative enterobacteria
Surgical wounds (dirty)	As above, plus anaerobes and enterococci
Burns	*Pseudomonas aeruginosa* *Staphylococcus aureus* Gram-negative enterobacteria Enterococci
Necrotising infections	*Streptococcus pyogenes* *Staphylococcus aureus* *Clostridium perfringens* Gram-negative enterobacteria Mixed anaerobes—e.g. *Bacteroides*, *Peptostreptococcus*
Intravascular catheters	*Staphylococcus aureus* Coagulase-negative staphylococci Gram-negative enterobacteria *Corynebacterium jeikeium*
Pressure sores	*Staphylococcus aureus* Group A streptococci Gram-negative enterobacteria Enterococci
Dog/cat bites	*Pasteurella multocida* *Capnocytophaga canimorsus* Oral anaerobes
Accidental wound (soil or faecal contamination)	Clostridia *Pseudomonas aeruginosa* *Bacteroides fragilis*

Surgical wound infections

Infections of surgical wounds are among the more common types of healthcare-associated infections (see Chapter 13) and are a major cause of morbidity in patients following surgery. These infections increase the length of hospital stay and increase the mortality risk by as much as tenfold. About 5–6 per cent of all surgical wounds become infected, but the infection rate varies considerably with the type of surgery, the body site involved and patient susceptibility.

The term **postoperative infection** is used for any infection that occurs as a result of surgery. It includes infection involving the operative field, specifically called **surgical-site infections** (**SSIs**), and other infections that may follow surgery but occur in a part of the body not involved in the surgical procedure. In this section our focus is on SSIs.

Infection of clean, closed wounds is most likely to be due to organisms introduced during surgery, since in most closed wounds the skin edges are sufficiently sealed within several hours to prevent the entry of microorganisms during postoperative care. However, in situations where skin closure is delayed, microorganisms acquired postoperatively become more important. If surgery involves a site that is normally heavily colonised, such as the colon, the number of organisms that can contaminate the wound from surrounding tissue can be very large, and there is a much greater risk of infection. Thus, in bowel surgery, for example, part of the preoperative preparation may involve the reduction of this microbial load with antimicrobial drugs.

The most common causes of SSIs are bacteria, particularly *Staphylococcus aureus* (including MRSA), *Escherichia coli* and other Gram-negative enterobacteria, *Pseudomonas aeruginosa*, enterococci and various anaerobes (e.g. *Bacteroides* spp.). Most surgical wound infections become apparent within several days of the surgery, and some can be extremely quick, occurring within hours. However, infection of some surgical wounds can have a delayed onset, as much as 30 days after surgery. In surgery involving an implant (e.g. prosthetic heart valve, joint prosthesis), an SSI may become apparent even later than this—up to a year after the operation.

Epidemiology of surgical wound infections

Most surgical wound infections are probably acquired at the time of the operation. Possible sources of the organisms include:

- the operating room environment
- the hands of operating personnel
- other body parts of operating room personnel
- the patient's own body.

Strict environmental control, stringent sterilisation and disinfection practices, preoperative scrubbing and the use of surgical gloves help to reduce infection. Dermatitis on the hands of surgical personnel increases the risk because of the heavy colonisation of these lesions by bacteria. Materials used for gowns and drapes may not be effective barriers for bacteria, especially if they become moist, since microbes on underlying skin may then penetrate the gown and enter the operative field.

However, the usual source of organisms in surgical wound infections is the patient's own skin and mucosal membranes. These include the patient's normal flora, as well as any organisms that the patient may have been colonised with since entering hospital. As explained in Chapter 13, hospital-derived organisms can be resistant to antibiotics, and thus the infections they cause can be difficult to treat.

Factors that increase the risk of surgical wound infection

It has been shown that the duration of the patient's preoperative stay in hospital significantly affects the risk of acquiring a surgical wound infection. Infection rates are approximately doubled if the patient spends a week, as opposed to a day, in hospital prior to surgery, and approximately trebled after two weeks. The longer patients are in hospital, the greater the possibility that they will be colonised by drug-resistant hospital bacteria.

If the patient already has an infection in another part of the body, there is an increased risk of the wound being infected. When organisms have access to the bloodstream, their spread to the surgical wound site is facilitated.

The duration of the operation is a major risk factor for surgical wound infection. Longer procedures have a higher risk of wound colonisation because of the length of time the tissues are exposed. Longer procedures also usually involve more extensive tissue damage.

Other factors that increase the risk of infection are:

- age, due to the waning of the immune system
- the presence of underlying debilitating disease (e.g. diabetes or cancer)
- obesity, because a large amount of fat tissue can prolong the operation
- surgery involving a normally colonised site of the body (gastrointestinal, respiratory, genital tracts)
- the implant of a foreign body during surgery (e.g. prosthetic heart valve, vascular graft)
- inappropriate use (or omission) of wound drains.

Classification of surgical wounds and surgical site infections

It is common to classify operations into one of four types. This allows the risk of infection for different types of surgery to be estimated. Actual infection rates can then be assessed. A typical scheme is detailed in Table 16.3.

Surveillance of SSIs is also important to provide information on the rates of infection, any clusters of infection, and infection trends over time. This data assists healthcare organisations to develop and assess SSI prevention and control strategies. In order to perform accurate surveillance, standard definitions are required. The definitions utilised by the most organisations, including the Australian Infection Control Association, are outlined in Table 16.4.

TABLE 16.3 Classification of surgical wounds—a typical scheme

CLASSIFICATION	DESCRIPTION
Clean	No infection encountered Gastrointestinal, respiratory and genital tracts not entered No break in aseptic technique
Clean–contaminated	Gastrointestinal, respiratory or genital tract entered Minimal spillage of contents
Contaminated	One of above tracts is entered with significant spillage of contents Or acute inflammation without pus encountered Or surgery involving a minimally contaminated traumatic wound Or a major break in aseptic technique
Dirty	Pus encountered Or perforated viscus found Or heavily contaminated traumatic wound involved

Preventive measures

Given the usual sources of infecting organisms and the factors that increase the risk of infection, the major measures for preventing surgical wound infections are:

- reducing the preoperative hospitalisation of patients
- eradication of other infections prior to surgery, where possible
- appropriate prophylactic use of antimicrobial drugs
- washing of the skin and the application of antiseptics at the incision site
- promoting adequate oxygenation of the wound with exercise and warm water cleansing
- strict aseptic technique when changing dressings, especially for open wounds.

Laboratory diagnosis

The most common specimen collected for the identification of the cause of surgical wound infection is a swab. The swab should be used to collect as much purulent material as possible from the base of the wound, and transported to the laboratory in transport medium. Where large amounts of pus are present, this should be collected into a syringe and then transferred to a transport vial. Where anaerobes are suspected, a tissue specimen should be collected and sent in a sterile container. These procedures are described more fully in Chapter 15. Blood cultures should be collected if the patient is febrile.

Cultures of wound specimens are often difficult to interpret. The main reasons are that potentially pathogenic organisms can simply colonise a wound without causing infection, and that common colonisers (e.g. coagulase-negative staphylococci) can sometimes cause infection. Also, mixed infections are common, and pathogens and colonisers may be difficult to differentiate. Poor specimen quality and insufficient clinical information on request forms can add to the problem.

Treatment

Superficial infections often do not require antimicrobial therapy; local measures, including drainage, debridement and irrigation, are often adequate. Basic wound cleansing is important because it reduces bacterial numbers and their toxins in the wound and also reduces the nutrients in debris and devitalised tissue that the infecting microbes utilise.

Antimicrobial drugs are generally used when the infection is extensive or involves deeper tissues, or if the patient has impaired immune defences. In these situations, empirical therapy with a broad spectrum agent is usually commenced when the clinical diagnosis is made, and then modified, if necessary, when the sensitivity of the causative agent(s) has been determined. Local measures should accompany this treatment, since pus and necrotic tissue can prevent antimicrobial drugs from reaching the organisms and can also inactivate the drugs.

Burns wound infections

Burns wounds are one of the most common forms of trauma and are highly susceptible to infection. They are moist and full of nutrients and necrotic tissue, ideal for microbial growth and proliferation. And not only has the skin barrier been lost but other defences may be impaired. Extensive and deep burns wounds lead to depressed immune function, with both humoral and cellular defences affected. There is evidence that cytotoxic T cell activity can be suppressed, serum immunoglobulin and complement levels may be reduced, and neutrophil activity may be impaired. Also, defence cells have difficulty in reaching the infection because of the damage to the vascular system.

Patients who survive severe burns may suffer fatal infections while in hospital. In patients with burns affecting more than 30 per cent of the body surface, infection is very common and is responsible for a large proportion of fatalities. The vast majority of burns wounds become infected after the patient is admitted to hospital, and are therefore appropriately regarded as hospital-acquired infections. Outbreaks of infection are a major challenge in burns units.

The thick crust or scab that forms over a burn is called an **eschar**. Microbes growing in or on the eschar are usually not a problem; however, if they invade beneath the eschar, infection of adjacent tissue occurs. If they then enter the blood vessels, septicaemia, with a potentially high mortality, ensues.

Causative organisms, sources and transmission

Like other wounds, burns wounds are generally colonised by bacteria, some of which may subsequently cause infection.

TABLE 16.4 Definitions of surgical site infections

TYPE OF SSI	DEFINITION
Superficial incisional	Infection involves only skin and subcutaneous tissue of the incision AND Occurs within 30 days after the operative procedure AND Patient has at least one of the following: a. purulent drainage from the superficial incision b. organisms isolated from fluid or tissue c. at least one of: pain or tenderness, localised swelling, redness or heat; and the incision is deliberately explored by surgeon and is culture-positive d. diagnosis or antimicrobial treatment of superficial incisional infection by the operating surgeon or registrar.
Deep incisional	Infection involves deep soft tissues (e.g. fascial and muscle layers) AND Occurs within 30 days after the operative procedure if no implant is left in place, or within one year if implant is in place AND Patient has at least one of the following: a. purulent drainage from the deep incision but not from the organ/space of the surgical site b. a deep incision spontaneously dehisces or is deliberately explored by a surgeon when the patient has a fever (>38°C) or localised pain or tenderness, and is culture positive or not cultured c. an abscess or other evidence of infection is found on direct examination, during re-operation, or by histopathologic or radiologic examination d. diagnosis of, or antimicrobial treatment of, a deep incisional SSI by the operating surgeon or registrar.
Organ/space	Infection involves any part of the body, excluding the skin incision, fascia or muscle layers AND Occurs within 30 days after the operative procedure if no implant is left in place, or within one year if implant is in place AND Patient has at least one of the following: a. purulent drainage from a drain that is placed through a stab wound into the organ/space b. organisms isolated from fluid or tissue in the organ/space c. an abscess or other evidence of infection involving the organ/space found on direct examination, during re-operation, or by histopathologic or radiologic examination d. diagnosis or antimicrobial treatment of an organ/space SSI by the operating surgeon or registrar.

Staphylococcus aureus (including MRSA), group A streptococci, and a variety of Gram-negative organisms, particularly *Pseudomonas aeruginosa* and enterobacteria, are the most common causes of burn infections. *Candida albicans* and filamentous fungi such as *Aspergillus* spp. cause a small percentage, but serious infections. In a given burns unit, certain organisms tend to predominate; however, the predominant types may vary over time.

Burns wounds quickly become colonised and thus constitute a huge reservoir of microbes for cross-contamination to other hospitalised patients. Organisms may be transmitted from patients with infected or colonised burns to new patients by hands, clothing, other fomites and air.

Hospital staff can carry pathogens on their hands and are also an important reservoir of organisms in burns infections. In addition, their hands are important vectors for the transmission of microbes from other sources to the wound. Hands of staff may become contaminated directly from contact with a patient, or indirectly from contaminated environmental surfaces. The importance of proper hand hygiene, especially immediately before and after contact with a patient, cannot be emphasised enough.

Diagnosis and treatment

A wound biopsy is the preferred specimen for the diagnosis of burns wound infection, because a quantitative culture can be performed and histologic evidence of infection (as opposed to colonisation) can be obtained. However, the invasiveness of the technique and the time taken for collection and processing of this specimen means that swabs or excised necrotic tissue are more often collected. Blood

cultures should be collected if systemic symptoms are present.

Blood-borne antimicrobial drugs do not readily reach the infection site, so antimicrobial therapy has only a supportive role in treatment. Debridement or surgical excision of dead tissue is the most important aspect of treatment, and this also aids in the delivery of antimicrobial drugs to the infected site. Because infection is usually acquired in hospital and the sensitivity of the infecting organisms cannot be predicted, antibiotic sensitivity tests are essential.

Prevention of burns wound infections

Survival rate of burns patients has improved substantially over the last decade, at least in part due to advances in care management, including infection control practices. Prevention of infection involves prompt excision of dead tissue, skin graft closure of the wound, good wound care, the use of topical antimicrobials to suppress the multiplication of organisms on the wound surface, and strict environmental control. Environmental control includes strictly enforced hand hygiene, the use of good aseptic techniques, and gowns and gloves, and fastidious sterilisation and disinfection of fomites.

Necrotising fasciitis

Necrotising fasciitis is an uncommon, but very severe, rapidly progressive infection and necrosis of subcutaneous tissues and fascia (the soft tissue below the dermis). Necrotising fasciitis moves along the fascial plane, with the infection spreading, in some cases, 2–3 cm per hour. The media has popularised the idea that this infection is caused by 'flesh-eating bacteria'; however, its rapid and destructive clinical course is most likely due to the secretion of highly damaging enzymes and toxins, often by multiple organisms (see Figure 16.18). Up to 20 per cent of cases are fatal.

Necrotising infections usually occur in tissue following trauma, surgery, ischaemia or other causes of tissue damage, or from haematogenous spread. However, it has occasionally developed in a healthy person suffering minor trauma, such as a sprain, without skin damage. Other conditions associated with necrotising fasciitis include diabetes mellitus, obesity, immunosuppression and intravenous drug use. Organisms spread along the fascial planes, causing vascular occlusion, ischemia, tissue necrosis, and ultimately septicaemia in many cases. Associated nerves may be damaged, producing the characteristic localised anesthesia. Gases are the end products of microbial metabolism, and they may accumulate in the tissues.

Three main forms of necrotising fasciitis have been identified. Type I is due to polymicrobial infection, usually involving a combination of aerobic and anaerobic bacteria. Group A streptococci and *Staphylococcus aureus,* alone or together, are often the initiating bacteria. Since there is often local hypoxia due to tissue damage, anaerobes, especially *Bacteroides* spp. and *Peptostreptococcus* spp., are often involved, in combination with Gram-negative enterobacteria, such as *Escherichia coli, Enterobacter, Klebsiella* or *Proteus.* Type II is usually caused by group A streptococci or *S. aureus,* including MRSA. Community-associated MRSA has emerged as a major cause of this

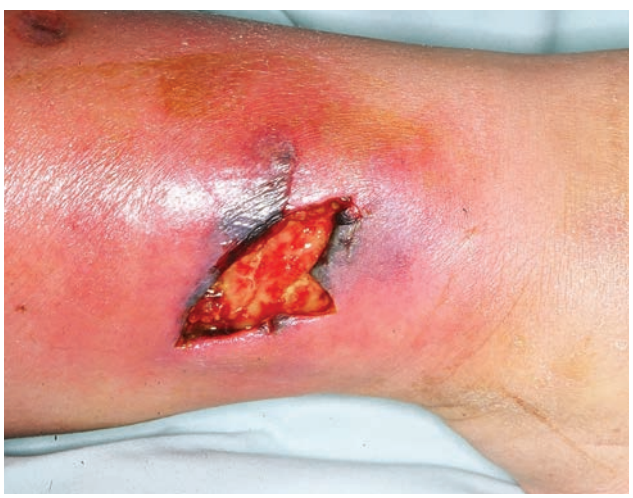


FIGURE 16.18

Necrotising fasciitis

Source: Reproduced with permission of New Zealand Dermatological Society Incorporated at DermNetNZ.org.

CASE HISTORY 16.4

Necrotising fasciitis

A 56-year-old male chef suffered a right-hand scald burn four days before attending the emergency department. His only underlying condition was that he had diabetes mellitus. He stated that his whole arm was painful, and erupted vesicles, hemorrhagic bullaes and oedema were observed. During examination his blood pressure dropped dramatically. A preliminary diagnosis of necrotising fasciitis was made and he was sent to theatre for emergency fasciotomy. Culture of tissue obtained during surgery and blood cultures confirmed the causative organism to be methicillin-resistant *Staphylococcus aureus*. Antibiotic testing indicated the organism was probably a community-associated MRSA. Empirical antibiotic treatment of penicillin, clindamycin and gentamicin was changed to vancomycin and gentamicin once the organism was identified. The man required two more fasciotomies, and a skin graft was performed during the last operation. He was discharged four weeks after admission.

Questions

1. Why was the man given empirical antibiotic therapy of penicillin, clindamycin and gentamicin?
2. Why was he sent for an 'emergency fasciotomy'?
3. What factors predisposed the man to developing necrotising fasciitis?
4. What did his sudden drop in blood pressure indicate?

type. Type III is **gas gangrene**, or clostridial myonecrosis, and is most often caused by *Clostridium perfringens* or occasionally by other clostridial species such as *C. novyi, C. septicum* and *C. bifermentans*. Spores of clostridia are found in soil and in human and animal faeces. They can be introduced into tissues by accidental puncture wounds, during surgery if improperly sterilised instruments are used, or if faecal contamination of the wound occurs. The major toxin, alpha toxin, is a lecithinase which hydrolyses lipids in cell membranes, causing cell lysis and death. This allows the organism to invade deeper into the tissues.

A preliminary diagnosis of necrotising fasciitis is based on clinical findings. Because of the infection's rapid progression and potentially fatal outcome, treatment is commenced before laboratory test results are available. Indeed, when the patient is seriously ill, necrotising fasciitis is a surgical emergency, often requiring extensive debridement. Repeated surgical debridement may be required, until the spread of the bacteria is halted and tissue necrosis ceases. If a limb is involved, amputation may be necessary because of irreversible necrosis. Aggressive antibiotic therapy is usually instituted—a typical empirical regimen would include a combination of penicillin G, an aminoglycoside and clindamycin. A more specific antibiotic regimen can be introduced after the results of culture and sensitivities are available. Gram staining of exudate or biopsied tissue may provide initial clues about the causative organism(s), but a definitive diagnosis usually depends on isolation of the organism(s) from tissue samples obtained at the time of surgical debridement.

INFECTIONS OF THE EYE

The eye is exposed to the outside world and is therefore vulnerable to microorganisms and other foreign particles. It has a number of highly effective defences which generally keep it free of infection. The exposed surface of the eye and the interior surface of the eyelid are covered by the conjunctiva, a thin, mucus-secreting epithelial membrane (Figure 16.19). As described in Chapter 9, mucous membranes are armed with both non-specific and specific defences. In addition, tears mechanically flush the eye and contain the antimicrobial substance lysozyme.

Infections of the eye most often involve the conjunctiva, and the resulting inflammation is called **conjunctivitis**. Bacteria and viruses are the most common infectious causes of conjunctivitis, but non-infectious causes are also common, particularly irritation by foreign particulate matter and allergic reactions.

Other infections of the eye may involve the cornea (**keratitis**), the eyelid (**blepharitis**), both the cornea and conjunctiva (**keratoconjunctivitis**), or both the eyelid and conjunctiva (**blepharoconjunctivitis**).

Bacterial eye infections

Common causative agents

A wide range of bacteria comprise the normal flora of the eye, including *Staphylococcus epidermidis, Propionibacterium,*

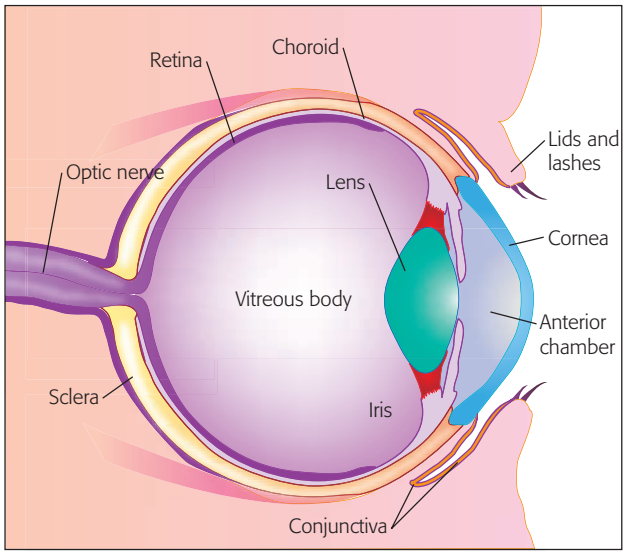


FIGURE 16.19

Structures of the eye

streptococci and corynebacteria. Some of these organisms can cause conjunctivitis, but usually only in the immunocompromised. Bacterial conjunctivitis primarily affects infants and young children. The most common causes are *Streptococcus pneumoniae, Staphylococcus aureus, Chlamydia trachomatis, Haemophilus influenzae* and *Moraxella catarrhalis.* Contact-lens-related conjunctivitis can be caused by *Pseudomonas* or other Gram-negative bacteria, especially in individuals using extended-wear soft lenses.

Bacterial conjunctivitis (see Figure 16.20) typically develops abruptly, with patients usually experiencing redness, watery eyes, a feeling of grittiness in the eyes, a burning or stinging sensation, and photophobia. The watery discharge usually becomes mucopurulent or purulent. On awakening the eyelids can be stuck together by this discharge. The infection usually starts in one eye but may spread to the other eye in 1–2 days. Many patients will recover completely without treatment.

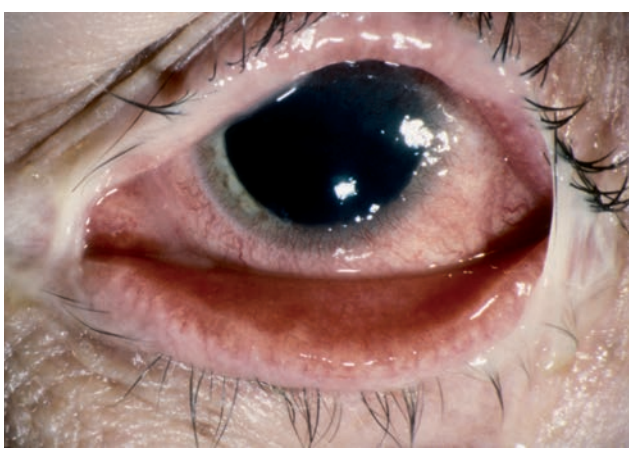


FIGURE 16.20

Bacterial conjunctivitis

Source: Medical Photographic Imaging Centre. © Eye and Ear Hospital, Melbourne.

CASE HISTORY 16.5

Conjunctivitis

Michael was an active 7-year-old who awoke with the eyelid of his left eye stuck to the lower lid. When he opened this eye he said it felt like he had sand in it, and that it felt a bit 'stingy'. His mother, who was very busy that day, took him to school, intending to take him to the doctor in the afternoon. Apart from his eye problem he felt all right. As soon as she got to work she had a phone call from Michael's teacher, asking her to take him home. The teacher said that he wasn't permitted to stay at school with an eye infection.

Questions

1. Was it reasonable that Michael be sent home from school? Why?
2. What is the likely cause of his infection?
3. What treatment would most likely be prescribed for Michael?

Conjunctivitis is extremely contagious, especially among children, and can spread rapidly through schools and daycare centres. Children rub itchy, running eyes and transfer organisms to their playmates.

Neisseria gonorrhoeae infections

In Australia and other developed countries, *Neisseria gonorrhoeae* is now an uncommon cause of conjunctivitis, but in underdeveloped countries the disease is still prevalent. It is a potentially serious problem in neonates, who usually acquire the infection from their mother during passage through the birth canal. It is an important cause of **ophthalmia neonatorum**, the term for a purulent conjunctivitis in an infant less than 14 days old.

It can also be spread among children, and a number of outbreaks have been recorded in Aboriginal communities in Central and Northern Australia. Sexual transmission is also possible. The infection requires prompt treatment, because it can cause keratitis (inflammation of the cornea); this can progress rapidly to ulceration and scarring of the cornea, and even to perforation, leading to blindness. Perforation can occur within three days of the onset of infection.

Chlamydia trachomatis infections

Chlamydia trachomatis serotypes D to K are able to cause conjunctivitis. Transmission is usually by contact, with fingers, towels and flies being important vectors. Some serotypes of *C. trachomatis* can infect the genital tract as well as the conjunctiva, so sexual transmission, autoinoculation from genital infection or infection of the newborn during birth can occur.

Trachoma (Figure 16.21), from the Greek word meaning 'pebbled' or 'rough', is caused by *Chlamydia trachomatis* serotypes A to C. It is the leading cause of preventable blindness worldwide; it is estimated that over 80 million people are infected, of whom 8 million are visually impaired. The disease is widespread in parts of Asia, Africa and South America, in some areas affecting up to 90 per cent of the population. It is especially prevalent in arid conditions and in communities with poor hygiene, poor water supply and crowded living conditions. It is still endemic in some remote Aboriginal communities, where infection rates among children can be up to 7 per cent. Flies are important mechanical vectors and close mother–child contact facilitates transfer.

Trachoma is a chronic follicular conjunctivitis, mainly affecting the upper eyelid. In chronic or repeated infection, fibrosis may occur, which can cause contraction of the eyelid tissue and a turning under of the eyelashes. The lashes abrade the cornea, leading to blindness.

Laboratory diagnosis and treatment

Laboratory tests are usually not necessary for diagnosis of bacterial conjunctivitis, except for severe cases, cases that do not respond to therapy, or when *N. gonorrhoeae* or *C. trachomatis* are suspected. Gonococcal conjunctivitis is confirmed by culture of a conjunctival swab. For suspected chlamydial infections, a swab is rolled firmly several times across the conjunctiva. A diagnosis of chlamydial infection can be confirmed by polymerase chain reaction (PCR) detecting chlamydial antigen in this swab. Sexually active patients with chlamydial conjunctivitis should also be assessed for systemic infection (e.g. urethritis, cervicitis, vaginitis).

As stated above, many cases of acute bacterial conjunctivitis are self-limiting and antimicrobial therapy is not always required. However, appropriate treatment will shorten the course of infection, relieve patient discomfort and limit the spread to other individuals. Topically applied antimicrobials, such as chloramphenicol, are the main form of treatment for bacterial conjunctivitis, except for gonococcal and

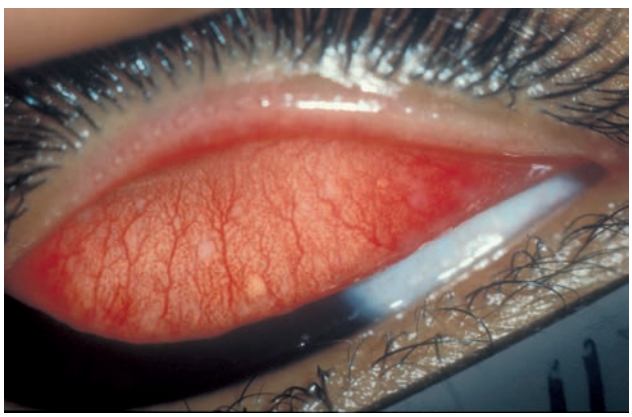


FIGURE 16.21

Trachoma

A conjunctivitis caused by Chlamydia trachomatis. *Note the follicles on the conjunctival membrane.*

Source: Medical Photographic Imaging Centre. © Eye and Ear Hospital, Melbourne.

chlamydial infections. Children should not return to school until their infection has cleared.

Treatment of *N. gonorrhoeae* conjunctivitis involves the use of a topical broad spectrum antibiotic such as a fluoroquinolone (e.g. ciprofloxacin) plus a systemic broad spectrum antibiotic such as ceftriaxone. Preventive measures have almost eradicated the disease in developed countries. Formerly, a 1 per cent solution of silver nitrate in the eyes of neonates was used to prevent infection, but this preventive measure has been replaced by the use of topical antimicrobial drugs.

Treatment of chlamydial infection is with oral azithromycin. When sexual transmission is possible, treatment should also be provided for the patient's sexual partner.

Viral eye infections

Viruses are a common cause of infectious conjunctivitis. Adenoviruses cause most of these infections, but other viruses—such as the herpes simplex virus, varicella zoster virus, enteroviruses, coxsackie viruses and cytomegalovirus—may also be involved. The typical history is of a recent upper respiratory infection or contact with someone with a red eye. Like bacterial conjunctivitis, viral infection usually begins in one eye but may spread to the other eye within days.

Adenovirus infections

Epidemic keratoconjunctivitis (**EKC**) is caused by adenovirus serotypes 8, 19 and 37. After an incubation period of about a week, the conjunctiva becomes inflamed and the patient usually complains of a burning irritation and a watery discharge. In contrast to bacterial conjunctivitis, the discharge usually remains watery. Within days the infection may spread to the corneal epithelium and sometimes to deeper corneal tissue.

Direct contact with infected individuals or contaminated fomites is the most common form of transmission. EKC epidemics tend to occur in institutions such as schools, hospitals, camps and nursing homes. EKC has often been transmitted in epidemic form in eye clinics and ophthalmologists' offices via contaminated equipment or hands. The reasons for this are that the virus can remain viable for many weeks on contaminated equipment, the virus is resistant to standard disinfectants, such as isopropyl alcohol, and an infected person can shed the virus three days before and 14 days after onset of symptoms. Patients should be informed of the extremely contagious nature of this disease, and advised to wash their hands frequently and to avoid touching their eyes, sharing towels and having close contact with other people for about two weeks.

Another less common disease, caused by adenovirus serotypes 3 and 7, is pharyngoconjunctival fever. This disease mainly affects children under 10 years of age and is characterised by pharyngitis, fever and conjunctivitis. This is also highly contagious, person-to-person contact disease. Outbreaks often involve children in holiday camps (see Case History 16.6: Pharyngoconjunctival fever) or infection from swimming pools or small lakes.

CASE HISTORY 16.6

Pharyngoconjunctival fever

On 19 October 2000 the principal of a North Queensland primary school contacted the Tropical Public Health Unit regarding a high rate of absenteeism. Investigations revealed that 34 students were unwell from 17 to 23 October. The most common symptoms were fever, headache, sore throat, nausea and eye irritation. Between 11 and 13 October, some children from the school had been to a school camp at a coastal resort in Far North Queensland. The camp had a large saltwater swimming pool. Of the 34 students who were ill, 25 (74 per cent) had attended the camp. The nine affected students who did not attend the camp were assumed to have acquired the infection from siblings or from the school community.

Laboratory investigations confirmed that adenovirus 3 was the cause of this outbreak. Although the virus could not be isolated from the swimming pool, it was strongly implicated as the source of the outbreak. There was evidence that the pool was not appropriately maintained, with an inadequate chlorine level.

Source: Adapted from D. Harley et al. 2001, A primary school outbreak of pharyngoconjunctival fever caused by adenovirus type 3. *Communicable Diseases Intelligence* 25(1): 9–12.

Questions

1. What does this outbreak indicate about the transmissibility of adenovirus 3?
2. What the two major sources of infection in this case?
3. What would be the most useful approach for preventing the spread of infection in such an outbreak?
4. Why can a related virus cause outbreaks of infection in patients attending eye clinics?

Viral conjunctivitis is typically self-limiting, normally resolving spontaneously within 2–6 weeks. There is no evidence that topical antiviral drugs improve outcomes. Supportive treatment may be provided, including cold compresses, topical vasoconstrictors and analgesics.

Herpes simplex eye infections

Ocular herpes infections are most often due to herpes simplex type 1 virus. It can produce a number of different clinical manifestations, the most serious being a keratitis, which can result in severe visual impairment. The peak incidence is in children between the ages of 1 and 10 years who have not previously been exposed to the virus and lack protective antibodies. Infection usually results from direct contact with a person who is shedding the virus from skin or mucous membrane lesions, or by self-inoculation from a primary infection elsewhere in the body.

As with other herpes simplex infections, there is the chance of recurrence due to the latency of the virus in a nerve ganglion, and factors that cause suppression of the immune system (e.g. HIV infection) increase the risk of recurrence. Herpes simplex type 2 virus can also cause conjunctivitis and is most commonly associated with neonatal disease after acquisition from the mother's genital tract.

Treatment of herpes conjunctivitis or keratitis is with topical antiviral agents such as aciclovir.

Diagnosis of viral eye infections

Viral isolation methods are difficult and are generally performed only by specialist virus laboratories. Fluorescent antibody tests can detect some viruses (e.g. herpes simplex and adenoviruses) in conjunctival scrapings.

Other eye infections

Keratitis, or inflammation of the cornea, can result in permanent damage and vision impairment. Normally, the cornea is quite resistant to infection but factors such as trauma, contact lens wear and topical corticosteroid use can predispose to keratitis. Keratitis can be caused by fungi, viruses and protozoa, but bacteria are the most common cause. A wide variety of bacteria can be involved, including all the common causes of conjunctivitis. *Pseudomonas* spp. and the protozoan *Acanthamoeba* are important causes associated with contact lens use. *Acanthamoeba* is ubiquitous in soil and water (including tap water) and therefore commonly contaminates lens cases and solutions. Corneal thinning and perforation are potential threats of severe infection.

Blepharitis is predominantly caused by *Staphylococcus aureus*. Other possible causes include herpes simplex virus and the crab louse, *Phthirus pubis*. **Endophthalmitis** is an inflammation of the fluid behind the cornea and usually follows intraocular surgery when bacteria are exogenously introduced. **Retinitis**, inflammation of the retina, can occur following congenital infection or in immunocompromised patients. Cytomegalovirus retinitis is one of the more common AIDS-associated infections.

A summary of infections of the eye is presented in Table 16.5.

TABLE 16.5 Eye infections

TYPE OF INFECTION	COMMON CAUSES
Bacterial conjunctivitis (inflammation of conjunctiva)	*Haemophilus influenzae* *Streptococcus pneumoniae* *Moraxella catarrhalis* *Staphylococcus aureus*
Trachoma (chronic follicular conjunctivitis)	*Chlamydia trachomatis*
Viral conjunctivitis	Adenovirus Herpes simplex virus types 1 & 2
Keratitis (inflammation of cornea)	All causes of bacterial conjunctivitis *Pseudomonas* spp. Herpes simplex virus *Acanthamoeba*
Blepharitis (inflammation of eyelid margin)	*Staphylococcus aureus*
Endophthalmitis (inflammation of fluid behind cornea)	*Staphylococcus epidermidis* *Propionibacterium acnes* *Actinomyces* sp.
Retinitis (inflammation of retina)	Cytomegalovirus Human immunodeficiency virus
Preseptal cellulitis (cellulitis of eyelid)	*Staphylococcus aureus* Streptococci *Haemophilus influenzae*
Orbital cellulitis (cellulitis of orbit)	*Staphylococcus aureus* Streptococci *Haemophilus influenzae*

SUMMARY

- Undamaged skin provides an effective barrier against invasion by most microorganisms.
- The eye has a number of highly effective defences which generally keep it free of infection.

INFECTIONS OF THE SKIN

- Tissue damage is the most common factor leading to infection of the skin.
- In some systemic infections (e.g. measles, rubella and chickenpox), a skin rash is the major manifestation.

Bacterial skin infections

- *Staphylococcus aureus* is a common cause of skin infections: folliculitis, furuncle, boil and carbuncle.
- Impetigo is a highly contagious, pyogenic infection caused by staphylococci, streptococci, or both.
- Scalded skin syndrome is caused by strains of *S. aureus* that produce the exfoliatin toxin.
- Erysipelas is a skin infection characterised by a bright red, swollen lesion with a sharply demarcated edge, followed by bullae that rupture and weep. It is usually caused by Group A streptococci.
- Cellulitis is an acute bacterial infection of the skin that spreads to subcutaneous tissues.
- Wound drainage and debridement are often all that is necessary for minor bacterial skin infections.
- Impetigo and other streptococcal infections are highly

contagious. They are usually treated with an oral antibiotic or intravenous antibiotics in severe infections.
- *Propionibacterium acnes* has been strongly implicated as a cause of acne.

Viral skin infections
- Rubella (German measles) is a viral infection in which a skin rash is the main symptom.
- Congenital rubella syndrome is an infection of a foetus when the virus crosses the placenta from the mother to baby.
- A rubella vaccine is a component of the MMR (measles-mumps-rubella) vaccine.
- Measles (rubeola) is a highly infectious skin disease, spread mainly by respiratory secretions.
- Measles encephalitis is the most serious complication of measles infection.
- Measles vaccine prevents major epidemics and is usually given in the form of the MMR vaccine.
- The varicella zoster virus causes chickenpox and shingles.
- Chickenpox is a highly infectious disease transmitted by respiratory secretions or by contact with skin lesions.
- Warts are caused by human papillomaviruses.
- Infection with herpes simplex type 1 virus usually involves the skin around the mouth or nose (cold sores).
- Herpes simplex type 2 virus predominantly involves the genitalia.
- Herpes viruses enter sensory nerves and travel to the sensory ganglion, where they remain latent until reactivated by various stimuli.
- Molluscum contagiosum is a common viral infection caused by pox viruses.

Fungal skin infections
- The fungi that cause tinea (ringworm) are called dermatophytes.
- The characteristic lesion of tinea is an annular, scaling patch of dry skin with a red, raised margin.
- Superficial infection of mucous membranes by *Candida albicans* appears as thrush—milky-white patches of inflammation.
- Tinea versicolor (pityriasis versicolor) is a skin infection caused by the yeast *Malassezia furfur*.

Arthropod infestations
- Scabies is caused by the mite *Sarcoptes scabiei* var. *hominis*.
- The female scabies mite burrows in the epidermis, laying eggs; the typical scabies lesion is the burrow.
- Norwegian (crusted) scabies is characterised by formation of thick crusts on the face, scalp, hands and feet.
- Pediculosis is a lice infection characterised by itching and a maculopapular rash.

WOUND INFECTIONS
- A wound is colonised if there are replicating microorganisms adhering to the wound surface without causing any tissue damage.
- Wound infection is the invasion of replicating microorganisms into the wound with evidence of tissue injury.
- Local signs of erythema, pain, oedema, odour and purulent exudate are suggestive of wound infection.
- Infections of surgical wounds are among the more common healthcare-associated infections.
- Burns wounds are highly susceptible to infection.
- Necrotising fasciitis is an infection of the soft tissue below the dermis.
- The rapid and destructive clinical course of necrotising fasciitis is due to the secretion of highly damaging enzymes and toxins, often by multiple organisms.

INFECTIONS OF THE EYE
- Infections of the eye most often involve the conjunctiva (conjunctivitis), but may also involve the cornea (keratitis) or eyelid (blepharitis).

Bacterial eye infections
- The most common causes of bacterial conjunctivitis are *Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis* and *Staphylococcus aureus*.
- *Neisseria gonorrhoeae* conjunctivitis can be acquired by babies during birth.
- *Chlamydia trachomatis* serotypes D to K cause conjunctivitis.
- Trachoma, caused by *C. trachomatis* serotypes A to C, is the leading cause of preventable blindness worldwide.

Viral eye infections
- Epidemic keratoconjunctivitis (EKC) is caused by adenovirus serotypes and is highly contagious.
- Pharyngoconjunctival fever is a highly contagious disease caused by adenovirus serotypes 3 and 7.
- Ocular herpes infections are most often due to herpes simplex type 1 virus.

Other eye infections
- *Pseudomonas* spp. and the protozoan *Acanthamoeba* are important causes of keratitis associated with contact lens use.
- Endophthalmitis is an inflammation of the fluid behind the cornea.

STUDY QUESTIONS

1. What are the characteristics of skin that make it such an effective barrier against infection?
2. What defences does the eye have to protect it from infection?
3. Name the common causes of (a) a boil, (b) a carbuncle, (c) impetigo, (d) erysipelas and (e) cellulitis.
4. Why is the spread of rubella difficult to control in an outbreak?

5. What is the congenital rubella syndrome?
6. How is rubella prevented?
7. What serious complication can result from measles infection?
8. Why do outbreaks of measles infection still occur despite the availability of an effective vaccine?
9. Explain the implications of latency of the varicella zoster virus.
10. What are the causative agents of warts and what is their link with cancer?
11. What are the common sites of infection of the herpes simplex 1 and 2 viruses?
12. How does the herpes simplex 1 virus cause recurrent infections?
13. What is a dermatomycosis and what are the causative organisms?
14. What is thrush, and what are the common predisposing factors for this infection?
15. What is scabies?
16. Why is Norwegian scabies potentially very contagious?
17. What is pediculosis and how is it transmitted?
18. Why are wounds so readily colonised by microorganisms?
19. What are the signs that indicate a wound is infected?
20. List the factors that can increase the risk of surgical wound infection.
21. List the procedures that are implemented to prevent infection of surgical wounds.
22. How are wound infections treated?
23. What are the common causes of burns wound infections and what are their usual sources?
24. List the procedures that help to prevent infection of burns wounds.
25. What is necrotising fasciitis?
26. What is conjunctivitis and what are the common causes?
27. What is ophthalmia neonatorum?
28. What is trachoma and what causes this infection?
29. What is epidemic keratoconjunctivitis and how is it transmitted?
30. Define the terms 'keratitis', 'blepharitis', 'endophthalmitis' and 'retinitis'.

TEST YOUR UNDERSTANDING

1. What is community-associated MRSA and what infections does it cause?
2. Describe the scientific basis for the link between the measles vaccine and autism.
3. Explain how the chickenpox virus causes shingles.
4. Explain why surgical wound infections are predominantly endogenous infections.
5. What are biofilms and how do they assist bacteria to cause infection?

FURTHER READING

Anderson, D.J. 2011, Surgical site infections. *Infectious Diseases Clinics of North America*, 25: 135–53. (Details the causes, pathogenesis, risk factors and prevention of surgical site infections.)

Breen, J.O. 2010, Skin and soft tissue infections in immunocompetent patients. *American Family Physician*, 81(7): 893–99. (Reviews the causes, diagnosis and management of bacterial skin infections.)

Johnson, R., J. McElhaney, B. Pedalino and M. Levin 2007, Prevention of herpes zoster and its painful and debilitating complications. *International Journal of Infectious Diseases* 11(Suppl 2): S43–S48.

Oliva, M. and H. Taylor 2004, Conjunctival conditions. *Australian Doctor*, 11 June: 39–46. (Reviews the signs and symptoms, diagnosis and management of common eye infections.)

Rafla, K. and E.E. Tredget 2011, Infection control in the burn unit. *Burns*, 37: 5–15. (A comprehensive review of the epidemiology, causes, acquisition, and prevention of burns wound infections.)

Wolcott, R. & S. Dowd 2011, The role of biofilms: Are we hitting the right target? *Plastic and Reconstructive Surgery*, 127(Suppl): 28S–35S. (Describes the role of biofilms in wound infections.)

CHAPTER

17

Respiratory tract infections

CHAPTER FOCUS

- What are the main defences that protect the respiratory system against infection?
- What are the main factors that predispose people to respiratory tract infections?
- What are the major types of upper respiratory tract infections, and what are their common causes?
- How are upper respiratory tract infections diagnosed and treated?
- What are the major types of lower respiratory tract infections, and what are their common causes?
- How are lower respiratory tract infections diagnosed and treated?
- What microorganisms cause chronic infection of the lower respiratory tract?
- How are chronic infections of the lower respiratory tract diagnosed and treated?

INTRODUCTION

Thousands of litres of air pass into and out of the respiratory tract of a healthy adult each day. This air can contain thousands of microorganisms per cubic metre. It is not surprising, therefore, that respiratory infections are among the most common that afflict humans. Most of the microbes in air are harmless, but potentially pathogenic microorganisms may be present.

People with respiratory infections may expel enormous numbers of pathogens when coughing or sneezing. In addition, a number of potentially pathogenic microbes may comprise part of the normal flora of the upper respiratory tract (see Table 17.1). These microbes are generally kept in check by other normal flora organisms, but they can cause infection if the host becomes susceptible, or when they are transferred to another individual whose immunity is compromised in some way. Most pathogens that enter the body via the respiratory tract infect only the respiratory tract, but the respiratory system is also a route of entry for some organisms that infect other parts of the body (e.g. measles, mumps and rubella viruses).

TABLE 17.1 Normal flora of the upper respiratory tract

■ Commonly present	Viridans streptococci
	Neisseria spp.
	*Moraxella catarrhalis**
	Staphylococcus epidermidis
	Corynebacteria (avirulent strains)
	Bacteroides spp.*
	Veillonella spp.
	*Candida albicans**
	Streptococcus mutans
	*Haemophilus influenzae**
■ Occasionally present	*Streptococcus pyogenes**
	*Streptococcus pneumoniae**
	Neisseria meningitides
	*Corynebacterium diphtheriae**
	*Klebsiella pneumoniae**
	Pseudomonas spp.*
	*Escherichia coli**

* Potentially pathogenic in the respiratory tract.

PREDISPOSING FACTORS OF RESPIRATORY INFECTIONS

To resist the constant threat of microbial invasion, the respiratory system has a number of important defence mechanisms for preventing organisms infecting the upper parts of the tract, or reaching and infecting the lower regions. These defences (summarised in Table 17.2) are responsible for keeping the lungs free of microorganisms and other foreign particles. The incidence of respiratory infection is greatly increased in people who have a defect in one or more of these mechanisms, or who have defences bypassed—for example, by an endotracheal tube. Figure 17.1 shows the ciliated epithelium of the upper respiratory tract, which is a particularly important component of the defences.

A number of factors predispose people to respiratory infections. The most important are:

- *Young age.* Young children are much more susceptible because their immune system is not fully developed, and because they have narrower airways that are more easily obstructed.
- *Old age.* Pulmonary defences in the elderly may be compromised by: (1) reduced effectiveness of the cough reflex, due to loss of elastic recoil in the lung and reduced respiratory muscle strength; (2) a waning immune system; (3) some chronic diseases; and (4) declining mobility.
- *Cigarette smoking.* Smoking inhibits ciliary action in the respiratory tract, increasing the risk of infection and its severity.
- *Chronic obstructive pulmonary disease (COPD).* The common types of COPD are asthma, chronic bronchitis, emphysema, cystic fibrosis and bronchiectasis. Lower respiratory infection is a common complication of COPD, due to factors such as reduced mucociliary clearance and diminished effectiveness of cough reflex.

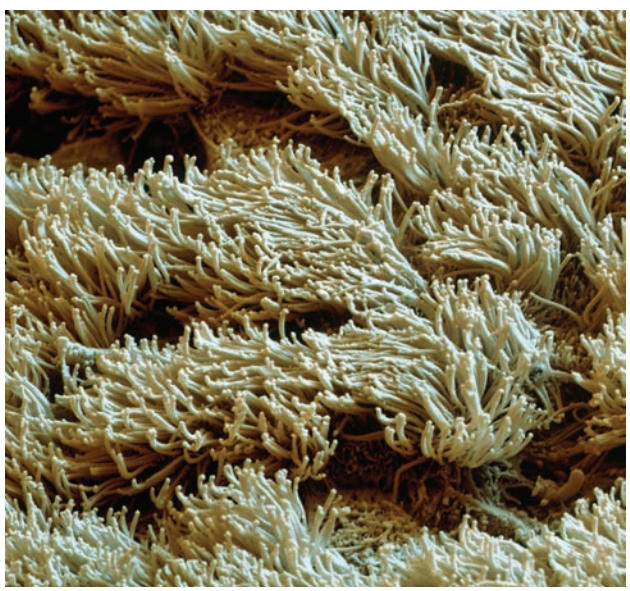


FIGURE 17.1

Cilia of the upper respiratory tract

Source: Eye of Science/Science Photo Library.

TABLE 17.2 Defences of the respiratory system

DEFENCE MECHANISM	ACTION
Normal flora of upper respiratory tract	Inhibit the establishment of potential pathogens by blocking their adherence to the mucosal surface
Nasal hairs	Trap large particles in air
Mucus secretions	Trap particles in air and prevent organisms attaching to tissue surface
Ciliated epithelium lining the larynx, trachea and bronchi	Moves mucus and particles trapped in it upwards away from the lungs, to be swallowed or expelled Together with mucus it is termed the mucociliary escalator
Non-specific antimicrobial substances in secretions (e.g. lysozyme, lactoferrin)	Destroy microbes or prevent their establishment in the respiratory tract
Secretory IgA antibodies	Present in secretions protecting against a variety of pathogens
Cough and sneeze reflexes	Expel large particles and purulent or excessive secretions from central airways and larynx
Alveolar macrophages	Phagocytosis of foreign materials and microbes that reach the lungs
Tonsils and lymph nodes	Line the respiratory tract and provide specific humoral and cellular immunity against organisms that enter the tissues

- *Poor living standards.* Low socioeconomic standards, including pre-existing illness, poor healthcare facilities and crowded living conditions, are major factors. Chronic respiratory disease is very common in the children of many rural Aboriginal communities. This, in turn, contributes to the high prevalence of chronic airway disease in the adults.
- *Alcoholism.* Alcohol intoxication interferes with virtually all respiratory tract defences, including cough reflex, ciliary motility, glottic reflex, and alveolar macrophage and NK cell activity.
- *Immunosuppression.* Immunosuppression can predispose a person to unusual, severe infections. For example, pneumocystosis (caused by *Pneumocystis jiroveci*) affects people with AIDS, but rarely causes infection in immunocompetent individuals.
- *Cancer.* Pneumonia is one of the common causes of death in patients with malignancy. Respiratory tract defences may be compromised by the malignancy itself, by associated chronic diseases, or as a result of cancer treatments that cause immunosuppression.

Acute respiratory infections are major causes of illness throughout the world. They are the most common form of illness in developed countries, the most common reason for consultation of general practitioners, and a major reason for hospital admission of children. In Australia, around 6 million visits to general practitioners a year are related to respiratory infections.

Although the respiratory tract is continuous from the mouth and nose to the lungs, it is convenient to consider it in two parts: the upper respiratory tract and the lower respiratory tract (see Figure 17.2). In this chapter, we first consider infections of the upper respiratory tract. This region is colonised by normal flora organisms and infections there are generally not life-threatening (although there are exceptions). In contrast, the lower respiratory tract, especially the lungs, is normally free of microorganisms and infections there are often serious. Pneumonia, especially in infants and the elderly, is one of the leading causes of death worldwide, and is still responsible for a large number of deaths in developed countries. While the incidence of upper respiratory tract infections is similar throughout the world, the incidence of lower respiratory infections, especially pneumonia, is at least ten times greater in undeveloped regions than in industrialised countries.

Most acute respiratory infections are caused by viruses, but the more severe diseases are generally caused by bacteria. Fungi and protozoa are important causes of pneumonia, but tend to occur mainly in immunocompromised people.

UPPER RESPIRATORY TRACT INFECTIONS

The upper respiratory tract consists of the nose, throat (pharynx), sinuses, eustachian tubes and middle ear. They are all closely interconnected, enabling infection to spread readily from one site to another. The epiglottis, larynx and trachea are also generally considered to be part of the upper respiratory tract. The majority of upper respiratory infections are due to viruses and are mostly self-limiting, mild infections. The infections of the upper respiratory tract are summarised in Table 17.3.

The common cold

The common cold is normally a mild illness, lasting about a week. It is mainly an infection and inflammation of the nasal passages (called **rhinitis**) and the oropharynx (**pharyngitis**). There are many different causes, but rhinoviruses are the most common, followed by coronaviruses. Other causes include parainfluenza viruses, respiratory syncytial

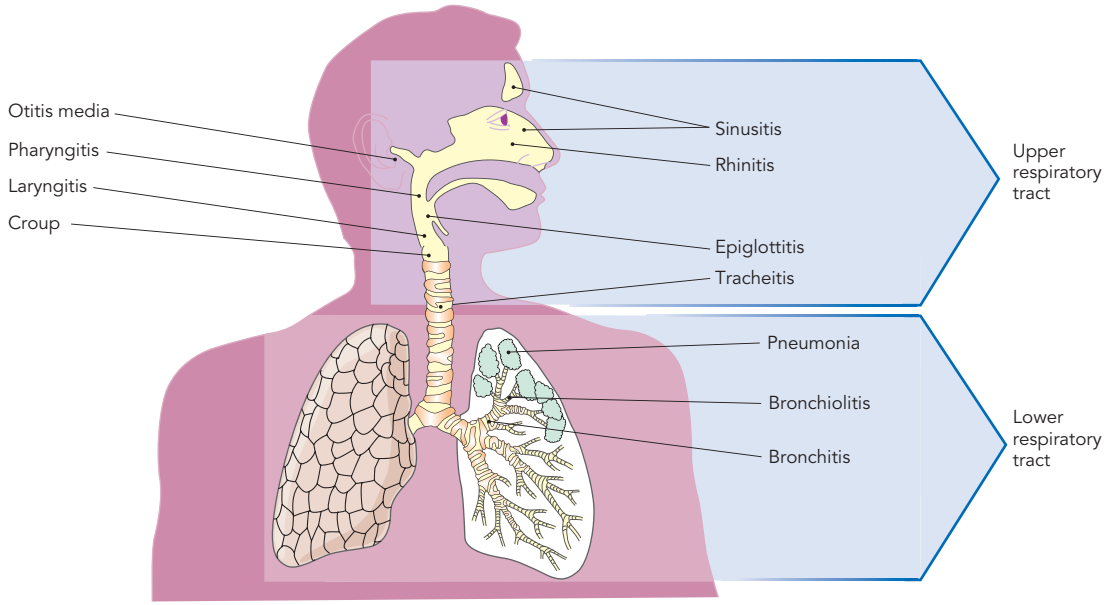


FIGURE 17.2

Possible sites of infection in the respiratory tract

TABLE 17.3 Common infections of the upper respiratory tract

DISEASE	CAUSATIVE AGENT(S)	MODES OF TRANSMISSION
Common cold	Rhinoviruses, coronaviruses, adenoviruses, plus other viruses	Respiratory secretions—airborne, via fomites
Otitis media	*Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, respiratory syncytial virus, parainfluenza and influenza viruses	Direct, as a complication of other upper respiratory infections
Acute sinusitis	As for otitis media	As for otitis media
Pharyngitis and tonsillitis	Adenoviruses, E-B virus, rhinoviruses, other viruses, *Streptococcus pyogenes*, *Neisseria gonorrhoeae*, *Candida albicans*	Respiratory secretions Genital secretions Part of normal flora, after antibiotic therapy or immunosuppression
Diphtheria	*Corynebacterium diphtheriae*	Respiratory secretions—cases and carriers
Acute epiglottitis	*Haemophilus influenzae*	Respiratory secretions—cases and carriers
Laryngotracheitis (croup)	Parainfluenza viruses, respiratory syncytial virus, influenza virus, human metapneumovirus	Respiratory secretions

virus and adenoviruses. Rhinoviruses have great antigenic diversity, with over 110 serotypes known. The large number of colds that a person can suffer is due mainly to the fact that infection with one serotype of the virus may not confer protection against another. Coronaviruses (see Figure 17.3) also show marked antigenic variation. Nevertheless, immunity to cold viruses does appear to accumulate, since colds occur most frequently in childhood and progressively less often in adulthood.

People with colds are the only reservoirs of infection, and their nasal secretions contain large numbers of virus particles. Cold viruses are transmitted by coughing and sneezing, but they are also readily transmitted by hands and fomites (e.g. door knobs, money, telephones) that have become contaminated with nasal secretions. Rhinoviruses are able to persist for some time on hands and fomites because they are resistant to drying. Touching of the nose or eyes with contaminated fingers enables the viruses to enter the body. A person starts shedding the virus within 24 hours of infection and may continue to shed viruses for some time after resolution of symptoms.

A cold is characterised by a blocked or runny nose with excessive nasal discharge and sneezing. A sore throat, headache and cough may also occur. The mild cough may

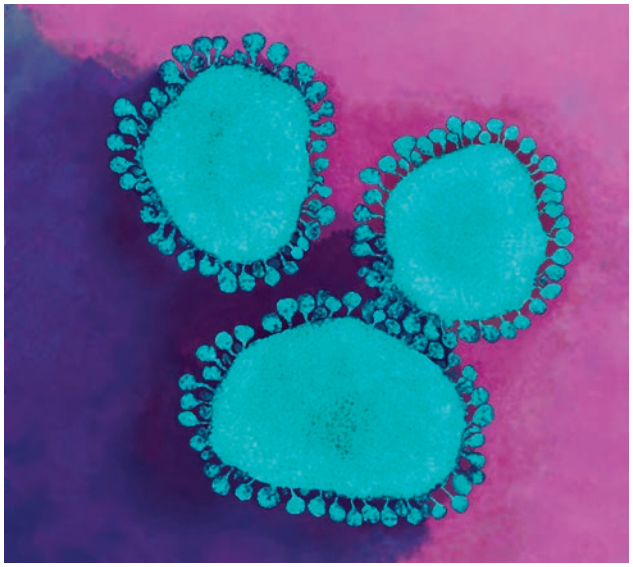


FIGURE 17.3

Coronavirus

One of the causes of the common cold.

Source: Dr Linda Stannard, UCT/Science Photo Library.

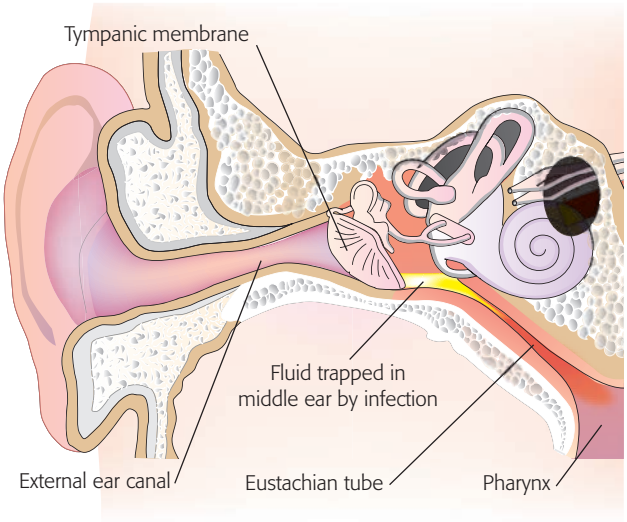


FIGURE 17.4

Middle ear infection

Accumulation of fluid due to inflammation may block the eustachian tube.

persist for a few weeks. A high fever does not occur in a cold (except if there is a secondary bacterial infection), which distinguishes it from other viral respiratory infections. The body's response to cold viruses is the major cause of cold symptoms. Rhinoviruses first attach to specific receptors on respiratory epithelial cells, invade the cells and then spread to adjacent epithelial cells. They cause little damage, but induce excessive mucus production and the other symptoms of colds by stimulating the release and local action of inflammatory mediators. Secondary bacterial infection of the paranasal sinuses and middle ear are possible complications of a cold when the sinuses or middle ear become blocked by the inflammatory exudate. Cold viruses have an important role in precipitating asthma attacks and exacerbating chronic bronchitis, especially in young children.

The common cold is usually diagnosed by clinical findings alone. Treatment with decongestants and analgesics may help to reduce symptoms. Antibiotic therapy for colds represents a misuse of these drugs, unless secondary bacterial infection is present. There is no definitive evidence that over-the-counter preparations such as vitamin C, zinc and echinacea have any clinical benefit.

Middle ear infections

Infection of the middle ear is called **otitis media**. It occurs most often in young children because of the narrow diameter and shape of their eustachian tube. Nasopharyngeal bacteria can reach the middle ear because of the anatomical connection between the sites. Blockage of the eustachian tube due to viral respiratory infection or allergy can interfere with normal mucociliary expulsion of upper respiratory bacteria (see Figure 17.4). The resultant proliferation of bacteria then causes further inflammation and blockage. Some children suffer from recurrent episodes of otitis media.

Streptococcus pneumoniae and *Haemophilus influenzae* are the most common causes of otitis media, followed by *Moraxella catarrhalis* and *Staphylococcus aureus*. Although the pneumococcal and *H. influenzae* type b vaccines have been introduced into the childhood immunisation schedule, non-vaccine serotypes continue to be responsible for infections. Viruses, including respiratory syncytial virus, parainfluenza virus and influenza viruses, also have a significant role. Chronic middle ear infections tend to be caused by organisms such as *Pseudomonas aeruginosa*, *Proteus* spp. or *S. pneumoniae*.

Some cases of otitis media are asymptomatic, but usually earache and fever are present. The eardrum becomes inflamed and swollen due to increased pressure in the middle ear. In severe cases perforation of the eardrum may occur. In some children, non-specific symptoms of fever, diarrhoea and vomiting occur. Diagnosis is usually based on the signs and symptoms in conjunction with pneumatic otoscopy. Laboratory diagnosis is not usually performed because of the pain associated with middle ear aspiration. Nasal administration of a vasoconstrictor, such as ephedrine, may be used to reduce congestion.

Antibiotic treatment of otitis media is a controversial issue. Most cases (70–90 per cent) resolve spontaneously, and research indicates that antibiotic treatment has only a slight advantage over no antibiotic therapy. Antibiotics are generally warranted if the patient is less than 6 months old, has a fever, or if symptoms persist for more than two or three days. The drugs of choice are usually amoxycillin or cefaclor, although antibiotic resistance is a growing problem. Even with appropriate therapy, fluid can persist in the middle ear (called 'glue ear'), impairing hearing for weeks or even months. Perforation of the eardrum may also lead to later problems with hearing. Impaired hearing has often led to children being wrongly labelled as inattentive or disobedient, and can lead to poor speech development.

Acute sinusitis

The pathogenesis of acute sinusitis is similar to that of acute otitis media, except that sinusitis is more common in adults than in children. The major causes of acute **sinusitis** are *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*. Viruses or environmental allergens or pollutants probably play a role in many cases of sinusitis by inducing inflammation and increased mucus production and congestion of the nasopharyngeal mucosa. Clinical features include facial pain, due to pressure caused by blockage and fluid accumulation in sinuses, a nasal discharge that is often purulent, and headache.

It may be possible to identify the causative bacteria by microscopy and culture of pus aspirated from the sinus, but sinus puncture is not often carried out. The patient is usually treated empirically with ampicillin or amoxycillin, plus an analgesic for the pain and a nasal vasoconstrictor to reduce congestion. Antibiotic resistance in the bacteria is an increasing problem.

Pharyngitis and tonsillitis

Cause, pathogenesis and clinical features

Pharyngitis, or an inflamed, sore throat, is a common condition that can be caused by many different organisms, although viruses are the usual causes (see Table 17.3). When the tonsils are affected, it is called **tonsillitis**. A sore throat may be due to infection restricted to the throat, or may be one of the early symptoms of a cold, influenza or certain systemic infections. Otitis media and sinusitis are possible complications.

Adenoviruses are particularly common causes of sore throat and may infect the conjunctiva as well, causing a syndrome known as **pharyngoconjunctival fever**. Epstein-Barr (EB) virus causes a pharyngitis as part of the syndrome called infectious mononucleosis, or glandular fever (see Chapter 19). Other viral causes of pharyngitis include rhinoviruses, coronaviruses, influenza viruses, herpes simplex virus and coxsackie A virus. Bacteria are less common causes of pharyngitis and tonsillitis, but the infections they cause can be more serious. The most important bacteria are:

- *Streptococcus pyogenes* (Group A streptococci), the cause of streptococcal sore throat
- *Corynebacterium diphtheriae*, the cause of diphtheria (see later section)
- *Haemophilus influenzae* type b, a major cause of severe epiglottitis (see later section)
- *Neisseria gonorrhoeae*, in pharyngeal gonorrhoea (see Chapter 21).

The yeast *Candida albicans* is an important cause of pharyngitis in immunosuppressed patients.

Acute pharyngitis caused by *S. pyogenes* is commonly referred to as 'strep sore throat' (see Figure 17.5). It occurs mainly in children. The oropharynx of humans is the natural reservoir of this organism. It can be found in low numbers in the throat and nose of normal people, and in large numbers in the throat of infected people. It is spread primarily by coughing and sneezing and can persist for long periods in dried mucus and dust. Although this organism causes less than 10 per cent of cases of acute pharyngitis, it is important because of the possibility of serious complications (see below). Some strains of *S. pyogenes* also produce an erythrogenic toxin, which causes the erythematous skin rash characteristic of **scarlet fever** (see Chapter 7, Figure 7.12). The toxin also causes the tongue to be reddened and enlarged, and it develops a strawberry-like appearance. A high fever is also characteristic.

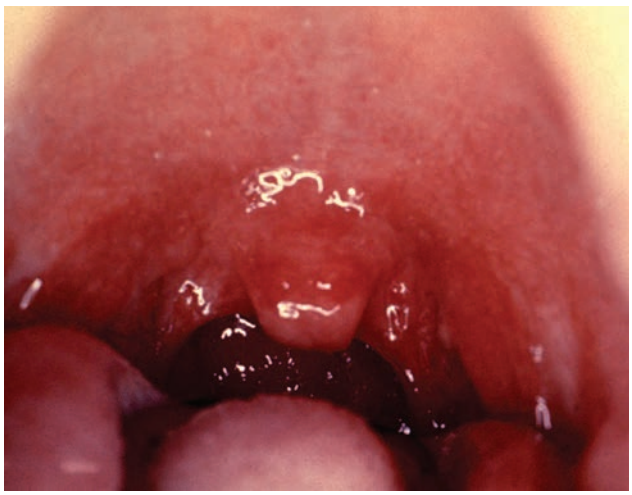


FIGURE 17.5

Streptococcal sore throat

Source: CDC.

Laboratory diagnosis and treatment

In most cases, the clinical condition of pharyngitis or tonsillitis is not serious enough to require a laboratory diagnosis and, because the vast majority are caused by viruses, no specific therapy exists. One viral infection, infectious mononucleosis, warrants identification and is diagnosed by serological means (see Chapter 19). When bacteria are suspected, a laboratory diagnosis may be warranted because of the potential seriousness and because they can be treated with antibiotic therapy. In such cases a throat swab is usually collected for culture or for rapid antigen detection tests. However, these methods have low sensitivity.

Possible sequelae of streptococcal sore throat

Two non-infectious complications of streptococcal sore throat occasionally occur. **Rheumatic fever** is a possible sequela of streptococcal sore throat in which antibodies, formed against antigens in the streptococcal cell wall, cross-react with the sarcolemma (muscle cell membrane) of human heart and other tissues. Rheumatic heart disease, representing damage to the heart valves by these antibodies, can result from repeated infections. **Acute glomerulonephritis** develops when antibodies combine with certain streptococcal antigens to form circulating immune complexes. If these complexes are deposited in glomeruli, local inflammation and kidney disease can result.

Rheumatic fever occurs in only a minority of people but is, nevertheless, a potentially serious consequence of streptococcal sore throat. It is a common disease in some rural Aboriginal communities in Australia where the rate of streptococcal infection is high. In susceptible people, each episode of untreated streptococcal sore throat will lead to an episode of rheumatic fever. Therefore, prompt diagnosis and treatment of streptococcal sore throat in individuals with a history of rheumatic fever are essential. Penicillin, amoxicillin or erythromycin is usually used for streptococcal sore throat. In severe cases, prophylactic antibiotics to prevent streptococcal infection may be warranted. Rheumatic fever is discussed in detail in Chapter 19.

In contrast, antibiotic treatment of streptococcal sore throat does not necessarily prevent post-streptococcal glomerulonephritis.

Diphtheria

Cause, pathogenesis and clinical features

Diphtheria is an acute infectious disease caused by toxin-producing strains of *Corynebacterium diphtheriae*. The infection was once a major cause of death, especially in children, throughout the world, but is now rare in developed countries due to the availability of an effective vaccine since the 1930s (see Figure 17.6). Diphtheria has been virtually eradicated in Australia, with only one case (in 2001) reported since 1992, but the possibility of resurgence exists if population immunity drops to a low level. It is still a major threat to children in developing countries where the vaccine is not readily available or administered.

C. diphtheriae are pleomorphic, Gram-positive, rod-shaped bacteria (see Figure 17.7). Avirulent strains, which do not produce the diphtheria toxin, are common colonisers of the skin and respiratory tract of humans and animals. Virulent *C. diphtheriae* produces the toxin and is usually transmitted by the airborne route from infected people or asymptomatic carriers. Once established in the respiratory tract, usually on the pharynx or larynx, the bacteria secrete the toxin that destroys the epithelial surface, forming an ulcer. A sore throat and fever are the first symptoms to develop after an incubation period of 2–5 days. The ulcer becomes covered by a greyish membrane (called a pseudomembrane), consisting of dead tissue cells, bacteria and inflammatory exudate. In severe cases the membrane can block the airway. If the larynx is infected, a life-threatening respiratory obstruction is possible.

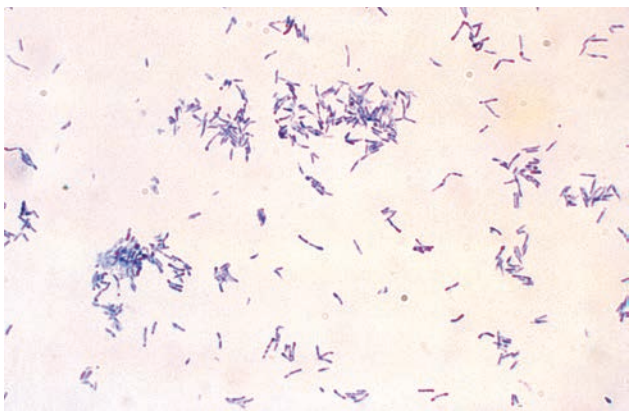


FIGURE 17.7

Gram stain of *Corynebacterium diphtheriae*

Note the pleomorphic Gram-positive rods.

Source: CDC.

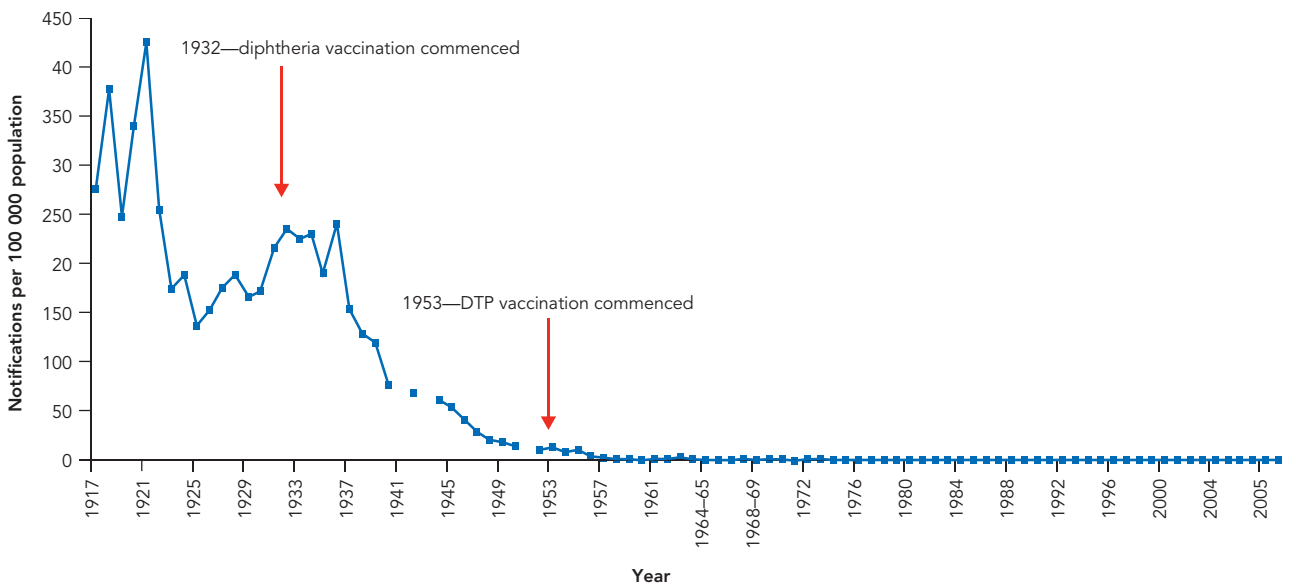


FIGURE 17.6

Annual rate of notifications of diphtheria in Australia, 1917–2005

Source: *Vaccine Preventable Diseases and Vaccination Coverage in Australia*, 2003 to 2005, 31, Graph 1 Diphtheria, 1917–2005, *Communicable Diseases Intelligence*, June 2007, s112, <www.health.gov.au/internet/main/publishing.nsf/Content/cda-cdi31suppl.htm~cda-cdi31suppl-apx1.htm>.

The bacteria multiply locally without invading deeper tissues or spreading to other sites in the body, but the toxin is absorbed into the bloodstream and then kills cells in the heart, kidneys and nervous system by interfering with protein synthesis. The toxin is highly potent—10 μg is sufficient to kill a healthy adult. Cardiac failure and death can result from the action of the toxin on the heart.

C. diphtheriae can also infect the skin, but usually only occurs in people in the tropics with poor skin hygiene. The organism infects existing skin lesions, causing poorly healing ulcerations covered by a grey membrane.

Diagnosis and treatment

Diphtheria is a life-threatening disease and has considerable public health importance. It therefore warrants urgent diagnosis and treatment. A definitive diagnosis of diphtheria is made by the isolation and identification of toxin-producing corynebacteria in a swab from under the membrane. Confirmation that the isolate is a toxigenic strain is necessary, and is performed in an immunodiffusion assay, called an Elek test. As soon as the diagnosis is suspected clinically, treatment with antitoxin, to neutralise the toxin, should be commenced. Penicillin or erythromycin is given in conjunction with antitoxin to eliminate the infection and prevent further toxin production.

Close contacts should be tested for carriage of toxigenic *C. diphtheriae* and have their immunisation status checked. Non-immunised contacts should be vaccinated and given penicillin or erythromycin as a prophylactic measure.

Prevention

Although diphtheria has almost disappeared from developed countries, the serious outbreak in Russia from 1991 to 1996, in which there were over 140 000 cases and 4000 deaths, emphasises the need for continued vigilance. The vaccine consists of formaldehyde-inactivated diphtheria toxin. It is part of the standard childhood vaccination schedule and is incorporated in the diphtheria-tetanus-pertussis (DTPa) triple vaccine, recommended at 2, 4 and 6 months of age (primary course) with a booster dose at 4 years. Further boosters with the lower-dose adolescent/adult formulation are recommended at 12–17 years and at 50 years. Travellers to high-risk countries (e.g. Russian Federation, Ukraine) should receive a booster dose if they have not had one within the last 10 years.

Acute epiglottitis

The epiglottis is a flap of cartilage that prevents food and fluids from entering the larynx. **Epiglottitis**, inflammation of the epiglottis, is most often caused by *Haemophilus influenzae* type b. In the 2–4 year-old age group it is one of the more serious types of upper respiratory infection. The organism probably spreads from an initial focus of infection in the nasopharynx. Bacteraemia is usually present and the patient is usually toxic and febrile. A swollen, red epiglottis may be seen when the child's mouth is opened.

There may be respiratory distress because of obstruction of the airway. The infected child often adopts a characteristic posture with the neck hyperextended and the chin pushed forward, to maximise airway diameter. It is a rapidly progressive disease which can result in death within a few hours due to airway obstruction. Older children and adults may be affected, especially if immunocompromised. Airway obstruction is not a prominent feature in these older groups because of their greater airway diameter.

CASE HISTORY 17.1

Epiglottitis

Timothy, a 3-year-old, had been suffering from symptoms of a cold for two days. His crying woke his parents in the middle of the night and they observed that he was feverish and having some difficulty breathing. They immediately took him to the casualty department of the local hospital.

On examination, his epiglottis was red and swollen, confirmed by X-ray of the neck. A diagnosis of epiglottitis was made and Timothy was immediately taken to theatre to have an endotracheal tube inserted. Blood cultures were taken and treatment with cefuroxime was commenced. In four days he had completely recovered.

Questions

1. Why did Timothy have an endotracheal tube inserted?
2. Why were blood cultures collected from Timothy?
3. *H. influenzae* typically causes infections in children less than 4 years old. Why is this age group so susceptible?

Young patients with epiglottitis must be treated as a medical emergency. An endotracheal tube is often inserted as a prophylactic measure and treatment with antibiotics is begun immediately. The clinical diagnosis is confirmed by isolating bacteria from blood and possibly the epiglottis. Extreme care must be taken when examining the throat, since the swollen epiglottis can be sucked into the airway and cause total obstruction. Treatment with antibiotics such as cefotaxime is usually effective.

Epiglottitis is the second most common infection caused by *H. influenzae* type b (Hib), after meningitis. Hib vaccines have been available in Australia since 1992 and have resulted in a dramatic reduction in the incidence of epiglottitis and other Hib disease in young children. (There were only nine notifications in children under 10 years in 2008.) Most cases of epiglottitis in Australia now occur in adults. Hib vaccines are discussed in more detail in Chapter 20.

Laryngotracheitis

Viral infections of the upper respiratory tract may spread downwards to involve the larynx and the trachea. **Laryngotracheitis** (inflammation of the larynx and trachea), or **croup**, is characterised by stridor (noisy respiration),

hoarseness, and a resonant cough that is often described as 'barking' or 'brassy'. These signs are due to partial obstruction of the larynx, due to oedema and spasm. In most cases, the symptoms become no more serious than this and recovery occurs within a week. However, in some children, respiratory failure can occur.

Croup is most common in children under 4 years old because of the narrowness of their larynx, and the ease with which it can be partially obstructed by inflammation. There is often a preceding cold, pharyngitis or cough. Some children suffer repeated episodes of croup, probably because they have hyperreactive airways. Laryngeal or tracheal infection in adults (**laryngitis** or **tracheitis**) causes hoarseness and a burning pain, usually without obstruction of the larynx.

Laryngotracheitis is usually caused by viruses, especially the parainfluenza group. Other less common causes include adenoviruses, respiratory syncytial virus and influenza viruses. *H. influenzae* and *C. diphtheriae* are rare causes.

The diagnosis of croup is generally based on clinical, and sometimes radiologic, examination. Patient management may involve: corticosteriod therapy (prednisolone) and adrenalin via a nebuliser for relief of symptoms, and, if serious, oxygenation to reverse hypoxaemia. Intubation or tracheostomy may be required if complete obstruction is likely. Antibiotics are administered if a bacterial cause is demonstrated.

LOWER RESPIRATORY TRACT INFECTIONS

In contrast to the upper respiratory tract, the lower respiratory tract is normally sterile, and infections involving it are potentially more serious. Lower respiratory tract infections include a spectrum of diseases ranging from acute bronchitis to pneumonia. The major syndromes discussed in this section are whooping cough, bronchitis, bronchiolitis, influenza and pneumonia. Chronic infections of the lower respiratory tract are dealt with later in this chapter.

Pathogenic microorganisms may reach the lower respiratory tract in a number of ways:

- inhalation of organisms suspended in air (aerosols or dust)
- aspiration of oropharyngeal contents (sometimes gastrointestinal contents) when asleep, intoxicated or unconscious
- spread of infection from the upper respiratory tract
- via the bloodstream, from a primary site of infection elsewhere in the body.

Pertussis

Causative agent and pathogenesis

Pertussis (**whooping cough**) is a severe, sometimes fatal, disease caused by the Gram-negative coccobacillus, *Bordetella pertussis*. It is a highly contagious disease, spread from person to person by respiratory droplets. Its incidence in developed countries has been markedly reduced through immunisation, but it is nevertheless the most common vaccine-preventable disease in developed countries, including Australia. In developing countries where immunisation is lacking, infection rates are high.

Despite the availability of a vaccine, epidemics tend to occur every 3–4 years (see Figure 17.8). In 2008 over 14 500 cases were notified, marking the beginning of an epidemic period that peaked in early 2009. Because of childhood and adolescent vaccination, a significant proportion of notifications now occurs in adults over the age of 30.

B. pertussis has specific adhesins that allow it to attach to the ciliated respiratory mucosa. It multiplies in the epithelial cells but does not invade further. The organism produces several toxins, which combine to produce the symptoms of disease. The pertussis toxin appears to be partly responsible for adhesion of the bacteria to the tracheal epithelium and the increase in respiratory secretions and mucus production that is characteristic of whooping cough.

Adenylate cyclase toxin inhibits vital functions of neutrophils, such as chemotaxis, phagocytosis and killing activity, and may also be involved in inducing the inflammatory response. Tracheal cytotoxin is a cell wall component that specifically kills tracheal epithelial cells, thereby partially immobilising the mucociliary escalator and causing an accumulation of mucus in the airway. No exact role for pertussis endotoxin has been established but, like the endotoxin of other Gram-negatives, it is likely to be involved in the production of fever and inflammation.

The major activities of *B. pertussis* toxins are illustrated in Figure 17.9.

Clinical features

After an incubation period of 7–20 days, there is an initial illness that resembles a cold, characterised by runny nose, cough and mild fever. This is the most infectious period. The cough, caused by an accumulation of mucus in the airway, gradually becomes more irritating and paroxysmal (abrupt,

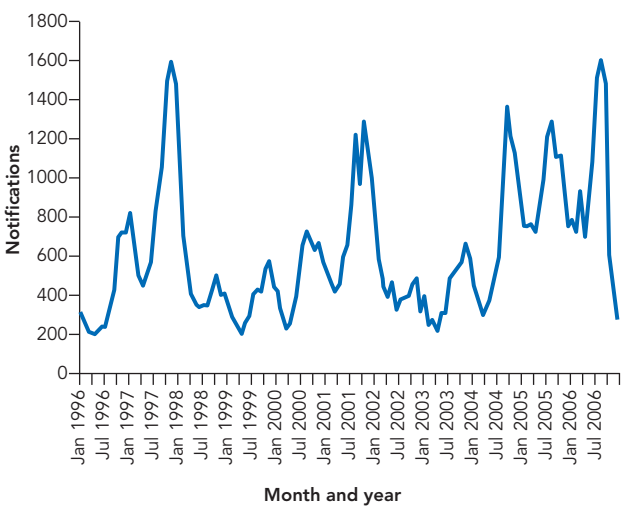


FIGURE 17.8

Notifications of pertussis in Australia, 1996–2006, by month of onset

Source: K. Begg et al. *Communicable Diseases Intelligence* 2008, 32(2): 179.

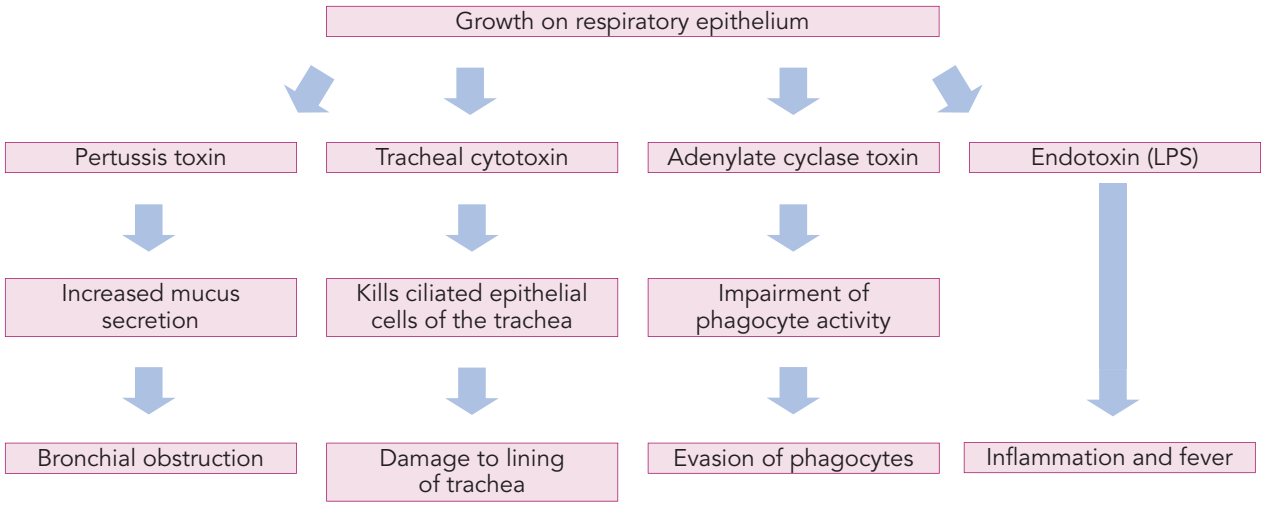


FIGURE 17.9

Mechanisms of action of the toxins of *Bordetella pertussis*

short attacks) over the next 1–2 weeks. A paroxysm is characterised by a series of short coughs followed by a 'whoop'—the characteristic sound produced by a gasp of air at the end of a coughing episode. The whoop represents the rush of air through the narrowed airway. The characteristic whoop may be absent in some patients, particularly in very young infants, older children and adults, in whom the infection is often mistaken for other conditions such as a cold, influenza or asthma. The cough may persist for up to three months. A person with pertussis is infectious for up to three weeks from the onset of illness.

Possible complications include cerebral hypoxia, which can result in brain damage, bronchopneumonia and secondary pneumonia due to invasion of the damaged respiratory tract by other bacteria (e.g. *Staphylococcus aureus*). Death, due to hypoxia, occurs in up to 3.5 per cent of infected babies under 6 months of age. Infection in adults is often asymptomatic or mild. Adults can become infected if never immunised, or once their vaccine-induced immunity has waned.

Diagnosis

B. pertussis is extremely fastidious and difficult to culture. PCR testing on a nasopharyngeal swab or aspirate is the gold standard laboratory method for diagnosis of whooping cough, although its sensitivity declines over the course of the illness. Serological testing by enzyme-linked immunosorbent assay (ELISA) is also useful.

Treatment

Supportive care, including oxygen and suctioning, is of prime importance. Antibiotics do not appear to reduce the severity or duration of infection. However, erythromycin or azithromycin reduce the numbers of organisms in the throat and are thus useful in helping to control the spread of infection. Erythromycin prophylaxis of close contacts of active cases may prevent infection or reduce the severity of subsequent infection. Infants are very susceptible prior to immunisation.

Prevention

The National Health and Medical Research Council of Australia recommends immunisation against pertussis from 2 months of age, unless there are contraindications. The primary course consists of three doses, two months apart. Booster doses are recommended at 4 years and again at 12–17 years of age. A further booster is recommended for adults planning a pregnancy and their other adult household members, grandparents and carers of young children, all healthcare workers, and people over the age of 50 years. Pertussis vaccine is usually combined with diphtheria and tetanus toxoids and administered as DTPa (called **triple antigen**) as part of the standard childhood immunisation schedule, or as dTpa (lower content of diphtheria and pertussis antigens) for adolescents and adults. Pertussis vaccine has been very effective in reducing the number of infections (up to 90 per cent reduction) in countries where uptake has been high.

Acute bronchitis

Acute bronchitis represents an inflammation of the tracheobronchial tree, and is usually a result of the spread of an infection from the upper respiratory tract into the bronchi. Viruses are the most common causes, including influenza viruses, respiratory syncytial virus, human metapneumovirus, common cold viruses and adenoviruses. *Mycoplasma pneumoniae* can also be responsible. A dry or slightly productive cough lasting 1–3 weeks is the major feature. Treatment is mainly symptomatic. In otherwise healthy individuals, there is usually a speedy recovery with few sequelae. Antibiotics are warranted only when secondary bacterial infection occurs. Chronic bronchitis is a condition which results from a combination of factors, with cigarette smoking the predominant cause. Bacteria such as *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* may be involved in acute exacerbations. Spread of bacteria into

CASE HISTORY 17.2

Whooping cough (pertussis)

In July 2004, six infants diagnosed with pertussis had all been born during the period 4–16 June at the same hospital. It was determined that an outbreak of pertussis had occurred among 11 newborn babies after direct exposure to a healthcare worker (HCW) with the disease. Not realising her cough was pertussis, the HCW had had symptoms while working from mid-June until mid-July. Now aged 24 years, she had been fully vaccinated for pertussis as a child. On 17 July, she was given leave for five days and treated with erythromycin.

During the period that the HCW exhibited symptoms, she had cared for 113 infants. Given that 11 were subsequently diagnosed with pertussis, this represents an attack rate of 9.7 per cent.

Source: Adapted from J.L. Hood, D.K. Murphey and J.J. Dunn 2008, Hospital-acquired pertussis among newborns—Texas, 2004. *Morbidity and Mortality Weekly Report*, 6 June, 57(22): 600–03.

Questions

1. Why is pertussis so easily transmitted from person to person?
2. Why did so many babies become infected?
3. How could this outbreak have been prevented?
4. Does pertussis pose a risk for a hospital physiotherapist planning to become pregnant?

the lungs can result in pneumonia. Antibiotics are used for the treatment of associated bacterial infections.

Bronchiolitis

Cause and epidemiology

Bronchiolitis is predominantly a disease of childhood, especially in children less than 2 years old, although it can occur at any age. The bronchioles of a young child are narrow and, if their lining cells are swollen by inflammation, the passage of air to and from the alveoli may be severely restricted. Infection results in necrosis of the epithelial cells that line the bronchioles and the infection may spread into the lung tissue and cause pneumonia. Most of these infections are caused by respiratory syncytial virus (RSV), although other viruses (e.g. human metapneumovirus, influenza and parainfluenza viruses) and *Mycoplasma pneumoniae* are occasionally responsible.

RSV is highly infectious and can cause community and hospital outbreaks. It is a frequent cause of lower respiratory tract infections in infants, but rarely produces significant illness in adult contacts. Between 50 and 90 per cent of hospital admissions for bronchiolitis are due to RSV infection. Infection is mainly transmitted by droplets, but also via hands. The only known reservoir is infected humans.

Clinical features

In older children and adults, RSV is restricted to the upper respiratory tract, causing a mild, cold-like illness. However, infection in infants can be severe if the virus invades the lower respiratory tract. Typical symptoms include fever, coryza (symptoms resembling a head cold), a cough and respiratory obstruction resembling asthma. The latter may lead to respiratory distress and cyanosis. Viral pneumonia can develop in some patients. Most cases begin to resolve within a few days, but some children may continue to show depressed pulmonary function or wheezing up to a year or two later. Recurrent infections are common, although they are generally less severe.

Diagnosis and treatment

The diagnosis of bronchiolitis is based mainly on clinical appraisal. RSV-specific antigens in nasopharyngeal washings are detectable by immunofluorescence or ELISA. As bronchiolitis is caused mainly by viruses, treatment options are limited and, once symptoms have developed fully, viral replication and load have already begun to decline. The major concerns in the management of a patient with bronchiolitis are the maintenance of adequate hydration and supplementary oxygen if necessary.

Influenza

Seasonal influenza virus infections cause worldwide epidemics annually, resulting in millions of infections with significant morbidity, mortality and economic burden. Yearly epidemics are estimated to cause up to 500 000 deaths globally.

Causative agents

The causative agents are the influenza viruses (orthomyxoviruses), shown in Figure 17.10. Influenza A viruses can infect a variety of animals and birds as well as humans, and cause epidemics and occasionally pandemics; influenza B viruses infect only humans and can cause epidemics but not pandemics.

The envelope of an influenza virus has two surface glycoprotein antigens that are used to classify the virus types: the haemagglutinin (H) and neuraminidase (N) spikes (see Figure 17.11). The H protein is used for attachment of the virus to the host cell, and the N protein facilitates spread of the virus progeny. Currently, there are 16 H and nine N subtypes. Influenza viruses are named according to their H and N antigens—for example, H1N1, H2N3 or H5N1.

Protective antibodies are made to both H and N antigens in infected people, but influenza viruses can vary these antigens over time by *antigenic drift* and *antigenic shift* (see Chapter 10). Antigenic drift represents small, constantly occurring mutations, affecting the H and N antigens. If the changes occur in areas where antibodies bind to the antigens, the antibodies may not bind as well, allowing the virus to reinfect communities repeatedly over time, even though the individuals have immunity from previous flu infections. Thus, antigenic drift is responsible for the generation of the new strains of virus that cause seasonal influenza epidemics.

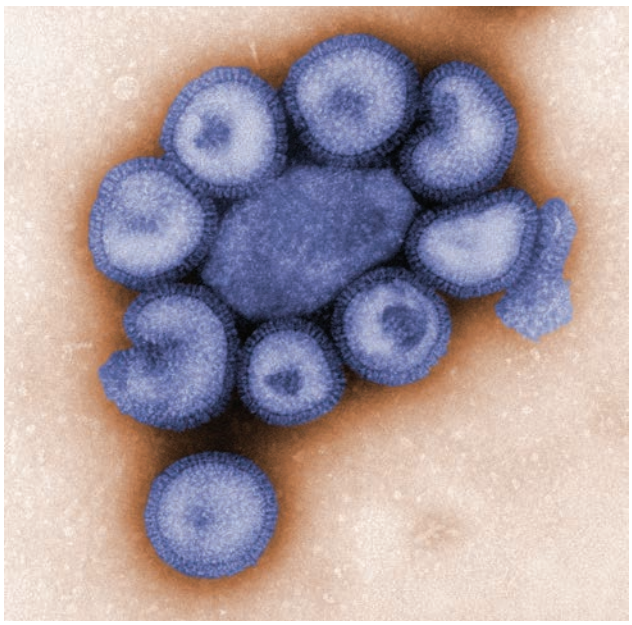


FIGURE 17.10

Electron micrograph of the influenza virus

Source: CDC/Dr F.A. Murphy.

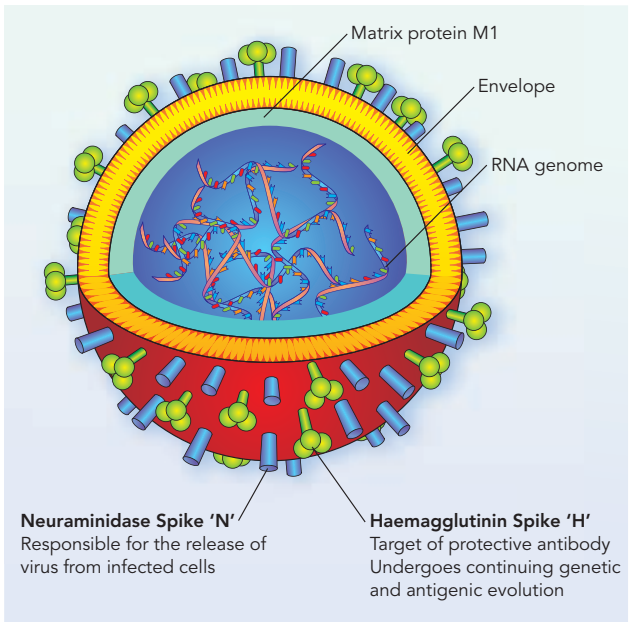


FIGURE 17.11

The spikes of the influenza A virus

Antigenic shift is a sudden, major change in the H or N antigens, based on a reassortment or mixing of genes from different virus strains infecting a single host. The new strain that develops is due to a mixing of genes from an animal and/or avian virus with a human virus. This results in completely different antigen structures, enabling the new strain to spread through populations in pandemic form. Only type A strains undergo antigenic shift.

Antigenic shift has resulted in five major pandemics: H1N1 Spanish flu (which killed 20–50 million people worldwide) in 1918; H2N2 Asian flu in 1957 (an estimated 2 million deaths); H3N2 Hong Kong flu in 1968 (an estimated 1 million deaths); H1N1 Russian flu in 1977; and the 2009 pandemic H1N1 flu ('swine flu') (see Spotlight box: The 2009 pandemic H1N1 influenza). These pandemics are believed to have been caused by viruses that had mixtures of genes from a human influenza virus and an avian influenza virus, plus genes from swine influenza virus in the case of the 2009 pandemic.

Transmission of influenza is by droplet inhalation and fomites, and infectivity can be very high. The viruses can survive on surfaces and on cloth and tissues for many hours. Influenza occurs throughout the world, typically during the coldest months of the year. This is largely because people spend more time inside buildings during cold weather and this favours virus transmission via aerosols and fomites. Outbreaks have occurred in healthcare settings, and healthcare workers are often involved, in terms of acquiring and transmitting infection.

Pathogenesis and clinical features

The virus enters the respiratory tract via droplets and attaches to receptors on respiratory epithelial cells via the H spikes on its envelope. One to three days after infection, symptoms such as fever, chills, malaise and muscle pain develop, due mainly to cytokines liberated from damaged cells and infiltrating leucocytes. As the virus spreads, symptoms of runny nose, sore throat and dry cough may occur. Subclinical or mild infections resembling a cold are common. Recovery usually occurs within 1–3 weeks, although the infection can be severe enough to develop into bronchitis or pneumonia. Viral shedding begins before the appearance of symptoms, and usually persists for about a week.

Mortality is highest in the elderly and the debilitated, especially in developing countries, and is usually due to secondary bacterial pneumonia or exacerbation of chronic cardiac or respiratory disease.

Diagnosis

A specific diagnosis can be made by serological means or by isolating the virus from nasopharyngeal aspirate or swab or sputum. Rapid diagnostic tests that detect viral antigen in respiratory samples and PCR tests have recently been developed. However, in most cases, these methods are impractical and unnecessary for an individual patient, but are important for public health authorities when following infection with a new virus strain.

Treatment and prevention

Rest, fluids and analgesics form the basis of treatment, and recovery is usually within a week. Antibiotics are required if secondary bacterial infection occurs. Amantadine and rimantadine have been used prophylactically to limit outbreaks in elderly, non-immunised residents of institutions such as nursing homes. However, these drugs are effective only

against influenza A and can cause neurological side effects, and viruses seem to become resistant to them fairly easily.

Zanamivir (Relenza®) and oseltamivir (Tamiflu®) are related drugs that are used to treat influenza caused by either influenza A or B viruses. They act by inhibiting viral neuraminidase, thus blocking the release of newly formed viruses from infected cells. There is some concern regarding the development of resistance to oseltamivir in some influenza viruses.

When given before the epidemic season arrives, influenza virus vaccine offers partial protection, but only against those strains covered by the vaccine. The vaccines are made from inactivated viruses and are multivalent, directed against several strains of the virus. The composition of annual vaccines in Australia is decided by the Australian Influenza Vaccine Committee and is based on the anticipated spread of active influenza A and B viruses, according to worldwide surveillance coordinated by the World Health Organization (WHO).

Vaccination is recommended for high-risk individuals such as the elderly and debilitated (e.g. residents of nursing homes and chronic care facilities), and people with chronic cardiopulmonary disease. Vaccination is also strongly recommended for healthcare workers. Because of antigenic drift and the short duration of protection afforded by the vaccine, annual immunisation is necessary. Efforts are

SPOTLIGHT ON

The 2009 pandemic H1N1 influenza

Cases of an atypical influenza-like illness were first reported in Mexico on 18 March 2009. The outbreak that ensued was confirmed as H1N1 influenza A, and the causative agent was subsequently referred to as '2009 pandemic H1N1 virus'. It was originally called 'swine flu virus' because it was thought to be predominantly a pig virus that had jumped to humans. It was ultimately determined to be a re-assortment virus comprising genes from swine, avian and human influenza viruses. On 11 June 2009, the WHO announced a global pandemic, because of spread infection beyond North America to other regions, including Australia. The first case in Australia, a traveller returning from the United States, was identified in early May 2009.

Between April 2009 and August 2010 nearly all countries in the world reported cases of H1N1 virus infection. Hundreds of millions of people were infected worldwide, with around 18 500 deaths. The WHO declared on 10 August 2010 that the pandemic was over, although the virus continued to circulate in some parts of the world as a seasonal virus.

Transmission of the virus appeared to be similar to that of seasonal flu viruses. It was spread by infected humans, not by pigs. Although this virus was initially thought to cause severe disease, the clinical manifestations were similar for the pandemic H1N1 strain as for other seasonal influenza A strains. However, whereas severe cases of seasonal influenza usually occur in frail, elderly people, greatest morbidity in pandemic H1N1 occurred in young children, people with underlying, chronic conditions, such as obesity, asthma and diabetes, and in Indigenous Australians. Pregnant women were identified as being at increased risk of severe infection. Bacterial co-infection, usually a pneumonia caused by *S. pneumoniae*, *S. aureus* or *H. influenzae*, was a major factor in many people who died of pandemic H1N1 flu.

Treatment is similar to that of seasonal flu—largely supportive, consisting of bedrest, increased fluid consumption, cough suppressants, and antipyretics and analgesics for fever and myalgia. Severe cases may require intravenous hydration and other supportive measures. During the pandemic the WHO recommended treating serious cases with oseltamivir or zanamivir. Oseltamivir, when given within 48 hours of the onset of symptoms, significantly decreased the risk of pneumonia and the need for hospitalisation. It was also recommended for patients with underlying medical conditions that increase the risk of severe disease. During the pandemic, oseltamivir-resistant strains were observed in a small number of patients; mostly in severely immunocompromised patients with prior exposure to oseltamivir.

Vaccines against pandemic H1N1 virus began to be available in September 2009, initially as a monovalent vaccine, and ultimately incorporated into trivalent seasonal vaccines in many countries. These vaccines provided significant protection against the infection.

Although there were grave concerns initially concerning the potential seriousness of the 2009 H1N1 pandemic, it was generally mild. Three factors are considered to have been responsible for this:

- The virus did not mutate to a more lethal form.
- Development of resistance to oseltamivir was low.
- The vaccine was effective and remarkably safe.

In addition, several years of international efforts aimed at controlling a potential avian flu pandemic facilitated the prompt detection, surveillance and management of the pandemic H1N1 strain when it appeared.

currently aimed at the development of a universal influenza vaccine, which would protect against all flu virus strains, and hence would not require annual renewal.

Avian influenza

Avian influenza is an infectious disease of birds caused by influenza virus type A strains. The natural reservoir of avian influenza viruses is wild aquatic birds. Given that these birds are migratory, they have the potential to carry viruses over great distances. Until recently, avian influenza viruses were considered to be a rare cause of infection in humans because of difficulties in crossing species barriers. However, avian influenza H5N1 has proven to be an exception, causing infection in humans and a variety of animals, including domestic cats and dogs. H5N1 is regarded as a high pathogenic avian influenza (HPAI) because it is associated with severe disease and mortality in wild birds and domestic poultry.

HPAI H5N1 was first isolated in 1996 from a farmed goose in Guangdong province, China. Soon after, outbreaks of H5N1 were reported in poultry farms and live animal markets in Hong Kong. In 1997, 18 people became infected with the virus, resulting in six deaths. H5N1 was increasingly being associated with diseased chickens, resulting in the total culling of all commercial poultry flocks in Hong Kong. This strategy worked well for several years, until in February 2003 two human cases of infection were confirmed in a Hong Kong family that had recently travelled to China.

Virtually all species of birds appear to be susceptible to avian influenza virus, with poultry among the most vulnerable. From late 2003, H5N1 spread to birds in many parts of the world. To date, outbreaks of infection have occurred in over 60 countries and over 400 million birds have died as a result of disease or culling. The virus has broadened its host range in birds and mammals, and as of October 2011 had caused over 560 human infections in 15 countries with a fatality rate of over 60 per cent (see Table 17.4). The exceptional virulence of this virus, together with its ongoing genetic evolution in birds, identifies it as a significant pandemic threat. The global trade in wildlife provides additional disease transmission opportunities for outbreaks in birds, humans, livestock and native animals.

Most human infections with H5N1 have been associated with the handling of sick or dead infected poultry. Although there appears to have been a few cases resulting from human-to-human spread, this mode of transmission is at present limited. Most people with H5N1 influenza present with symptoms of fever, cough and shortness of breath, and radiological evidence of pneumonia. Besides respiratory symptoms, many patients complain of gastrointestinal symptoms such as diarrhoea, vomiting and abdominal pain. In severe cases, H5N1 induces the release of excessive amounts of pro-inflammatory cytokines, leading to a cytokine storm in the lung, which can cause tissue necrosis and haemorrhage. Complications include acute respiratory distress syndrome, renal dysfunction and multi-organ failure. The primary cause of death is usually a fulminant pneumonia. If H5N1 mutates into a virus capable of transmission between humans, it is likely to spread very rapidly throughout the world and perhaps result in millions of deaths, similar to the influenza pandemic of 1918.

TABLE 17.4 **Cumulative number of confirmed cases of avian influenza (H5N1) reported to WHO to 10 October 2011**

	TOTAL 2003–11	
COUNTRY	CASES	DEATHS
Azerbaijan	8	5
Bangladesh	3	0
Cambodia	18	16
China	40	26
Djibouti	1	0
Egypt	151	52
Indonesia	179	147
Iraq	3	2
Lao People's Democratic Republic	2	2
Myanmar	1	0
Nigeria	1	1
Pakistan	3	1
Thailand	25	17
Turkey	12	4
Vietnam	119	59
Total	**566**	**332**

Source: Adapted from WHO GIP data: <www.who.int/influenza/human_animal_interface/EN_GIP_20111010CumulativeNumberH5N1cases.pdf>.

The WHO recommends the use of oseltamivir (Tamiflu®) for treatment of H5N1 infections. The demonstration of low-level oseltamivir resistance in a few cases is a concern.

Pneumonia

Pneumonia is an infection and inflammation of the lungs. It is a major cause of death throughout the world, especially in the elderly, and in children and the malnourished living in crowded and unhygienic conditions. It is one of the most life-threatening types of infection in the immunocompromised. Worldwide, as many as 5 million people are thought to die each year from pneumonia.

A wide variety of microorganisms are capable of causing pneumonia. Bacteria and viruses cause most cases, but fungi (e.g. *Aspergillus* and *Pneumocystis jiroveci*) can also be responsible, especially in the immunocompromised. Pneumonia is often classified as community-acquired or hospital-acquired, because the latter are usually more difficult to treat and are therefore associated with greater morbidity and mortality. The common causes of community-acquired infections are somewhat different from those that cause hospital infections (see Table 17.5). The cause also depends on a number of risk factors such as age, underlying disease, and particular

CASE HISTORY 17.3

H1N1 influenza

On 1 June 2009, a 79-year-old man went to the emergency department of a Melbourne hospital after having been ill for a week with dyspnoea and a productive cough. His relevant underlying conditions were chronic obstructive airways disease and type 2 diabetes mellitus. He was admitted, and from a nasal swab was diagnosed, two days later, as having pandemic (H1N1) 2009 influenza. He was then moved from the four-bed ward he was in from admission to a single room, commenced on oseltamivir, and droplet precautions were instituted.

Twenty-one people in the hospital who had had high-risk contact with the man (spending more than 15 minutes within 1 metre of the patient without wearing appropriate personal protective equipment) were identified, comprising nine medical staff, six nurses, three allied health staff and three patients. All were given oseltamivir prophylaxis. Three of the medical staff reported the onset of an influenza-like illness (ILI) within the previous two days. They were therefore given treatment doses of oseltamivir.

It was assumed that the three medical staff with ILI had pandemic 2009 influenza and a second round of contact tracing was initiated. A further 17 medical staff and seven patients were identified and received oseltamivir prophylaxis.

Four days later, the swab results from the three symptomatic medical staff showed they were negative for influenza A. None of the other 18 people who had significant contact with the index patient developed ILI.

Source: Adapted from U. Devi and K.L. Buising 2010, Infection control of pandemic (H1N1) 2009 influenza in hospitals—a logistic challenge. *Medical Journal of Australia* 192(3): 164–65.

Questions

1. What does this case illustrate about the potential for spread of respiratory infections in a hospital?
2. Why were the staff members not offered vaccination against the pandemic influenza strain?
3. What are the possible explanations for the non-infection of the people who had significant contact with the index patient?

TABLE 17.5 Common causes of pneumonia: community-acquired versus hospital-acquired infections

COMMUNITY-ACQUIRED INFECTION		HOSPITAL-ACQUIRED INFECTION	
Organism	% of cases	Organism	% of cases
Streptococcus pneumoniae	15–40	*Pseudomonas aeruginosa*	15–30
Respiratory syncytial virus	10–40	*Staphylococcus aureus*	10–30
Mycoplasma pneumoniae	10–40	*Klebsiella* species	5–15
Haemophilus influenzae	5–10	*Acinetobacter* species	5–15
Chlamydophila pneumoniae	5–10	*Escherichia coli*	5–15
Enteric Gram-negative bacteria	5–10	*Enterobacter* species	5–10
Influenza viruses	5–10	*Proteus* species	5
Legionella species	5	*Streptococcus pneumoniae*	< 5
Staphylococcus aureus	< 5	*Candida* species	< 5
Moraxella catarrhalis	< 5	*Legionella* species	< 5
Parainfluenza virus	< 5	*Moraxella catarrhalis*	< 5

exposure to pathogens through occupation, travel or contact with animals.

Pneumonia is the most commonly fatal type of hospital-associated infection. As many as 15 per cent of all deaths in hospitals may be attributed to pneumonia acquired in hospital. Most of these occur in postsurgical patients and patients in intensive care with acute respiratory failure and who are receiving mechanical ventilation. Ventilator-associated pneumonia (VAP) occurs in around 20 per cent of mechanically ventilated patients. These patients are more susceptible because they are usually critically ill and have multiple risk factors. Also, an endotracheal tube negates

normal defence mechanisms, such as mucociliary clearance, provides an unnatural connection between the upper and lower airways, and can become colonised with potential pathogens. Early onset VAP tends to be caused by community type pathogens, whereas late onset VAP (>5 days) tends to be caused by hospital types.

Clinical features

Patients with pneumonia usually present with a fever, cough and chest pain. The cough is generally unproductive at first, but later may become purulent and sometimes blood-stained. As the disease progresses, the patient experiences increasing breathlessness and shallow, rapid breathing. Inspiratory rales (crackling sounds) are usually heard over the affected area of the lung. Some infections result in symptoms confined mainly to the chest, whereas others, such as Legionnaires' disease, have a wider systemic involvement, and the patient may present with mental confusion, diarrhoea, and evidence of renal or liver dysfunction.

A chest X-ray confirms the presence and distribution of shadows (consolidation) in the lung. Severe pneumonia can quickly lead to respiratory failure, requiring ventilation and intensive care. Antibiotics reduce the mortality rate, but deaths still occur despite appropriate treatment.

Complications of infection include spread of the infecting organisms directly to extrapulmonary sites such as the pleural space, giving rise to empyema, or indirectly via the bloodstream to other parts of the body.

Pathogenesis

Microorganisms may reach the lungs by inhalation of aerosolised particles or by the downward movement of mucus, which carries normal flora organisms or pathogens from the upper respiratory tract. Micro-aspiration of oropharyngeal and gastric secretions may also occur, especially in the critically ill, the elderly, stroke victims, and in people with an altered mental state (e.g. following seizure). The mucociliary escalator normally functions to move mucus and the foreign material trapped in it upwards away from the lungs. But this function can be impaired in people with an upper respiratory viral infection, and in those with damaged cilia due to inhalation of pollutants (e.g. cigarette smoke), alcoholism, or the presence of a tumour or foreign body. Sometimes, the lungs become infected by organisms that have spread, via the bloodstream, from another infected site in the body.

In response to infection or the action of microbial toxins, inflammation occurs, resulting in the filling of the affected alveoli—first with a serous exudate and then later with pus cells and sometimes red blood cells. Once the alveoli fill with infectious exudate, an overflow of the fluid occurs and there is a progressive spread of infection to adjacent alveoli.

Pneumonia has traditionally been classified into types according to differences in clinical signs and radiological findings. **Lobar pneumonia** is an infection and consolidation (solidification of the tissue due to inflammatory exudate in the alveoli) confined to one or two lobes of the lung. Most cases are caused by *Streptococcus pneumoniae*. **Bronchopneumonia** is a more diffuse, patchy inflammation of the lungs with numerous small foci of consolidation occurring throughout the lungs. A large variety of bacteria such as *S. pneumoniae*, *Staphylococcus aureus*, *Mycobacterium tuberculosis* and *Haemophilus influenzae*, or viruses, can be responsible.

Outcomes common to both types of pneumonia are respiratory distress resulting from the interference with air exchange in the lungs, and systemic effects typical of any severe infection. A **pleural effusion** occurs if the inflammatory exudate enters the space between the lung tissue and pleural membranes. **Empyema** is the term used to describe infection of the pleural space, and **pleurisy** means an inflammation of the pleural membranes.

Diagnosis

Usually, a chest X-ray is performed when pneumonia is suspected. This may indicate that infection and inflammation exist, but laboratory investigations are required to identify the causative organism. Making a specific laboratory diagnosis of pneumonia is often a problem, because of the variety of causes. Blood and sputum culture are the main methods of diagnosing bacterial pneumonia, although they are not especially sensitive methods. More invasive techniques, such as transtracheal aspiration, bronchoscopy and bronchoalveolar lavage, and lung biopsy, may yield more useful results. Specimens should be collected before antibiotic therapy is started, if possible.

Sputum samples are best collected in the morning, because sputum tends to accumulate while the patient is lying in bed, and before breakfast, to reduce contamination by food particles and bacteria from food. It is critical that the specimen collected is truly sputum with minimal oropharyngeal contamination, since some oropharyngeal flora (e.g. *S. pneumoniae* and *H. influenzae*) are potential causes of pneumonia and may be incorrectly identified as the responsible agent.

Antigen detection tests have been developed for some pathogens, most notably *S. pneumoniae* and *Legionella*. Multiplex PCR, which can detect multiple pathogens in a single test, and other molecular methods are being developed and becoming more widely used for diagnosis of pneumonia. These methods potentially have greater sensitivity, a rapid turnaround time, and can detect organisms that are difficult to culture.

Bacterial causes

Pneumococcal pneumonia. The most common bacterial cause (approximately 30 per cent of cases) of community-acquired pneumonia is *Streptococcus pneumoniae* (the 'pneumococcus'), a normal inhabitant of the upper respiratory tract of some people. It is also one of the common causes of early onset VAP in hospitalised patients. There are over 90 different serotypes of pneumococcus, but only about 30 of these are known to cause disease in humans. Although pneumococcal vaccines have been available since 2001 (see

later), they cover only some of the serotypes, albeit the most common ones. As a result, the prevalence of infection with non-vaccine serotypes has increased, and has partly offset the reductions in infections due to vaccine serotypes. The emergence of non-vaccine serotype 19A is of particular importance, since it has a worldwide prevalence and is multi-drug resistant.

The virulence of pneumococcus is primarily due to an extracellular polysaccharide capsule, which makes it resistant to phagocytosis (see Chapter 4) and aids in colonisation. However, the organism also produces an exotoxin, called pneumolysin, which appears to have a number of effects, including impairment of mucociliary clearance, plus the bacterium has a number of surface proteins that facilitate its adherence to respiratory epithelium.

Examination of a Gram-stained smear of sputum can give a quick, presumptive diagnosis of pneumococcal pneumonia if it demonstrates polymorphs and Gram-positive diplococci (see Figure 17.12). Detection of pneumococcal C-polysaccharide antigen by agglutination of antibody-coated latex particles can be used both on sputum and urine specimens. (The antigen is excreted in the urine.) Use of this rapid technique can provide a result within an hour of receipt of the specimen, but false positives occur and antibiotic susceptibility tests cannot be performed unless the organisms are isolated.

First-line treatment of community-acquired pneumonia before results are available is usually with amoxycillin and/or doxycycline or clarithromycin, but treatment failure is possible because of the wide range of potential causes and their differing drug sensitivities. Increasing resistance of *S. pneumoniae* to a variety of drugs, including penicillin, is of great concern. Amoxycillin is currently the drug of choice for pneumococcal pneumonia. The patient with pneumonia may also require oxygen, and physiotherapy should be given if there is significant sputum. In the severely ill, direct suction with an endotracheal tube may be necessary. Even with appropriate antibiotic therapy, pneumonia can be fatal, usually because of rapidly progressive disease or because of its interaction with some other underlying disorder.

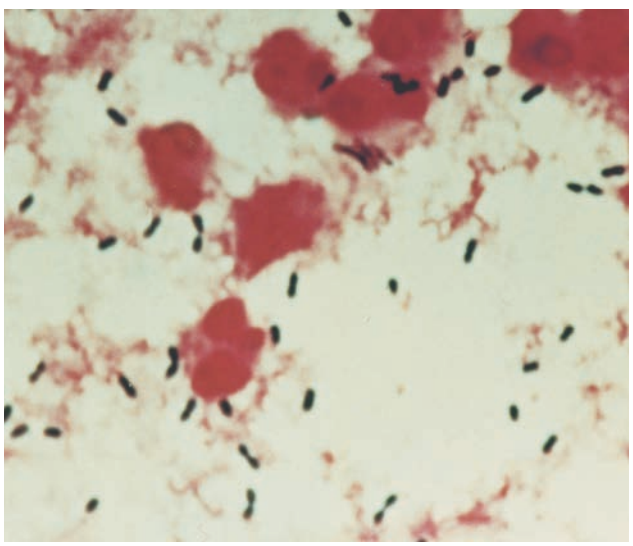


FIGURE 17.12

A Gram stain of a smear of sputum can provide a rapid presumptive diagnosis

This slide shows Gram-positive diplococci and pus cells suggestive of Streptococcus pneumoniae *infection.*

Source: Dr Gary Lee.

There are two types of pneumococcal vaccine currently in use in Australia. Prevenar®, a seven-valent pneumococcal conjugate vaccine (7vPCV) introduced in 2001, was replaced in July 2011 by Prevenar 13® (a 13-valent conjugate vaccine) on the National Immunisation Program in all states and territories except the Northern Territory. From October 2011 the Northern Territory replaced Synflorix® (a ten-valent conjugate vaccine) with Prevenar 13®. This 13-valent vaccine provides protection against an additional six serotypes that were not covered by Prevenar®, including the increasingly predominant serotype 19A. Pneumovax 23®, a 23-valent pneumococcal polysaccharide vaccine, which contains polysaccharides derived from the 23 most frequent or virulent capsular types of *S. pneumoniae*, is used in adults and high-risk groups.

Prevenar 13® is recommended for children at 2, 4 and 6 months of age, with a fourth dose recommended for medically at-risk children at 12 months of age. Aboriginal and Torres Strait Islander children living in the Northern Territory, Queensland, South Australia and Western Australia should receive a supplementary dose of Prevenar 13® at 12–35 months of age, plus a dose of Pneumovax 23® between the ages of 18 and 24 months. Pneumovax 23® is also recommended for:

- all people aged >65 years
- Aboriginal and Torres Strait Islander people >50 years of age and those 15–49 years of age who are at high risk of invasive pneumococcal disease.

The National Health and Medical Research Council has further recommendations for other high-risk groups. For complete details, consult the *Australian Immunisation Handbook* (details of which are provided at the end of this chapter).

Staphylococcus aureus

Staphylococcal pneumonia is most common as a complication of viral respiratory infection such as influenza or measles. Intravenous drug users may develop a staphylococcal endocarditis and pneumonia, following use of a contaminated needle or syringe. Elderly people, the immunocompromised, and patients with diabetes or cystic fibrosis are also susceptible.

Gram-negative enterobacteria

Gram-negative enterobacteria, such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* spp. and *Serratia marcescens*, are particularly associated with pneumonia acquired in hospital. These organisms can colonise the upper respiratory tract of hospitalised people, especially after antibiotic therapy, and then be aspirated into the lower airways.

Ventilators and humidifiers provide the necessary moisture for these organisms to persist and are well recognised as potential sources of infection in hospitals. As stated earlier, mechanical ventilation is a major risk factor. These organisms can also infect the lungs via the bloodstream, from infectious foci in the genitourinary or gastrointestinal tracts. The endotoxins in the cell wall of these bacteria are potent inducers of inflammation. Gram-negative enterobacteria can also cause community infections in alcoholics, the elderly and the immunocompromised.

Appropriate antibiotic therapy is based on the culture results and sensitivity testing. Drug resistance is a problem in hospital-acquired infections caused by Gram-negative enterobacteria.

Haemophilus influenzae

H. influenzae causes pneumonia, often as an exacerbation of epiglottitis, chronic bronchitis, bronchiectasis, septicaemia or meningitis. In general, type b strains of *H. influenzae* cause most cases, but non-type b strains are also possible causes. *H. influenzae* mainly affects non-immunised children under the age of 4 years.

Infections caused by *H. influenzae* have classically been treated with ampicillin, but the emergence of resistant strains has led to increased use of third-generation cephalosporins (e.g. cefotaxime or ceftriaxone). Effective *H. influenzae* vaccines have been available in Australia since 1992 and have substantially reduced the incidence of all types of invasive disease caused by *H. influenzae*, although non-vaccine types still cause infections.

Pneumonia in cystic fibrosis

Several of the organisms described above are involved in recurrent or chronic lung infections in patients with cystic fibrosis (CF). The organisms most often encountered are *H. influenzae*, *S. aureus*, *S. pneumoniae*, *P. aeruginosa* and other *Pseudomonas* species.

Of these, *P. aeruginosa* is the most important, causing a chronic lung infection that is almost impossible to eradicate in most CF patients. Over time, the infection and, more particularly, the inflammation it induces lead to permanent lung damage and often death. The predisposition of CF patients to lung infections is related to the production of overly viscid bronchial secretions, and the suppressive effect this has on mucociliary clearance.

Once established, the *Pseudomonas* protects itself against other defence mechanisms and antibiotics by growing in microcolonies coated with a film of alginate, a mucoid barrier through which many substances cannot pass.

Legionellosis

Legionella pneumophila (serogroups 1–6) and other species, particularly *L. longbeachae*, *L. bozemanii* and *L. micdadei*, cause a pneumonia referred to as **legionellosis** (or **Legionnaires' disease**). *L. pneumophila* causes the majority of infections worldwide, although *L. longbeachae* tends to predominate in Australia. These aerobic, Gram-negative, rod-shaped organisms are widely distributed in warm, wet habitats, such as air-conditioning cooling towers, reticulated water supplies, spa baths and environmental sources (e.g. creeks, lakes, compost and soil). The organism is spread in airborne droplets mainly from man-made water systems, and occasionally from environmental sources, particularly hot springs where the water temperature is at 35–40°C, or from soil or compost.

Dispersion from cooling towers can lead to outbreaks of infection as occurred in the first recognised incident, involving 182 people (29 deaths) at a conference of the American Legion (war veterans) in Philadelphia in 1976. The high fatality rate in that outbreak was due partly to the delay (six months) in identifying the causative agent.

Person-to-person transmission of legionellosis has not been reported. In Australia there are, on average, fewer than 400 cases per year, with the highest rates of notification in the over-60 age groups (see Figure 17.13).

The incubation period of Legionnaires' disease is usually 2–10 days. Together with symptoms of pneumonia, there may be systemic manifestations, including diarrhoea; abdominal pain; neurological signs, such as clouded consciousness and ataxia; and renal failure, with blood and protein in the urine.

The cough in Legionnaires' disease is usually only slightly productive, if at all. Fever is almost always present. The disease is more likely to affect the elderly, but other major risk factors are cigarette smoking, underlying respiratory disease (e.g. chronic obstructive pulmonary disease),

CASE HISTORY 17.4

Legionnaires' disease

On 19 January 2011 Australia's chief medical officer, Professor Jim Bishop, issued an alert, on behalf of the Department of Health and Ageing, to travellers in Bali and those who have recently returned. It was recommended that anyone who experiences 'flu-like' symptoms such as fever and cough should consult their GP or hospital emergency department. Health authorities had been made aware of 11 cases of Legionnaires' disease detected in Victorian and Western Australian residents returning from holidays in the Kuta area of Bali between August 2010 and January 2011. A number of those who contracted the disease stayed in the same hotel in Kuta (The Ramayana Resort and Spa) and most had visited the same local shopping centre.

Questions

1. How is Legionnaires' disease usually acquired?
2. Why are the sources of infection in Legionnaires' disease often difficult to identify?
3. What precautions should healthcare workers take when attending to a patient with Legionnaires' disease?

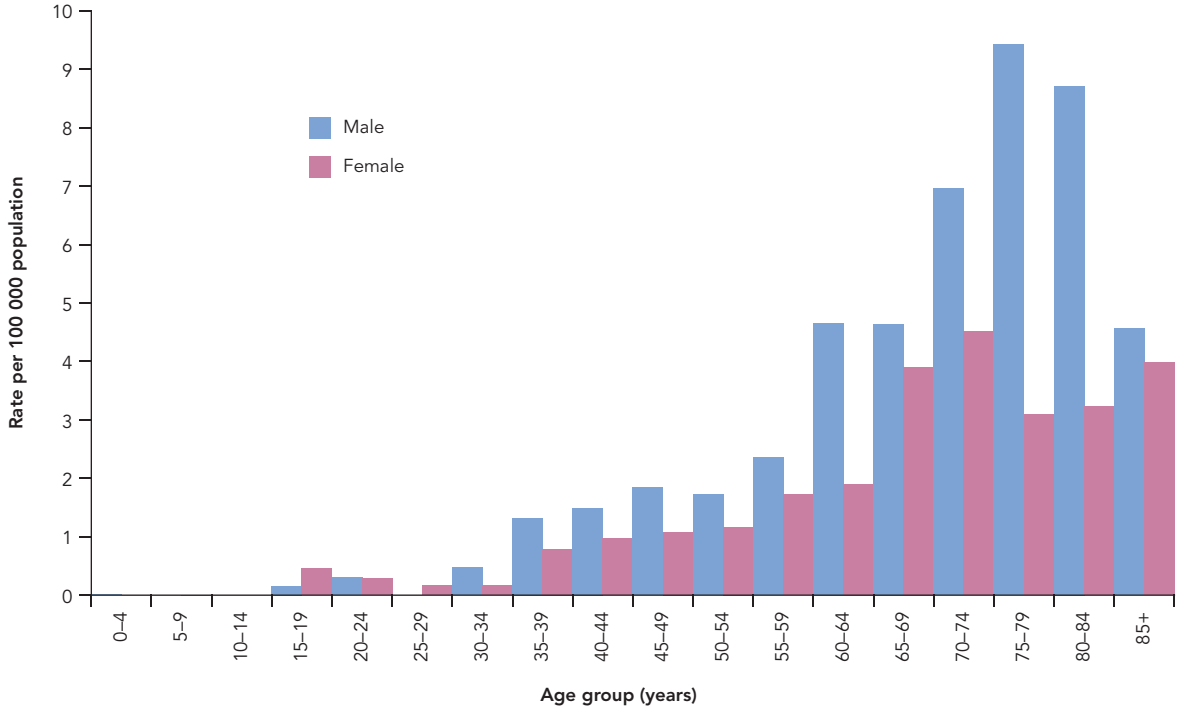


FIGURE 17.13

Notification rates of legionellosis in Australia, 2009, by age group and sex

Source: NNDSS Annual Report Writing Group, Australia's Notifiable Disease Status, 2009. Annual report of the National Notifiable Diseases Surveillance System, *Communicable Diseases Intelligence* 2011, 35(2): 121, <www.health.gov.au/internet/main/publishing.nsf/Content/cda-cdi3502-pdf-cnt.htm/$FILE/cdi3502.pdf>.

immunosuppression (e.g. malignancy, corticosteroid use) and excessive alcohol intake. However, legionellosis can occur in apparently healthy people of all ages.

Pontiac fever is a mild form of *Legionella* infection, characterised by influenza-like symptoms and rapid recovery.

Hospital-acquired legionellosis can occur when water supplies in hospitals become contaminated, particularly when hot water lines are kept at low temperatures (less than 55°C). Below this temperature, *Legionella* are able to survive and multiply in water supplies and can be spread from shower roses and taps. *Legionella* have also been isolated from humidifiers and vaporisers. An important factor in hospitals is the many people who are very susceptible and at high risk of infection. Mortality rates in the immunosuppressed can be very high.

The diagnosis of legionellosis is not straightforward. Culture of sputum is the definitive method, but it is not highly sensitive and has the disadvantage of taking 3–5 days for growth to occur. A fairly sensitive, rapid urinary antigen test is now the most widely used method, but it can only detect *L. pneumophila* serogroup 1. Fluoroquinolines or azithromycin usually provide effective treatment.

The contamination of cooling systems and hot-water supplies with legionellae has been the subject of intense study, and regulations are now in force to provide guidance for maintenance engineers (see Spotlight box: Legionnaires' disease, in Chapter 8, page 172).

Atypical pneumonia

A variety of microorganisms, including bacteria, viruses and fungi, cause what is sometimes referred to as **atypical pneumonia**. It is so named because of the diffuse, patchy nature of the infection and the insidious onset and non-productive cough. The major causes are *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Legionella* (see previous section) and *Pneumocystis jiroveci* in people with AIDS.

M. pneumoniae is transmitted in airborne droplets. It affects mainly the upper respiratory tract, and causes clinical pneumonia in 3–30 per cent of infected people, especially in children and young adults. It occasionally causes epidemics of pneumonia.

C. pneumoniae is recognised as an important pathogen of both the upper and lower respiratory tracts, causing as much as 10 per cent of community-acquired pneumonia. Perhaps of greater significance is the possible role of *C. pneumoniae* in atherosclerosis. The organism has been shown to be present in coronary atherosclerotic lesions. However, a causal role for the organism in this disease has yet to be proven.

Mycoplasma and *Chlamydophila* can be cultured in the laboratory, but they are very slow-growing and can take up to two weeks to form visible colonies. Thus, diagnosis is usually based on PCR and serological tests. It is also possible to detect *C. pneumoniae* directly in a smear of sputum, using fluorescent-labelled antibodies (see Figure 17.14). Azithromycin or tetracyclines are used for the treatment of mycoplasmal and chlamydial pneumonia.

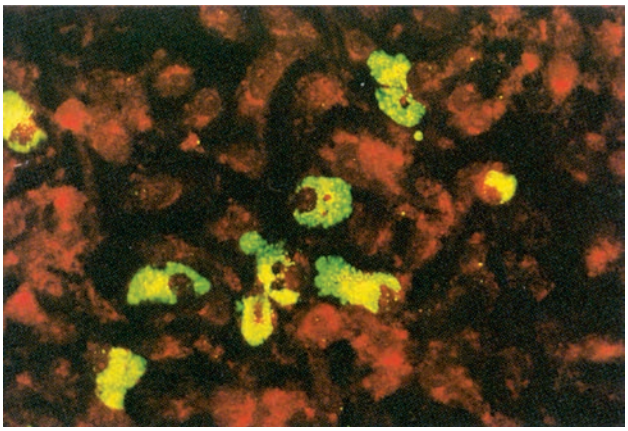


FIGURE 17.14

***Chlamydophila pneumoniae* identified with a fluorescent-labelled antibody**

Source: Photography courtesy of Dr A. Smithyman, Cellabs Pty Ltd, Sydney.

Q fever

The bacterium *Coxiella burnetii* is the cause of **Q fever**, a pneumonia in which chronic infection of the liver, heart valves and other organs may occur. It was originally recognised in the 1930s by Edward Derrick in Australia, when it was found to be infecting workers in a Brisbane abattoir. It was given the name 'Q [for query] fever', because the cause was unknown until Sir Frank Macfarlane Burnet identified the causative agent as a rickettsia.

The organism is found in a variety of wild animals including kangaroos, and in goats and sheep and, especially, in cattle. It is spread from animal to animal via ticks. It is shed in the faeces, milk and urine of infected animals. Humans usually acquire Q fever by inhalation of contaminated aerosols or dust. Outbreaks have occurred in people working in the livestock industry, such as stockyard workers, abattoir and dairy workers, and meat packers. This zoonotic infection has been reported in most countries in the world, with New Zealand the notable exception.

The organism is very resistant to drying and can survive for months in dust or on wool, animal hides and straw. On average, there are 450 notifications per year in Australia, with the highest numbers of notification usually in Queensland and New South Wales. *C. burnetii* is removed from milk by pasteurisation.

Many cases of Q fever are asymptomatic. When symptomatic, it has an incubation period of 1–4 weeks and produces an influenza-like illness of severe headache, fever and muscle pain lasting 1–2 weeks. Pneumonia or mild hepatitis may subsequently develop. The disease can also result in a fatigue syndrome that can last weeks, months or years. Endocarditis, which may occur years later, is one of the more serious possible complications.

Diagnosis of Q fever is usually by serological means. PCR tests have been developed but are not readily available. Treatment with doxycycline is currently the common approach. A Q fever vaccine is available and recommended for those at risk (e.g. farmers, abattoir workers and veterinarians). To improve

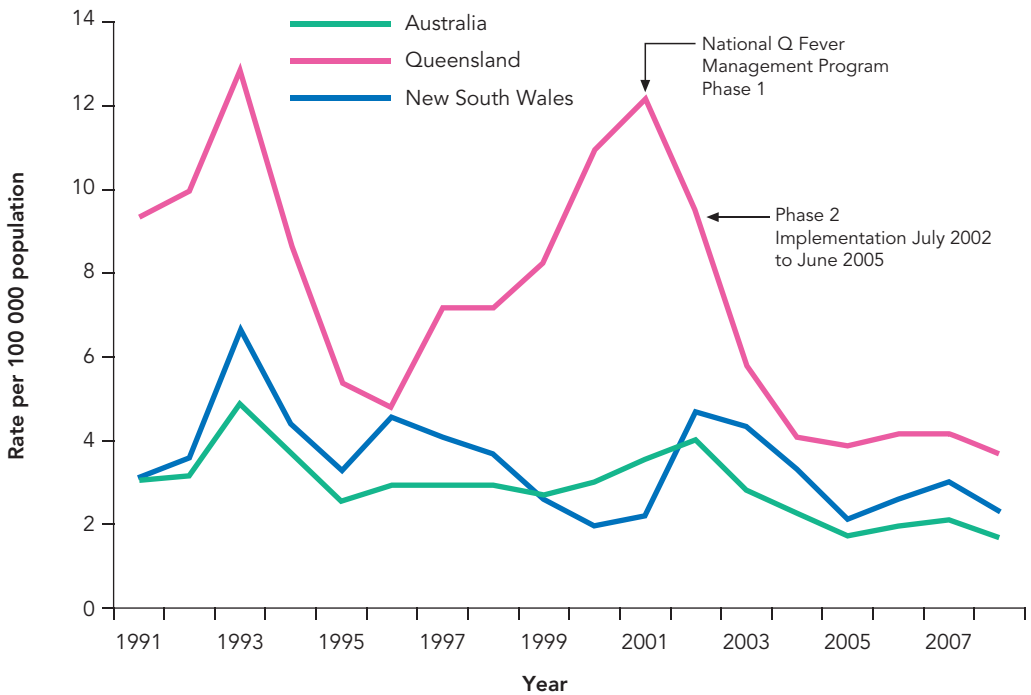


FIGURE 17.15

Notification rates of Q fever in Australia, New South Wales and Queensland, 1991–2008

Source: *Communicable Diseases Intelligence*, September 2010, 34(3), p. 211, <www.health.gov.au/internet/main/publishing.nsf/Content/cda-cdi3403-pdf-cnt.htm/$FILE/cdi3403.pdf>.

vaccination coverage in the at-risk population, the Australian government funded the National Q fever Management Program, which was implemented throughout the country during 2001–02. The impact of this program on the incidence of Q fever in Australia is shown in Figure 17.15. Severe reactions to this vaccine can occur in individuals already immune, so it is essential that a person's immunity and skin sensitivity to the vaccine antigens is tested before immunisation.

Ornithosis

Sir Frank Macfarlane Burnet also identified *Chlamydophila psittaci* in the 1930s, in Australian parrots. This bacterium causes a pneumonia called **ornithosis** (or **psittacosis**), mainly acquired by inhalation of dust particles from bird droppings, especially parrots, but potentially any bird. Sometimes, the birds themselves suffer from a diarrhoeal or respiratory illness. Infections in humans are found mainly in people who keep birds as pets. Person-to-person transmission is rare.

There is an average of 170 cases reported per year in Australia. Most cases are mild and self-limiting, but severe pneumonia occurs in some patients, with a need for prolonged hospitalisation.

Diagnosis of ornithosis is usually by serological testing or by antigen detection in sputum, or by PCR or culture. Tetracyclines are generally effective in the treatment of ornithosis.

Melioidosis

Melioidosis is caused by the bacterium *Burkholderia pseudomallei*, which is found in soil and surface water. It is a disease found mainly in tropical areas, including South-East Asia and Northern Australia. Most cases in Australia occur in the wet season in the Northern Territory and Queensland. In the Northern Territory, around 20–30 cases are reported each year, with several deaths. In Australia it is sometimes referred to as Nightcliff gardener's disease—Nightcliff being a suburb of Darwin.

Humans usually become infected via skin lesions in contact with contaminated soil or water, or by inhalation of dust or aerosolised polluted water. The organism can spread via the bloodstream to almost any organ of the body. Infection may be subclinical, but the most common form of acute illness is pneumonia. Abscesses can form in the skin, spleen, prostate and other organs. Severe illness and mortality are usually associated with other factors, such as alcoholism, diabetes and renal disease.

Laboratory diagnosis is by culture of sputum, blood, pus or other specimen from the likely site of infection. Growth can take up to 72 hours. Direct immunofluorescent microscopy of an appropriate specimen is only 70 per cent sensitive, but provides a positive diagnosis in less than one hour. The organism is intrinsically resistant to many antimicrobial drugs. Ceftazidime is often the drug of choice, followed by maintenance therapy with doxycycline and cotrimoxazole for several months to prevent relapse.

Viral causes

A number of viruses can cause pneumonia, including several newly identified viruses, such as human bocavirus and coronavirus NL63. Viruses tend to cause an interstitial pneumonia—that is, one that involves the interstitium of the lung. Co-infection with at least one other pathogen is common. Some viruses do not themselves cause pneumonia but may, by damaging tissues and defences, predispose the patient to secondary bacterial pneumonia.

Respiratory syncytial virus

Respiratory syncytial virus (RSV) is recognised as a major cause of pneumonia, especially in children and in adults with heart or lung disease. The virus has variable shape and size (see Figure 17.16), and has surface spikes that fuse infected host cells together to form syncytia—multi-nucleated masses of fused cells, hence the name of the virus. The illness in children may begin with URT symptoms and progress rapidly over 1–2 days to the development of diffuse small airway disease characterised by cough, coryza, wheezing and rales, and low-grade fever. In adults and children over the age of 3 years it is usually restricted to the upper respiratory tract. However, it can cause severe pneumonia in immunocompromised adults.

Epidemiological studies have shown that RSV is an important cause of severe lower respiratory tract infections in infants and children, and is the most common cause of lower respiratory tract infection in children less than 1 year of age. The CDC estimates that in the US it is responsible for around 100 000 hospitalisations per year of children under 1 year of age. It is the only agent of the three major causes of pneumonia (the other two being *S. pneumoniae* and *H. influenzae*) for which there is no vaccine.

Severe acute respiratory syndrome virus (SARS virus)

Coronaviruses have been known to infect humans since the 1960s. **SARS** coronavirus (SARS-CoV) is a new coronavirus that appeared in late 2002. The virus is thought to have originated from wild animals in wet markets in southern China. The large numbers and varieties of animals in overcrowded cages in these markets provide the opportunity for the virus

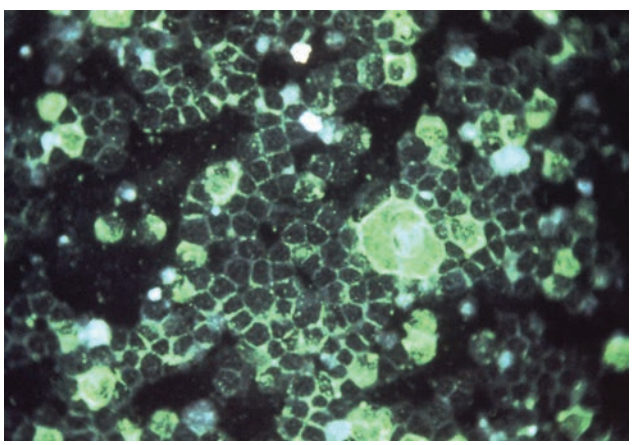


FIGURE 17.16

Photomicrograph of respiratory syncytial virus (RSV) using indirect immunofluorescence technique

Source: CDC/Dr Craig Lyerla.

to cross the species barrier from animals to humans. Within a few months the virus had spread to 32 countries worldwide. In March 2003 the WHO issued a global alert, recommending worldwide surveillance for this disease.

SARS appears to be spread by respiratory droplets (coughing and sneezing) of an infected person, or by contaminated hands or objects. People infected with the SARS virus can display a range of symptoms. After an incubation period of 2–14 days a person may demonstrate virtually any combination of fever, chills, cough and breathing difficulties, headache, muscle ache, dizziness, diarrhoea or sore throat. Approximately 20 per cent of cases develop severe lung injury and acute respiratory distress syndrome, often accompanied by a watery diarrhoea. Overall more than 8400 people were infected during 2002–04, with a mortality rate of around 11 per cent, mainly in patients over 65 years of age. The last cases of SARS were diagnosed in April 2004.

The vague symptoms and the lack of a diagnostic test for SARS are partly why it was able to spread so quickly, and why so many healthcare workers became infected. The SARS epidemic was contained by a highly effective global response coordinated by the WHO. Although several years have passed since the last cases, similar viruses are still believed to exist in animal reservoirs in southern China, and it is thus considered that SARS could return if conditions become appropriate for its re-introduction and transmission in humans.

Human metapneumovirus virus

The human metapneumovirus was first identified in 2001 and has since been found to have a worldwide distribution. It causes similar respiratory infections to RSV, ranging from mild, self-limiting illnesses to respiratory failure. Metapneumovirus is believed to cause lower respiratory tract infection in the very young, in hospitalised children, and in the elderly. It is the second most common cause of bronchiolitis in early childhood, after RSV. Infection in adults usually presents with mild cold-like respiratory symptoms, although more severe infection, such as pulmonary haemorrhage and respiratory failure, may occur in patients with underlying medical conditions such as cardiopulmonary disease, and in the immunocompromised. However, the incidence and epidemiologic features of metapneumovirus remain largely unknown.

Hendra virus

Hendra virus is a paramyxovirus, related to the Nipah virus. Fruit bats (flying foxes) are the only known natural reservoirs. Antibodies to the virus have been found in 20–50 per cent of fruit bats in mainland Australia. Transmission of the virus from flying foxes to horses is rare, and is thought to be due to contamination of horse feed with body fluids of infected bats. Humans are most likely infected through exposure to the respiratory secretions or blood of an infected horse.

Infected people develop symptoms 5–16 days after infection. Fever, cough, sore throat, headache and tiredness are the usual initial symptoms. Meningitis or encephalitis can then develop and lead to a fatal outcome.

Serological tests can detect infection, but require substantial time. PCR tests provide the quickest evidence of infection. Supportive care is all that is currently available for Hendra virus infection, because there is no specific treatment.

Other viruses

Other potential viral causes of pneumonia include adenoviruses and influenza viruses, as well as parainfluenza viruses. Measles virus can cause pneumonia in individuals with impaired immune defences. In children in developing countries, secondary bacterial pneumonia is a frequent complication of measles infection. Cytomegalovirus is not normally involved in respiratory illness, but in immunocompromised patients (e.g. transplant recipients and AIDS patients) it can cause an interstitial pneumonia. Measles and cytomegalovirus infections are discussed more fully in Chapters 16 and 19, respectively.

A laboratory diagnosis of viral pneumonia is often only undertaken for epidemiological or public health reasons, and even when attempted, a specific diagnosis is made in only around 50 per cent of cases. Virus culture of a suitable respiratory sample was the gold standard for laboratory diagnosis, but is a slow and highly specialised technique. Rapid antigen assays have been developed for many of the pathogens, but they can be lacking in sensitivity and specificity. Molecular methods, especially PCR and NASBA (nucleic acid sequence-based amplification) assays, are now the most common techniques being used and developed. Multiplex PCR assays allow simultaneous testing for multiple possible causes. Laboratory diagnosis of RSV is usually made by RT-PCR, direct examination of respiratory secretions using immunofluorescence or enzyme immunoassay techniques. A number of rapid ('point of care') diagnostic kits, which identify RSV antigen in specimens, are now available. Diagnosis of human metapneumovirus infection is difficult, and usually done by PCR on respiratory specimens.

Treatment options for viral pneumonia are very limited. Supportive care is the mainstay in most cases. Ribavirin and cidofivir are effective against some viruses, but for many (e.g. human metapneumovirus) there is currently no effective antiviral treatment available.

CHRONIC INFECTIONS OF THE LOWER RESPIRATORY TRACT

Tuberculosis

Tuberculosis, or consumption, as it was formerly called, has plagued the world for thousands of years. It is believed to have caused up to 25 per cent of deaths in Europe during the 19th century. It is still one of the major diseases affecting people in the developing world, ranking as the second-most common cause of death due to infection.

Incidence

According to the WHO, one-third of the world's population has tuberculosis. Approximately 8–9 million new cases occur in the world each year, with around 2 million deaths,

98 per cent occurring in the developing world. The highest incidence is in sub-Saharan Africa, associated with the high incidence of HIV infection there (see Figure 17.17). Other regions with a high incidence are parts of South-East Asia, the eastern Mediterranean and the western Pacific. In developed countries, the incidence has declined significantly since the early 20th century, although there was a disturbing trend of increased occurrence in the United States and some European countries in the late 1980s. Rates in these countries are now falling again, due mainly to increased investment in tuberculosis control measures. In 1993 the WHO declared the disease a global emergency.

In Australia, an average of 1100 cases of tuberculosis are reported each year. The notification rate in Australia has remained relatively stable since 1986, and the overall rate of fewer than 6 cases per 100 000 population per year is one of the lowest in the world. The highest rates of infection are in Aboriginal and Islander populations and migrants, especially those from South-East Asian countries and the Philippines.

Apart from its persistence worldwide, the other main concern with tuberculosis is the worldwide emergence over the past two decades of drug-resistant strains. First, strains that are resistant to at least two of the standard anti-tuberculous drugs, isoniazid and rifampicin, appeared and are referred to as multi-drug-resistant tuberculosis (MDR-TB). Then, extensively drug-resistant TB (XDR-TB), defined as MDR-TB also resistant to a quinolone and at least one of the second-line anti-TB drugs (amikacin, kanamycin and capreomycin), began to appear. This form of TB, with a high mortality rate, has been found in 69 countries to date, including Australia. Since 2007, strains that are considered resistant to all anti-tuberculous drugs have been reported. The WHO estimates that, globally, around 500 000 new cases of MDR-TB and 50 000 cases of XDR-TB occur each year.

Cause and transmission

Tuberculosis is caused mainly by the rod-shaped, acid-fast bacterium *Mycobacterium tuberculosis*, but other species of mycobacteria—*M. bovis*, *M. microti*, *M. canettii* and *M. africanum*—can also be responsible. The usual source of infection is a person, who can emit enormous numbers of mycobacteria when coughing. The organisms are transmitted in aerosols, droplet nuclei or dust. They can survive for long periods in air and dust because of their waxy outer coat, which allows them to resist drying.

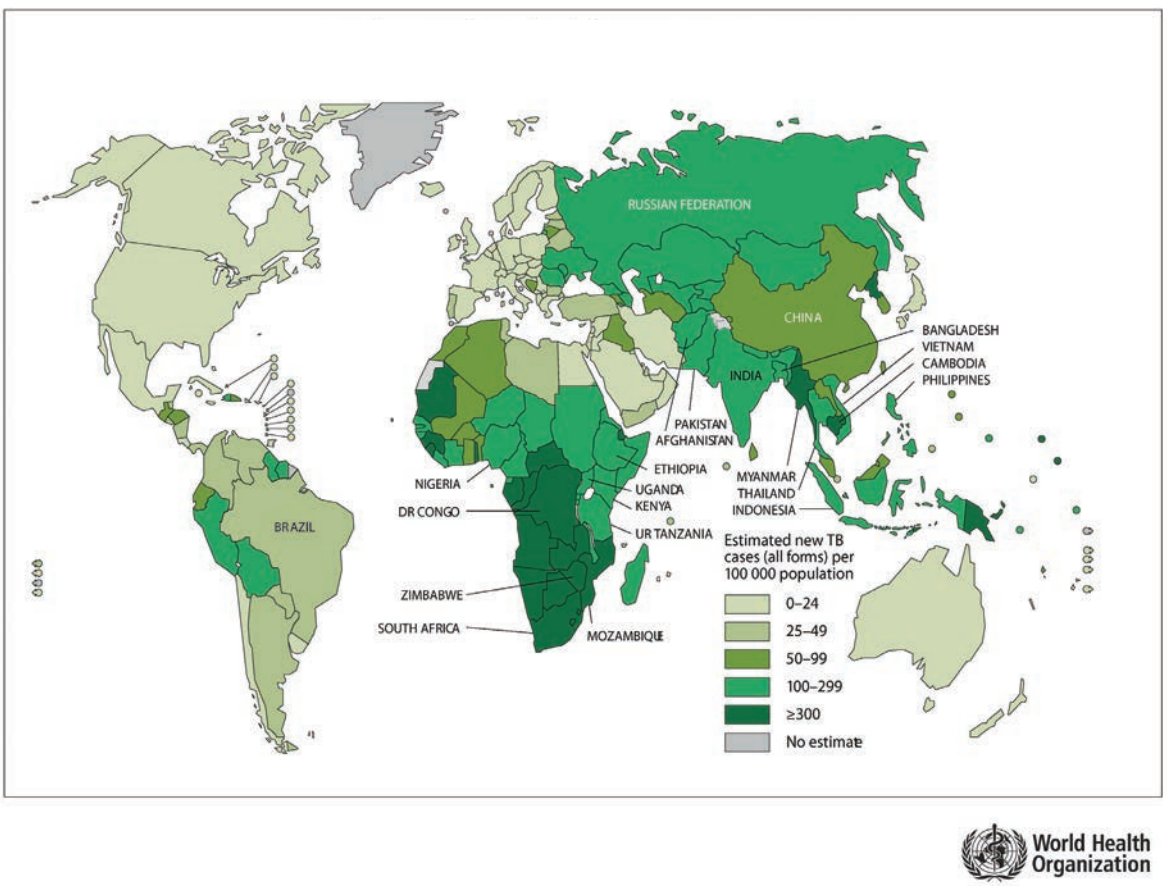


FIGURE 17.17

Estimated global incidence of tuberculosis, 2010

Source: Global TB Control, 2011, World Health Organization. Reproduced with permission.

M. tuberculosis grows very slowly, with a generation time of 15–20 hours, compared with 20–30 minutes for most bacteria.

Pathogenesis

In primary infection the organisms are engulfed by the alveolar macrophages. However, the bacteria can survive and multiply in these cells. The macrophages eventually carry the bacteria via the lymphatics to the local lymph nodes, where an immune response, predominantly cell-mediated immunity (CMI), is stimulated. The CMI response acts to prevent the spread of the bacteria by causing the release of lymphokines from sensitised T cells, which activate macrophages and increase their ability to destroy the bacteria. In addition, an infiltration of cells (lymphocytes, macrophages and epithelioid cells) to the sites of infection serves to contain the organisms within **tubercles**, or small granulomas. A tubercle in the lung plus enlarged lymph nodes is called the **Ghon** (or primary) **complex**.

After a time, the material within the granuloma becomes necrotic and caseous (cheesy). In people who are otherwise healthy, the tubercles may heal spontaneously, become fibrotic or calcified, and persist as such for a lifetime. They will show up years later on a chest X-ray. However, in a small percentage of people with primary infection, particularly in the immunocompromised, the mycobacteria are not destroyed or contained within the tubercles. Instead, they invade the bloodstream and cause a potentially fatal disseminated disease (called **miliary tuberculosis**). Tubercles may then form in many organs, including the liver, spleen, kidneys and meninges.

In some people, the activated macrophages never completely eliminate these bacteria, even though the patients recover clinically from the primary infection. The bacteria can remain dormant within granulomas for many years because the macrophages have controlled their multiplication but not killed them. Secondary tuberculosis occurs in a small percentage of these people, often many years later, due to the reactivation of dormant mycobacteria. Factors influencing the reactivation of these bacteria include old age, impaired immune function resulting from factors such as malnutrition, infection (e.g. AIDS), chemotherapy for treatment of malignancies, and corticosteroids for treatment of inflammatory diseases. Reactivation usually occurs in the apex of the lungs.

The pathogenesis of tuberculosis is illustrated in Figure 17.18.

Clinical features

Primary tuberculosis is completely asymptomatic in 90 per cent of immunocompetent people. In the asymptomatic there may be only a small lesion in the periphery of the lung and minor enlargement of draining lymph nodes.

In the 10 per cent who develop active disease, the onset of symptoms is often insidious, infection proceeding for some time before the patient develops fever, weight loss and malaise. Without treatment, pneumonia-like symptoms may develop, with a chronic cough productive of sputum which may be blood-stained. The infection can resolve spontaneously or the organisms may spread locally to cause pleurisy or bronchopneumonia, or via the bloodstream to cause meningitis or disseminated disease. Factors that increase the probability of a person becoming symptomatic include co-infection with HIV, immunosuppression, alcoholism, renal failure and diabetes mellitus. HIV and tuberculosis are a lethal combination because each accelerates the other's progress in a person suffering from both diseases.

Diagnosis

A presumptive diagnosis of tuberculosis is usually based on clinical signs and symptoms, supported by chest X-ray findings and a positive tuberculin (Mantoux) skin test (see

CASE HISTORY 17.5

Tuberculosis

A cluster of TB infections acquired in the Hunter area of New South Wales was first suspected in February 2005, as a result of interviews with contacts of a hospitalised patient with pulmonary TB. Epidemiological investigation indicated that some of the TB cases diagnosed in the Hunter area between 1994 and 2005 could have been linked. Genotype testing of stored bacterial isolates found that nine cases were caused by an identical genotype, indicating they were part of a cluster. Two other cases, for which isolates were not available, were epidemiologically linked to the cluster and regarded as probable cluster cases. Members of the cluster were relatively young (median age 35 years; range 21–57 years), and eight were women. Investigation of the cluster revealed complex social networks between cases, with a likelihood of several waves of transmission between 1994 and 2005. It was concluded that transmission probably occurred at a small, poorly ventilated recording studio, and possibly in social and workplace settings.

Source: Adapted from T.D. Merritt et al. 2007, An outbreak of pulmonary tuberculosis in young Australians. *Medical Journal of Australia* 186(5): 240–42.

Questions

1. What are the possible modes of transmission of tuberculosis in this case?
2. What further steps would you expect health authorities to take as a result of identifying this cluster?
3. What infection control procedures should a healthcare worker who attends to any of these people take?
4. What advice would you give to close family contacts of the cluster people regarding TB testing and vaccination?

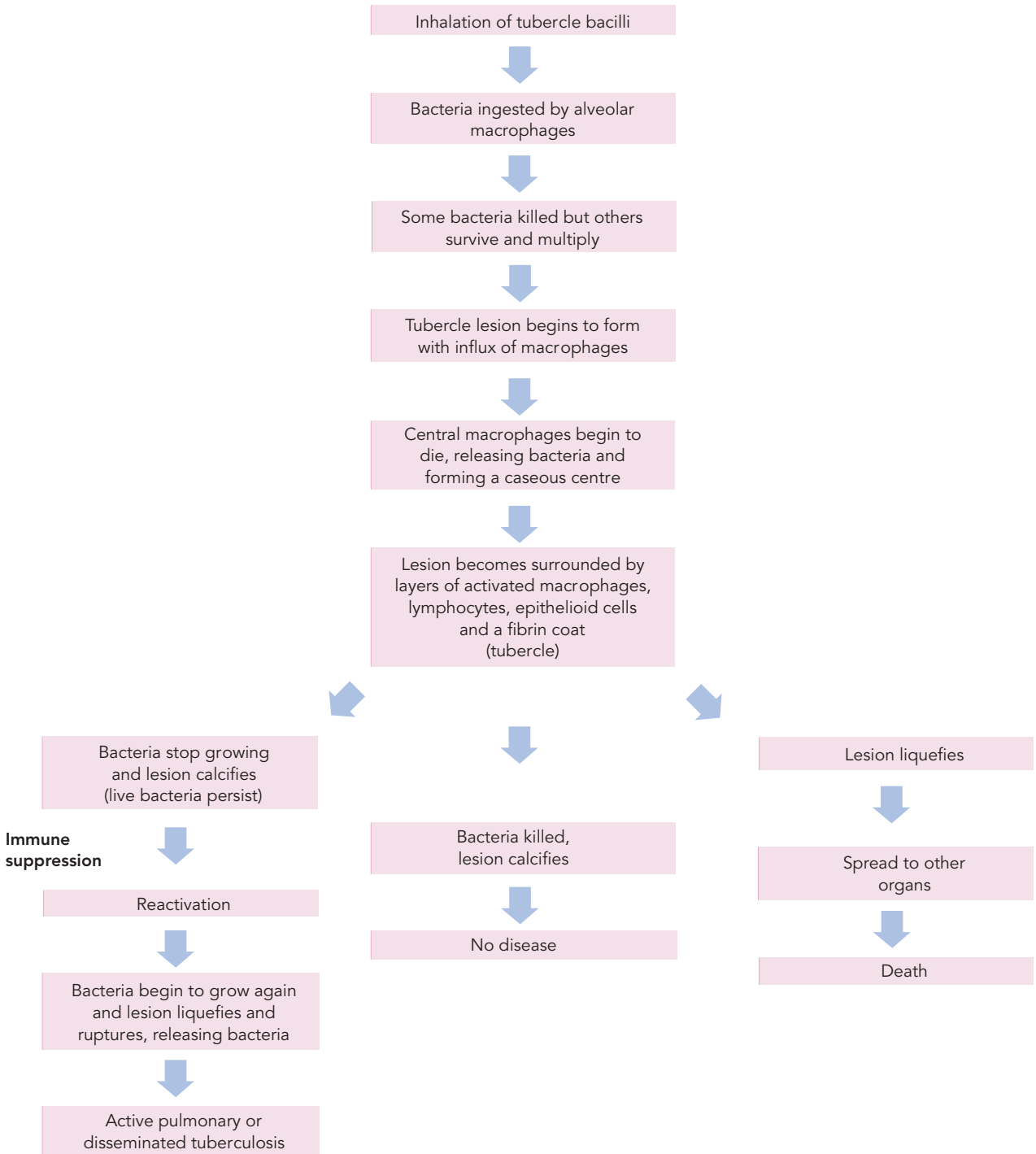


FIGURE 17.18

The pathogenesis of tuberculosis

below). Confirmation is by demonstration of acid-fast rods in sputum stained by Ziehl-Neelsen's method (see Figure 17.19), and culture of *M. tuberculosis* from sputum. With microscopic examination of a smear of sputum, a result can be obtained within hours of receipt of the specimen by the laboratory, but this method only detects about 60 per cent of cases. A positive smear also indicates that the patient is infectious.

Microscopy is important because *M. tuberculosis* can take up to six weeks to grow in standard solid media culture. Liquid culture systems and drug susceptibility testing have been recently developed and have become the gold standard for TB diagnosis. They are substantially faster, requiring days rather than weeks. Molecular methods that amplify and detect *M. tuberculosis* nucleic acids in specimens (e.g. PCR) are the most promising developments in TB diagnosis. They

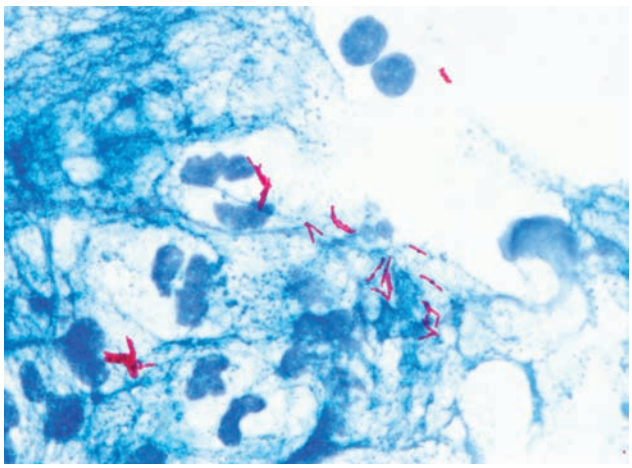


FIGURE 17.19

Ziehl-Neelsen stain of sputum

Acid-fast (pink) rods of Mycobacterium tuberculosis *against a blue background.*

Source: © Richard Lumb.

are very quick and have high specificity, and also allow for detection of some antibiotic resistance.

Tuberculin (Mantoux) skin test

A skin test is performed by intradermal injection of tuberculin, a purified protein derivative (PPD) of *M. tuberculosis*, and examination 48–72 hours later for a reddening and thickening (induration) of the skin at the site of injection. This is a delayed hypersensitivity reaction to the antigen (see Chapter 9). In general, if the diameter of induration is less than 5 mm it is considered to be negative. The interpretation of positive reactions is complex and dependent on many factors. A strongly positive reaction is suggestive of active or recent infection. A weak reaction may reflect past infection or vaccination. False negative reactions can occur when a person's cellular immunity is depressed by such factors as immunosuppressive therapy and infections (e.g. HIV). *In vitro* assays for assessment of immunity to *M. tuberculosis* have been developed and are increasingly being used in clinical laboratories.

Treatment

In 2010 the WHO revised its international guidelines for the treatment of tuberculosis. The standard regimen for new patients is a daily dose of isoniazid, rifampicin, pyrazinamide and ethambutol in combination for two months (intensive phase), followed by isoniazid and rifampicin for four months (maintenance phase). The regimen for the treatment of multi-drug-resistant tuberculosis is at least four drugs with certain, or almost certain, effectiveness, for a minimum of 18 months after the patient has become negative in sputum culture tests. Where possible, drug sensitivity testing should be performed in all cases. *M. tuberculosis* rapidly develops resistance to anti-tuberculosis drugs, but this is minimised with combination therapy.

With good compliance, the cure rate of drug-sensitive tuberculosis approaches 100 per cent; however, the treatment success rate in cases with MDR-TB is only around 50–60 per cent. Patient compliance with prolonged therapy can be difficult to achieve, especially after they start to feel well. Failure to complete the full drug treatment can result in incomplete elimination of the organism and the development of resistance. For this reason the WHO introduced the DOT (directly observed treatment) strategy in 1991, which involves the supervised administration of anti-tuberculosis drugs to patients.

Prevention and control

Prevention of tuberculosis in areas of low prevalence is based on case finding and effective treatment, and contact tracing and chemoprophylaxis. The BCG (Bacille Calmette *et* Guerin) vaccine, a suspension of live, attenuated *M. bovis*, was developed in the 1920s by the French bacteriologists Albert Calmette and Alphonse Guerin of the Pasteur Institute. The BCG offers significant protection against tuberculosis in children, but it has less certain protectiveness for adults (reported to be 0–80 per cent). In Australia, BCG vaccination is recommended for:

- children under the age of 5 who are going to live in countries of high incidence
- Aboriginal and Torres Strait Islander neonates in areas of high incidence
- neonates born to parents with leprosy or a family history of leprosy (to protect against leprosy, see Chapter 20)
- embalmers
- healthcare workers involved in autopsies.

The general use of BCG in low-risk areas is usually not recommended because of its variable efficacy. Furthermore, in low-risk countries it is considered to be a disadvantage because it makes the interpretation of the tuberculin skin test difficult, and hence the ability to identify recent infection is lessened. A tuberculin test should be performed before vaccination with the BCG, and the vaccine is given only to those who are negative for the test.

In hospitals, airborne precautions (see Chapter 13) should be instituted for patients with active tuberculosis. Certain types of healthcare workers have a high risk of exposure, particularly staff of respiratory and chest clinics, high dependency or emergency departments, staff who work with tuberculosis or HIV-infected patients, mortuary staff, physiotherapists, most medical and nursing staff of public hospitals, and microbiology laboratory staff. Such people, if Mantoux-negative, should have regular tuberculin skin tests. The use of BCG in these people is controversial and advice should be sought from local health authorities.

Rapid diagnosis and properly implemented infection control procedures are important approaches, since the greatest risk in hospitals comes from undiagnosed cases. The incidence of *M. bovis* infections is controlled by pasteurisation of milk, and the screening and eradication of infected cattle. Other major control measures in Australia are based on entry screening of migrants and contact tracing of infected people.

Globally, there is a Stop TB Partnership, which is a network of organisations (including the WHO and the United Nations), countries and individuals that are working together to eliminate TB as a public health problem. The partnership has developed 'The Global Plan to Stop TB: 2006–2015', which aims to halve the prevalence and mortality associated with TB by 2015, based on improved diagnostics, simpler treatment and more effective vaccination. While some progress in TB control has been made, it has been modest, with the incidence currently falling at less than 1 per cent per year. The greatest challenge is undoubtedly TB associated with the HIV epidemic.

Atypical mycobacteria infections

Other species of *Mycobacterium* can cause tuberculosis-like illnesses. *M. avium* and *M. intracellulare*, often referred to as the *Mycobacterium avium* complex (MAC) or *M. avium-intracellulare*, are the most important of these but there are other species, including *M. kansasii*, *M. fortuitum*, *M. chelonei* and *M. scrofulaceum*. MAC has come to prominence as a result of the AIDS epidemic but, curiously, it is not very common in people immunosuppressed by factors other than the human immunodeficiency virus. It is also rare in immunocompetent people.

In people with AIDS, the gastrointestinal tract is often the primary site of infection, but disseminated disease is common in the later stages of AIDS. Blood culture is the most reliable method for diagnosis. These mycobacteria are atypical in being resistant to most anti-tuberculous drugs.

Fungal pneumonia

A variety of fungal spores is usually present in air, but these organisms are much less frequent causes of lower respiratory tract infection than bacteria and viruses. In the main, fungi are opportunistic pathogens and so most infections are seen in immunocompromised patients. Given the increasing number of people who are immunocompromised as a result of treatment (e.g. chemotherapy, corticosteroids, radiotherapy), infection (e.g. AIDS) and other diseases (e.g. cancer), the number of infections has also increased significantly over the last two decades. Several fungi can cause a chronic type of pneumonia.

Pneumocystis pneumonia

Pneumocystis jiroveci (formerly *Pneumocystis carinii*) is a ubiquitous organism that was originally classified as a protozoan, but is now recognised to be a fungus. This organism has come to prominence with the AIDS epidemic. It is a common infectious complication of HIV-infected persons who are either not receiving appropriate therapy or not responding to it. It can also infect other immunocompromised individuals, as well as infants and the elderly.

P. jiroveci infects the tissue surrounding the alveoli, in which a foamy exudate forms, containing large numbers of the thick-walled cyst form of the organism. Typical symptoms include fever, dyspnoea and cyanosis. Laboratory diagnosis is by microscopic demonstration of the organism in a specimen from the patient (Figure 17.20). Sputum is rarely positive, so lung biopsy or bronchial alveolar lavage may be necessary. The organism is usually sensitive to cotrimoxazole, but treatment frequently fails because of the associated immune deficiency. Strains resistant to cotrimoxazole have been isolated in other countries (e.g. the United States and in Europe).

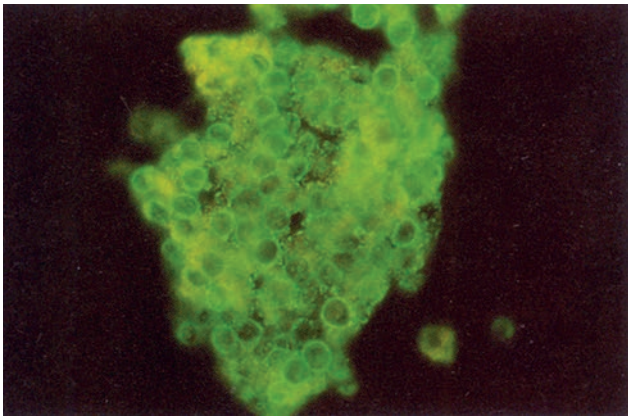


FIGURE 17.20

***Pneumocystis jiroveci* (fluorescent antibody stain)**

Found by microscopic examination of sputum, lung biopsy or bronchial alveolar lavage.

Source: Photography courtesy of Dr A. Smithyman, Cellabs Pty Ltd, Sydney.

Cryptococcal pneumonia

Cryptococcus neoformans is an encapsulated yeast that is widely distributed in nature. A number of serotypes exist, belonging to two subspecies: *C. neoformans* var. *neoformans* and *C. neoformans* var. *gatti*. Like other fungi, these organisms mainly cause infection in the immunocompromised. The major route of infection is the respiratory tract, where these organisms cause pneumonia. However, in most patients, the organisms quickly spread to the brain and thus the presenting symptoms often reflect a meningitis, rather than pneumonia. In lung infection, the organism can be observed in sputum. A microscopic examination of the specimen treated with India ink stain shows the yeast cell with its characteristic large capsule. Chapter 20, which covers central nervous system infections, has further information on *C. neoformans*.

Other causes

A number of other fungi can cause pneumonia. Exposure of immunosuppressed individuals to the widespread, air-borne spores of *Aspergillus fumigatus* and other *Aspergillus* species can result in pulmonary infection. Similarly, exposure to spores of *Rhizopus* and *Mucor* can lead to infection.

Parasitic infections of the lung

Various parasitic infections may involve the lung. Certain nematodes, namely *Ascaris* and some hookworms, transiently infect the lungs as their larvae migrate through the body on their way to the intestine. The tapeworm *Echinococcus granulosis* can infect the lungs and form hydatid cysts in the lung tissue. The cysts can become quite large and cause considerable respiratory dysfunction. These helminth infections are discussed more fully in Chapter 18.

SUMMARY

- Respiratory infections are among the most common that afflict humans.
- Respiratory pathogens may be expelled in enormous numbers by coughing and sneezing.
- Some potentially pathogenic microbes are part of the normal flora of the upper respiratory tract.

UPPER RESPIRATORY TRACT INFECTIONS

- Most upper respiratory infections are due to viruses, and most are self-limiting, mild infections.
- Rhinoviruses and coronaviruses are the most common causes of the common cold.
- Cold viruses are transmitted by coughing and sneezing, and by hands and objects contaminated with nasal secretions.
- Infections involving the middle ear are called otitis media.
- *Streptococcus pneumoniae* and *Haemophilus influenzae* are the most common causes of otitis media.
- Pharyngitis, or sore throat, is mainly caused by viruses.
- *S. pyogenes* pharyngitis is important because of the possibility of the serious complications of rheumatic fever and acute glomerulonephritis.
- Diphtheria is an acute infection caused by toxin-producing strains of *Corynebacterium diphtheriae*.
- Cardiac failure and death can result from the action of diphtheria toxin on the heart.
- Diphtheria vaccine is currently incorporated in the diphtheria, tetanus and pertussis triple vaccine.
- Epiglottitis is almost always caused by *H. influenzae* type b.
- Laryngotracheitis, or croup, is characterised by stridor, hoarseness and a resonant cough.
- Croup is most common in children under age 4 and is usually caused by viruses.

LOWER RESPIRATORY TRACT INFECTIONS

- Pathogenic microorganisms may reach the lower respiratory tract by inhalation, aspiration or spread of infection from the upper respiratory tract or via the bloodstream.
- Pertussis (whooping cough) is a severe disease caused by *Bordetella pertussis*.
- The incidence of pertussis has been markedly reduced by immunisation.
- Viruses are the most common causes of acute bronchitis.
- Bronchiolitis occurs mostly in children under 2 years and is caused mainly by the respiratory syncytial virus (RSV).
- Influenza A viruses cause flu epidemics and occasionally pandemics. Influenza B viruses cause flu epidemics.
- Influenza viruses can vary their antigens by antigenic drift and antigenic shift.
- Influenza vaccine offers protection against strains covered by the vaccine.
- Pneumonia is an infection and inflammation of the lungs and is a major cause of death throughout the world.
- Bacteria and viruses cause the vast majority of cases of pneumonia.
- Lobar pneumonia is an infection and consolidation confined to one or two lobes of the lung.
- Bronchopneumonia is a diffuse, patchy inflammation of the lungs, with numerous small foci of consolidation.
- Empyema is an infection of the pleural space.
- Pleurisy is an inflammation of the pleural membranes.
- The high pathogenic avian influenza H5N1 can potentially cause a pandemic in humans.
- The most common bacterial cause of community-acquired pneumonia is *Streptococcus pneumoniae*.
- *Haemophilus influenzae* can cause pneumonia, usually following epiglottitis, chronic bronchitis, brochiectasis, septicaemia or meningitis.
- *Pseudomonas aeruginosa* and other organisms cause chronic lung infections in patients with cystic fibrosis.
- *Legionella pneumophila* and other species of Legionella cause Legionnaires' disease (or legionellosis).
- The bacterium *Coxiella burnetii* is the cause of Q fever.
- *Chlamydophila psittaci* is a bacterium that causes a pneumonia called ornithosis (or psittacosis).
- Melioidosis is a type of pneumonia caused by the soil organism, *Burkholderia pseudomallei*.
- Respiratory syncytial virus, coronaviruses and human metapneumovirus are major causes of viral pneumonia.

CHRONIC INFECTIONS OF THE LOWER RESPIRATORY TRACT

- Tuberculosis is mainly caused by *Mycobacterium tuberculosis*.
- Lung disease is the most common form of tuberculosis.
- Pneumonia caused by *Pneumocystis jiroveci* is the most common infectious complication of AIDS.
- *Cryptococcus neoformans* is a yeast that can cause a lung infection, mainly in the immunocompromised.
- The tapeworm *Echinococcus granulosus* can infect the lungs and form hydatid cysts in the lung tissue.

STUDY QUESTIONS

1. List the major defences of the respiratory tract.
2. List the important factors that predispose people to respiratory infections.
3. What type of microorganisms are responsible for the majority of upper respiratory infections?
4. Why do people suffer repeatedly from the common cold?
5. What is otitis media, and what are the common causes?
6. What are the common causes of pharyngitis?
7. What are the important sequelae of *Streptococcus pyogenes* pharyngitis?
8. What is the cause of diphtheria, and what are the major features of this disease?
9. Describe the treatment for diphtheria.

10. Why is acute epiglottitis a potentially life-threatening disease?
11. What is croup?
12. How do microorganisms gain access to the lower respiratory tract?
13. Which adults should receive a booster vaccine for pertussis?
14. What is acute bronchitis, and what are the common causes?
15. What is bronchiolitis, and what is the usual cause?
16. What is meant by 'antigenic drift' and 'antigenic shift' in influenza viruses?
17. What is pneumonia?
18. Distinguish between lobar pneumonia and bronchopneumonia.
19. Define the terms 'pleural effusion', 'pleurisy' and 'empyema'.
20. What specimen(s) is/are commonly collected for the laboratory diagnosis of pneumonia?
21. What are the major risk factors associated with Legionnaires' disease?
22. What is Q fever, and how is it acquired by humans?
23. What viruses most commonly cause pneumonia?
24. What are the common clinical features of pneumonia?
25. What are the major aspects of patient management for a person with pneumonia?
26. What sites in the body does *Mycobacterium tuberculosis* infect?
27. Define the terms 'tubercle', 'miliary tuberculosis' and 'secondary tuberculosis'.
28. How is tuberculosis diagnosed?
29. What is a Mantoux test, and what does it detect?
30. How is MDR-TB treated?
31. What is the cause of pneumocystis pneumonia, and what is the major risk factor?
32. In a patient with fungal pneumonia, what is the most likely predisposing factor?

FURTHER READING

Australian Government Department of Health and Ageing 2008, *The Australian Immunisation Handbook*, 9th ed., <www.immunise.health.gov.au/internet/immunise/publishing.nsf/Content/Handbook-home>. (Comprehensive information on vaccine-preventable infections, vaccines and the National Immunisation Program.)

Guyard. C. and D.E. Low 2011, *Legionella* infections and travel associated legionellosis. *Travel Medicine and Infectious Disease* 9: 176–86. (Details the epidemiology, diagnosis and treatment of *Legionella* infections.)

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Vincent, J-L., D. Barros and S. Cianferoni 2010, Diagnosis, management and prevention of ventilator-associated pneumonia. *Drugs* 70(15): 1927–44. (Describes in detail the aetiology, management and preventative strategies of ventilator-associated pneumonia.)

CHAPTER
18

Gastrointestinal tract infections

CHAPTER FOCUS

- What microorganisms cause acute diarrhoeal disease in humans?
- What is the role of microorganisms in peptic ulcer disease?
- What microorganisms cause typhoid and paratyphoid fevers?
- What helminths are associated with gastrointestinal infections?
- What viruses cause hepatitis?
- What are the common modes of transmission of gastrointestinal infections?
- What are the important clinical features of gastrointestinal infections?
- How are gastrointestinal infections treated, and how may they be prevented?

INTRODUCTION

Intestinal infections are a major health problem throughout the world, but particularly in developing countries. Numerous microorganisms may enter the gastrointestinal tract (GIT) in foods and beverages but, fortunately, relatively few microbes are capable of causing intestinal disease. To establish and cause infection, the microbes have to be ingested in sufficient numbers, and then be able to circumvent a variety of highly effective antimicrobial defences, which include:

- gastric acidity
- digestive enzymes
- bile salts
- intestinal motility
- the normal flora of the intestine (see Figure 18.1)
- specific immune defences provided by the lymphoid tissues of the GIT.

These defences are described in detail in Chapter 9. It should be noted that the gastrointestinal tract is a hostile environment for most microorganisms, the majority of which are killed by the acid of the stomach and do not even reach the intestine alive. Certain pathogens, however, have properties that enable them to survive these defences and ultimately cause disease.

Most intestinal pathogens remain localised in the GIT, multiplying and/or producing toxins there and causing local symptoms. There are some, however, that cause more generalised symptoms by invading intestinal tissues and then spreading to other parts of the body. Other microbes cause gastrointestinal disease by growing and secreting toxins (exotoxins) in the food before it is consumed. These diseases have a very short incubation period, because symptoms are produced soon after the preformed toxins are ingested and absorbed.

Most gastrointestinal infections manifest as an acute diarrhoeal disease. In this chapter we first consider the major causes of acute diarrhoeal disease, which include bacteria, viruses and protozoa. There are other types of gastrointestinal infection in which diarrhoea is not a major symptom, and these include *Helicobacter pylori* infections (gastritis and peptic ulcers) and helminth (worm) infections. Other infections discussed in this chapter are the enteric fevers, typhoid and paratyphoid fever, caused by bacteria that invade the GIT and spread to other parts of the body, and hepatitis, an infection of the liver caused mainly by viruses.

FIGURE 18.1

***Escherichia coli* in the intestinal tract**

This organism is a major part of the normal flora of the intestine.

Source: EM Unit, VLA/Science Photo Library.

ACUTE DIARRHOEAL DISEASES

The terminology used to describe infections of the GIT can be very confusing. Some terms are used interchangeably and some are are given different meanings by different authors. In this text, **food poisoning** (or **food-borne illness**) is used in a general sense to refer to any gastrointestinal disease related to the consumption of food, including microbial and non-microbial factors. A **gastrointestinal infection** is a disease of the gastrointestinal tract caused by the establishment and multiplication of microorganisms in the GIT. **Food intoxication** is used for diseases caused by the presence of preformed toxins (microbial or non-microbial) in food. Other terms in common use and their meanings as applied in this text are given in Table 18.1.

A range of microbial pathogens is capable of producing symptoms of diarrhoeal disease. They include those that multiply in the GIT, as well as those that secrete toxins in food (food intoxications). Gastrointestinal pathogens are acquired mainly by the faecal–oral route, from contaminated food, fluids or hands. The major symptom of infection is **diarrhoea**, a disruption in bowel habits in which normally formed stools are replaced by more frequent, liquefied movements. Diarrhoea is the result of increased secretion of fluid and electrolytes into the lumen of the intestine, and represents a non-specific response of the intestine to a number of different factors, including infection, the action of toxins, sensitivity to drugs, and ischaemia. However, the most common causes are microbial infections due to bacteria, viruses or protozoa.

TABLE 18.1 Common terms used to describe gastrointestinal infections

TERM	DEFINITION
Food poisoning or food-borne illness	Gastrointestinal disease related to the consumption of food containing pathogenic microorganisms or their toxins, or other non-microbial toxins
Gastrointestinal infection	A disease of the gastrointestinal tract caused by the establishment and multiplication of microorganisms in the gastrointestinal tract
Food intoxication	Gastrointestinal disease caused by the consumption of food containing toxins (microbial or non-microbial)
Gastroenteritis	Inflammation of the gastrointestinal tract, including the stomach and intestine
Gastritis	Inflammation of the mucosa of the stomach
Diarrhoea	A disruption in bowel habits characterised by more frequent passage of loose and watery stools
Dysentery	An inflammatory disorder of the gastrointestinal tract characterised by severe diarrhoea with blood and pus in the stools

Acute diarrhoeal infections can result in a wide range of illnesses, from a mild attack of loose stools lasting one to several days to a severe illness with considerable fluid loss, which can be fatal. Overall, they are extremely common throughout the world, second only to upper respiratory infections.

In developing countries, diarrhoeal diseases are a major cause of morbidity and mortality. It has been estimated that they may be responsible for up to 3 million infant and childhood deaths per year. Diarrhoeal disease also contributes to the malnutrition that is prevalent in the children of developing countries. The magnitude of the problem of diarrhoeal disease in developed countries has declined with economic and public health improvements. But this type of disease is still a common complaint, although usually mild and self-limiting, except in the very young, the elderly and immunocompromised patients.

Notification rates of diarrhoeal diseases generally underestimate the true incidence, since many people do not seek medical attention and many cases are not identified by laboratory diagnosis.

Epidemiology of acute infectious diarrhoea

Acute infectious diarrhoea is usually acquired through ingestion of pathogenic microorganisms. Common sources of the organisms include:

- food or water contaminated with faeces
- faecally contaminated hands
- food contaminated with faecal organisms spread by flies.

Many pathogens of the intestinal tract are able to survive for at least a short time in the environment and on the surface of fomites. Some can survive for long periods in polluted natural water courses.

The highest incidence of acute diarrhoeal disease is in areas of the world where there are poor facilities for sanitation and sewage disposal. Transmission is mainly via contaminated food or water. In developed countries, food-borne transmission is the most common, but acute diarrhoeal disease also occurs in international travellers, hospitalised people and immunocompromised people. Outbreaks sometimes occur in hospital nurseries and daycare centres. Certain microbes tend to be associated with each of these particular epidemiological settings (see Table 18.2).

Food-borne gastroenteritis

Most cases of food-borne gastroenteritis (inflammation of the GIT) are due to bacteria, but viruses and parasites are also potential causes. Food-borne illness can occur when contaminated meats or seafood are eaten raw or are undercooked, or when food is improperly preserved, allowing significant multiplication of organisms in the food before its consumption. Generally, slightly contaminated food poses little risk if cooked properly and eaten fresh. Different foods tend to harbour certain types of organisms, as shown in Table 18.2.

TABLE 18.2 Epidemiological associations with microbes that cause diarrhoea

EXPOSURE	COMMON ORGANISMS
■ Food-borne: beef and pork	*Salmonella*, *Staphylococcus aureus*, *E. coli*, *Clostridium perfringens*, *Campylobacter*, *Yersinia*
poultry	*Salmonella*, *Staphylococcus aureus*, *Clostridium perfringens*, *Campylobacter*
milk and cheese	*Salmonella*, *E. coli*, *Campylobacter*, *Yersinia*
vegetables	*Clostridium botulinum*, *Salmonella*, *Shigella*, *Bacillus cereus*
shellfish	Vibrios, norovirus, hepatitis A
water	*E. coli*, vibrios, *Shigella*, *Giardia*, *Cryptosporidium*
■ Schools, nurseries and daycare centres	*Shigella*, *rotavirus*, *Campylobacter*, *Giardia*, *Cryptosporidium*, *Salmonella*, *Aeromonas*, *Plesiomonas*
■ Travellers	*E. coli*, *Shigella*, *Campylobacter*, *Giardia*, *Aeromonas*, *Salmonella*, *Entamoeba histolytica*, *Vibrio cholerae*
■ Hospitalised patients receiving antibiotics	*Clostridium difficile*
■ Immunocompromised (e.g. AIDS)	*Cryptosporidium*, *Isospora*, *Salmonella*, *Microsporidium*

Most cases of food-borne gastroenteritis are self-limiting, resolving in a few days with supportive care only. However, in certain situations, severe and even life-threatening disease can occur. Young children, the elderly and the immunocompromised are particularly susceptible. Children can dehydrate very rapidly and may require hospitalisation and intravenous fluids.

Hospital nurseries

Many pathogens can cause diarrhoeal disease in neonates. Infants can become infected by bacteria or viruses during passage through the birth canal, or after birth from parents, siblings, hospital personnel, or contaminated formula or water. Because of the immaturity of their immune system, they are highly susceptible to any pathogens encountered. Nursery outbreaks sometimes occur, often initiated by an asymptomatic infant shedding the organism.

Daycare centres

The incidence of diarrhoeal disease in children attending daycare centres can be up to twice that in children at home. Factors promoting spread include the presence of non-toilet trained children, oral exploration of objects, contamination of hands, close contact, and lack of appropriate infection control measures. *Shigella* spp., *Giardia intestinalis*, rotavirus and *Cryptosporidium* have frequently been associated with outbreaks. These organisms typically require only a low inoculum in order to cause infection in infants.

Faecal–oral spread and environmental contamination are the most common means of transmission. The most important interventions to prevent outbreaks of infection in this setting are good hygiene and handwashing practices.

Traveller's diarrhoea

This is the name given to acute diarrhoea that frequently occurs in people visiting a foreign country. Travellers may be exposed to unfamiliar organisms for which they have no specific immunity, and which may cause acute diarrhoea. Some pathogens are contracted by consumption of faecally contaminated food or water, especially when visiting countries with poor sanitation and sewage disposal, or by eating unwashed fruit and vegetables in areas where sewage is used as a fertiliser. Certain countries in Asia, the Middle East, Africa and South America are well recognised as regions where there is a high risk of developing gastroenteritis. Enterotoxigenic *E. coli* is the predominant cause of traveller's diarrhoea; other common causes are shown in Table 18.2.

Traveller's diarrhoea is generally a benign, self-limiting disease, although some of the more virulent agents may cause severe illness, such as dysentery (e.g. *Shigella*) or chronic diarrhoea (e.g. *Giardia*).

Hospitalised patients

People sometimes develop diarrhoea while in hospital. Mild diarrhoea is a common side effect of antibiotic therapy, but a more serious form, also associated with antibiotic therapy, is due to the bacterium *Clostridium difficile*. Broad spectrum antibiotics can predispose a patient to a pseudomembranous colitis, caused by *C. difficile*, by altering the intestinal microbial flora that normally inhibit its establishment in the intestine.

People with AIDS

Almost all AIDS patients experience at least one episode of diarrhoea during their illness. The suppression of their immune system makes them susceptible to some unusual, opportunistic organisms, as shown in Table 18.2.

Clinical features of acute diarrhoeal disease

It is difficult to differentiate between the causative agents of acute diarrhoea on the basis of signs and symptoms alone. Most acute diarrhoeal infections have an incubation period of 1–4 days, but shorter or longer incubations can occur (see Table 18.3). Most diarrhoeal infections are self-limiting, but the illness can be as short as one day or as long as three weeks, depending on the cause. Infectious diarrhoea can be divided into one of two syndromes based on the nature of the diarrhoea produced:

1. *non-inflammatory diarrhoea*—characterised by watery stools without blood, mucus or pus
2. *inflammatory diarrhoea (or dysentery)*—characterised by stools containing blood, mucus and pus.

Both types can be serious, although inflammatory diarrhoeas are generally more severe than the non-inflammatory types. The characteristics of these two forms of diarrhoea are summarised in Table 18.4.

Inflammatory diarrhoeas are caused by invasive microorganisms or by organisms that liberate toxins. Mainly the colon is affected, resulting in disruption of the mucosal lining.

TABLE 18.3 Diarrhoeal diseases: Incubation period and duration of illness

PATHOGEN	INCUBATION PERIOD	DURATION OF ILLNESS
Staphylococcus aureus	1–6 hours	12–24 hours
Bacillus cereus	1–24 hours	12–48 hours
Clostridium perfringens	6–24 hours	12–24 hours
Norovirus	1–2 days	24–48 hours
Campylobacter spp.	2–5 days	3 days to 3 weeks
Salmonella spp.	1–2 days	2–7 days
Vibrio parahaemolyticus	1–2 days	1–3 days
Vibrio cholerae	1–3 days	5–7 days
Rotavirus	1–4 days	4–7 days
Enterotoxigenic *E. coli*	1–3 days	5–10 days
Shigella spp.	1–4 days	1–3 days
Yersinia enterocolitica	4–7 days	1–2 weeks
Cryptosporidium parvum	1–2 weeks	1–3 weeks
Giardia intestinalis	1–2 weeks	weeks to months

Damage to the colonic lining leads to the oozing of red cells, serous fluid and white cells into the lumen of the intestine. Typically, the patient has a small-volume, bloody diarrhoea, often with mucus. The patient also frequently complains of tenesmus (straining without passing stools), faecal urgency (inability to delay passing stools) and abdominal cramps. The patient usually has a fever. Microscopic examination of the stool reveals the presence of numerous leucocytes. The microorganisms that usually cause an inflammatory diarrhoea are listed in Table 18.4.

Non-inflammatory diarrhoea is caused by microorganisms that primarily affect the small intestine. They may adhere to the intestinal epithelium, secrete toxins or invade the cells, but they generally don't cause significant damage to the tissue. They produce diarrhoea by causing excessive secretion of fluids from the lining cells. Typically, the patient has a profuse, watery, non-bloody diarrhoea and often experiences nausea and abdominal cramping as well. In viral infections, vomiting is common. Leucocytes and red cells are not generally found in the stools, because of the absence of mucosal destruction and leakage of cells from the bloodstream. Typical causes are listed in Table 18.4.

Bacterial causes of acute diarrhoea

Campylobacter

Campylobacter are comma-shaped Gram-negative rods. Since the mid-1980s they have emerged as the most common causes of diarrhoea in humans in many developed countries. In Australia, an average of 16 000 cases are reported annually. A number of *Campylobacter* species are associated with human disease, including *C. coli*, *C. concisus* and *C. upsaliensis*, but *C. jejuni* is by far the most common.

A large reservoir of *Campylobacter* bacteria exists in animals such as cattle, sheep, poultry and wild birds. Human infections are thus usually acquired by consumption of contaminated and poorly cooked foods of animal origin, especially poultry and red meat, and unpasteurised milk. Infection can also occur if foods that are eaten raw or lightly cooked (e.g. vegetables) are contaminated with fluid from raw chicken meat (e.g. via a cutting board). Symptoms develop 2–5 days after infection.

Campylobacter enteritis is due to ulceration and inflammation of the mucosal surface in the jejunum, ileum and colon. Several toxins of *C. jejuni* have been identified. Some strains produce a heat-labile, cholera-like enterotoxin, which is responsible for the diarrhoea. The role of other toxins has not been fully elucidated. Invasion and bacteraemia can occur, especially in neonates and debilitated adults. Some infected people excrete the organism asymptomatically. Guillain-Barre syndrome, an immune-mediated disease characterised by neuromuscular paralysis, is a rare post-infectious complication of *C. jejuni* infection.

Salmonella

Salmonellae are Gram-negative bacilli whose natural habitat is the intestinal tract of a variety of animals. In Australia, 7000–9000 cases are reported annually, making it the second most common cause of diarrhoea, after *Campylobacter*. The highest rates of notification occur in children under 4 years of age and in the northern parts of the country. There are numerous serovars (serotypes) of *Salmonella* that cause gastroenteritis, the most common being *Salmonella typhimurium*. Other serotypes commonly encountered in Australia include *S. enteritidis*, *S. virchow* and *S. saintpaul*. In the US, large multi-state outbreaks of **salmonellosis** have occurred and caused the recall of large amounts of various types of meat products from retailers.

The major source of diarrhoea-causing salmonellae is the intestinal tract of animals. In developed countries the organisms are transmitted to humans mainly via contaminated foods, especially poultry and eggs, meats such as pork, lamb and beef, and dairy products. Typically, salmonellae cause infection when the food is improperly stored and/or cooked. Takeaway chickens and foods containing eggs, such as baby formulas, have caused outbreaks of infection. Salmonellae can also be transmitted from infected people and asymptomatic carriers to other people, such as when food handlers who are carriers do not observe proper hygiene standards.

TABLE 18.4 The differences between inflammatory and non-inflammatory diarrhoea

CHARACTERISTIC	INFLAMMATORY DIARRHOEA	NON-INFLAMMATORY DIARRHOEA
Leucocytes in stools	Present	Absent
Blood in stools	Present	Rare
Mucus	Present	Rare
Stool volume	Small (normal)	Greatly increased
Abdominal pain	Severe—lower left quadrant	None to slight
Body temperature	May be elevated	Usually normal
Site of infection	Colon	Small intestine
Common causes	*Shigella*, *Salmonella*, *Yersinia*, *Campylobacter*, *Clostridium difficile*, invasive and enterohaemorrhagic *E. coli*, *Entamoeba histolytica*, *Vibrio parahaemolyticus*, *Aeromonas*	Viruses, *Vibrio cholerae*, *Giardia*, enterotoxigenic and enteropathogenic *E. coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens*, *Cryptosporidium*, *Isospora*, *Cyclospora*

CASE HISTORY 18.1

Salmonella gastroenteritis

On 26 March 2007 a Sydney hospital notified the Public Health Unit of the local Area Health Service that five people had presented to the emergency department with severe gastroenteritis, and that they had eaten food from the same bakery in the inner west of Sydney. An investigation was initiated involving telephone interviews of suspected cases and surveillance for cases via hospitals, general practitioners, pathology laboratories and the public health network. *Salmonella typhimurium* phage type 9 was identified as the cause of the outbreak.

There were 319 cases identified in the outbreak, with 221 of these cases presenting to a hospital emergency department and 136 requiring admission. Environmental samples from the bakery revealed widespread contamination throughout the premises. It was considered that raw egg mayonnaise was the most likely source of the *Salmonella* organisms.

Source: Adapted from T. Mannes et al. 2010, A large point-source outbreak of *Salmonella typhimurium* phage type 9 linked to a bakery in Sydney, March 2007. *Communicable Diseases Intelligence* 34(1): 41–48.

Questions

1. What are the possible ways that the mayonnaise could have become contaminated with *Salmonella*?
2. What are the possible reasons for hospitalisation of some people with Salmonella gastroenteritis?
3. How should the majority of the infected people be treated?
4. What infection control procedures are required in the hospitals treating patients with *Salmonellosis*?

Salmonellae invade the intestinal mucosa of the ileum and colon to produce disease. They replicate in the lamina propria, causing diarrhoea with blood and mucus. Most cases of salmonellosis are self-limiting diarrhoeal diseases. After clinical recovery, many patients become asymptomatic carriers for weeks to months and continue to shed the organisms in faeces. In some cases, especially in the young, elderly and immunosuppressed, the organisms invade mesenteric lymph nodes from which they can spread via the bloodstream to cause arthritis, osteomyelitis, pneumonia or meningitis.

Escherichia coli

Escherichia coli are Gram-negative rod-shaped bacteria that can survive in both aerobic and anaerobic environments (i.e. they are facultative anaerobes). Some strains are members of the normal GIT flora in humans and animals, where they can actually benefit their host in a number of ways. Other strains possess virulence factors that enable them to cause infection in the intestinal tract and sometimes other organs. The strains that cause diarrhoeal disease in humans have been classified into five main groups according to their pathogenic mechanism.

1. **Enterotoxigenic *E. coli*** (**ETEC**) is a major cause of diarrhoea in children in developing countries (an estimated 650 million cases per year), and of traveller's diarrhoea. This organism binds to specific receptors on the intestinal cell membranes where it releases potent enterotoxins. The heat-labile toxin (LT) of ETEC is similar to cholera toxin (see Chapter 10). Like cholera toxin, it causes an increase in levels of cyclic AMP in intestinal epithelial cells, resulting in a massive outflow of water and electrolytes into the lumen of the intestine, manifested as profuse diarrhoea. In addition, the heat-stable toxin (ST) of ETEC causes diarrhoea by a similar mechanism.
2. **Enterohaemorrhagic *E. coli*** (**EHEC**) are also known as Shiga toxin-producing *E. coli* (STEC) or Vero toxin-producing *E. coli* (VTEC). They are normal inhabitants of the intestine of ruminant animals, including cows, goats and sheep. Some serotypes are important causes of gastrointestinal disease in humans, ranging from mild diarrhoea to a life-threatening haemorrhagic colitis, which can sometimes develop into **haemolytic uraemic syndrome** (**HUS**). HUS is a serious complication, characterised by a haemolytic anaemia (see Figure 18.2), uraemia, thrombocytopaenia and renal failure, which can occur within the first few days of onset. Renal failure causes death in some patients. HUS can occur in any age group but is most common in children under 4. Throughout the world, *E. coli* O157 is the serotype most often associated with HUS, but other serotypes (e.g. *E. coli* O111) may also be responsible. The 'O' in the name refers to the cell wall antigen, followed by the number that the particular serotype has—e.g. 157 or 111. There are over 700 'O' serotypes. Sometimes an additional antigen is identified in the name, such as *E. coli* O157:H7. The 'H7' indicates that the organism has serotype 7 flagella antigen. In 2011, *E. coli* O104:H4 caused a large outbreak of bloody diarrhoea and HUS in Germany (see Spotlight box: Outbreak of haemolytic uraemic syndrome).

   The main virulence factor of EHEC is Shiga toxin (actually two toxins called Shiga toxins 1 and 2, or Verotoxin), which causes destruction of the colonic epithelium and haemorrhage. It is very similar to the Shiga toxin that was first identified in *Shigella* (see later in this chapter). HUS occurs when Shiga toxin and probably other toxins of the bacterium together cause small blood vessel damage in various sites, including the kidney. Red cells are fragmented when passing through these damaged vessels, resulting in anaemia. Platelets, which cluster in the vessels, are destroyed, causing a thrombocytopaenia. The diagnosis of EHEC infections can be difficult because the organisms are rarely present in stools after the initial diarrhoeal phase. The need for

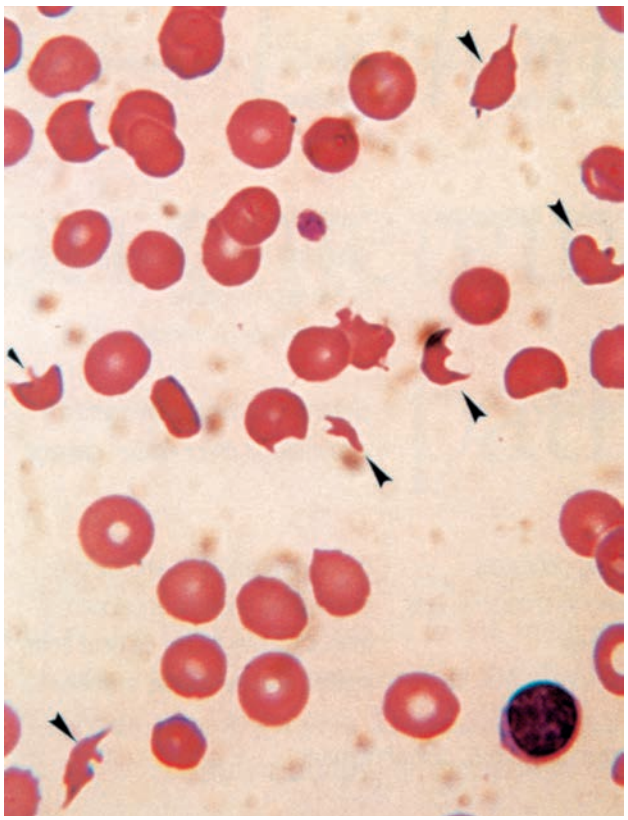

FIGURE 18.2

Smear of peripheral blood from a patient with haemolytic uraemic syndrome, showing red cell fragments (arrowheads)

treatment of HUS is urgent and may require intensive care, haemodialysis and prolonged hospitalisation.

Sporadic cases of HUS associated with EHEC have been reported in Australia, but the first outbreak due to a common source was recorded in South Australia in early 1995, when 20 children were affected and required hospitalisation. Some suffered permanent kidney damage, and one child died. HUS became notifiable in Australia in late 1998, and around 100–150 cases are currently notified each year. Large outbreaks have occurred in the United States and other developed countries. This highly virulent organism has a low infectious dose of about 10–100 bacteria.

3. **Enteropathogenic *E. coli*** (**EPEC**) is a leading cause of infantile diarrhoea in developing countries, but also occasionally occurs in developed countries. EPEC appear to adhere intimately to enterocytes and cause destruction of the microvilli at that site.
4. **Enteroinvasive *E. coli*** (**EIEC**) cause a gastrointestinal disease that is similar to the dysentery caused by *Shigella* spp. EIEC secrete toxins and actively invade epithelial cells of the large intestine and spread to adjacent cells, causing cell death, ulceration of the mucosa and inflammation. The inflammatory response causes an outpouring of pus, blood and mucus into the lumen.
5. **Enteroaggregative *E. coli*** (**EAEC**) is a subgroup that appears to cause disease by adhering to the intestinal surface by biofilm formation (in an aggregative manner) and releasing toxins that damage intestinal cells. EAEC cause acute and persistent diarrhoea, mainly in children in less-developed countries, and in travellers and the immunocompromised.

Cholera

Cholera is caused by the comma-shaped Gram-negative bacterium *Vibrio cholerae*. The toxin-producing serogroups O1 andO139 (called the Bengal strain) cause the rapidly dehydrating diarrhoeal disease. Cholera is endemic in many areas of the world, particularly in parts of South-East Asia, Africa, and Central and South America. The global burden of the disease is estimated at 3–5 million cases per year, with 100 000 to 130 000 deaths. Seven pandemics of cholera have occurred in the past 170 years, the latest and current one affecting West and Central Africa, Haiti (see Case History 18.2) and South America. In Australia, fewer than five cases are reported annually and these infections are almost always acquired in other countries.

V. cholerae causes infection only in humans. Symptomatic people and asymptomatic carriers are the reservoirs of infection. The organism is able to survive for long periods in water and thus occurs most frequently in areas where clean drinking water and adequate sewage disposal are not available (see Figure 18.3). Transmission via food (e.g. seafood)

Outbreak of haemolytic uraemic syndrome

SPOTLIGHT ON

On 1 May 2011, an outbreak of bloody diarrhoea and haemolytic uraemic syndrome (HUS), caused by Shiga toxin-producing *E. coli* O104:H4, began in Germany. Almost 4000 cases occurred during the outbreak, which progressed until 26 July 2011. The majority of cases occurred in Germany, but some were reported in over 15 other European countries. HUS occurred in approximately 20 per cent (850) of these cases, and 46 people died of the infection. Epidemiological studies identified contaminated fenugreek seeds used for growing sprouts as the probable vehicle.

The unusually high proportion of HUS cases suggests that the outbreak strain was highly virulent. Apart from the Shiga toxin, the outbreak strain also had extra virulence genes characteristic of another group, called enteroaggregative *E. coli*, giving it the capability to cause a more serious disease. Another unusual aspect of the outbreak was that the majority of HUS cases were adults.

CASE HISTORY 18.2

Cholera

On 12 January 2010 a massive 7.0 magnitude earthquake struck the Caribbean nation of Haiti. The centre of the quake was about 15 kilometres south-west of the capital, Port-au-Prince, and was followed by two strong aftershocks of 5.9 and 5.5 magnitude. The quake left tens of thousands of people homeless, and led to the establishment of numerous, rudimentary camps throughout the country.

No cases of cholera had been seen on the island for over a century, but in October 2010, a cholera outbreak began and was still raging 12 months later. Within that year 470 000 cases had been recorded, resulting in over 240 000 hospitalisations and 6500 deaths. Containment of the outbreak was severely hindered by the chronic deficiency of clean water and sanitation, especially in country areas.

Questions

1. What does this outbreak indicate about how cholera can be transmitted?
2. In cholera outbreaks, what factors/conditions would lead to high fatality rates?
3. What precautions should health professionals take when attending to patients with cholera?
4. Why is vaccination against cholera not generally recommended for travellers to endemic countries?

contaminated with human faeces also occurs. The infectious dose is between 100 and 1 million organisms. The disease is often associated with natural disasters (e.g. floods, earthquakes) as well as situations of social unrest and upheaval.

V. cholerae causes disease by secreting the cholera toxin. This toxin causes the levels of cyclic AMP in intestinal epithelial cells to increase, resulting in a massive outflow of water and electrolytes into the lumen of the intestine, manifested as profuse, watery diarrhoea. Chapter 10 gives a more detailed description of its action. Many cases are mild or asymptomatic. In severe cases, the patient may lose up to 30 litres of fluid in a day, and the stools are primarily a mixture of water and mucus, having the classic appearance of 'rice water stools'. Without fluid and electrolyte replacement, mortality can be as high as 40–60 per cent.

An oral cholera vaccine, composed of killed *V. cholerae* O1 organisms and the non-toxic B subunit of cholera toxin, is currently available in Australia (Dukoral®). A bivalent oral vaccine (O1 and O139) is in use in some endemic parts of the world. Most travellers are at low risk of contracting cholera, even in endemic areas, so routine vaccination is not recommended. Water and food hygiene and handwashing are considered far more important for prevention.

FIGURE 18.3

A typical scenario for cholera transmission

Source: Centers for Disease Control (CDC).

Immunisation might be considered for people at increased risk of severe or complicated diarrhoeal disease, such as those with inflammatory bowel disease, HIV/AIDS or other conditions resulting in impaired immunity. It may also be considered for workers in disaster areas.

Shigella

Shigella diarrhoea is also known as bacillary dysentery. There are four species: *Shigella sonnei* causes mostly mild infections, *S. flexneri* and *S. boydii* usually cause moderate disease, and *S. dysenteriae* is the most serious. *Shigella* infections are most common in children under 10 years and are often reported in schools and daycare centres. Daycare staff and family members may also become infected. Shigellosis is more common in disadvantaged communities and is much more prevalent among Aboriginal children than other Australian children. On average, 650 cases of shigellosis are notified annually in Australia. The highest rates of notification occur in the Northern Territory and in children under 4 years of age.

Shigella are strictly human pathogens, so the source of infection is always a symptomatic person or carrier of the organisms. Faecal–oral spread is the major mode of transmission. Prolonged excretion of *Shigella* may occur in some patients for several weeks after recovery. Handwashing and good hygiene are considered to be the most important means of preventing transmission of these organisms.

Shigella are readily transmitted and highly virulent—as few as 10–100 organisms may cause disease. Their pathogenesis is due to their enteroinvasive properties, as well as their secretion of the Shiga toxin. In the intestine, the bacteria are phagocytosed by colonic epithelial cells. They then lyse the phagocytic vesicle, multiply intracellularly and invade adjacent cells. The Shiga toxin acts by cleaving ribosomal RNA in colonic cells, halting protein synthesis and killing the cell. The Shiga toxin damages some endothelial cells (e.g. in the kidneys) and in some cases causes haemolytic uraemic syndrome similar to the enterohaemorrhagic *E. coli*.

CASE HISTORY 18.3

Shigellosis

In August 2007, Queensland Health investigated an outbreak of 11 cases of *Shigella sonnei*. Most of the cases reported either consuming imported baby corn from Thailand or eating at a venue where imported baby corn was served. Four of the cases were part of a larger outbreak among a film production crew which involved another 43 probable cases. Two more cases were infected while in hospital, and a further two ate at a particular holiday resort. The median age of cases was 31 years (range 18–76 years).

Epidemiological investigation indicated that 8 of the 11 cases may have eaten baby corn that was part of a consignment imported in July by a wholesaler in Queensland from an agent in Thailand. An outbreak of shigellosis had recently been reported in Denmark, also associated with the consumption of imported baby corn from Thailand. This outbreak in Queensland was thought to be possibly linked to the Danish outbreak through a common source in Thailand.

Source: Adapted from R. Stafford et al. 2007, An outbreak of multi-resistant *Shigella sonnei* in Australia: Possible link to the outbreak of shigellosis in Denmark associated with imported baby corn from Thailand. *Eurosurveillance* 12(37), Article 3266.

Questions

1. What is the usual means of transmission of *Shigella*?
2. Why was this organism transmitted to so many people in this outbreak?
3. Why does this organism predominantly affect children?

Clostridium difficile

Diarrhoea can arise whenever the normal intestinal flora is disrupted or altered. When antimicrobial drugs, particularly broad spectrum agents, are used in the treatment of infectious diseases, they can affect the intestinal normal flora. A change in the balance of the normal flora can lead to a mild diarrhoea. Since the late 1970s it has been recognised that antibiotic therapy can sometimes lead to much more serious gastrointestinal disease; this is characterised by severe diarrhoea and a form of inflammation in the colon that results in the formation of yellow-white raised plaques or pseudomembranes on the colonic mucosa.

The Gram-positive anaerobic rod, *Clostridium difficile*, is the cause of this colitis. The normal colonic flora of adults and children over the age of 12 months inhibit colonisation by *C. difficile*, but antibiotics can disrupt the flora, allowing *C. difficile* to become established, multiply and produce disease. *C. difficile* infection (CDI) may present in a variety of ways, ranging from mild diarrhoea without abdominal discomfort to the potentially life-threatening pseudomembranous colitis. Some people carry the organism asymptomatically.

The organism is able to survive for long periods in the hospital environment, and is resistant to alcohol and many other disinfectants because of its spores. It has been cultured from floors, toilets, bedding, mops and furniture, and also from the hands of hospital personnel. Heavy contamination can be found in rooms that have been occupied by infected patients. Thus, transmission of the organism to an individual who is susceptible because of antibiotic therapy can readily occur in a hospital. Asymptomatic carriers may also contribute to spread within a healthcare facility.

Although pseudomembranous colitis was initially related to clindamycin usage, almost all broad spectrum antibiotic agents have now been implicated in this disease; the main offenders are clindamycin, ampicillin, cephalosporins and aminoglycosides. While the use of antibiotics is recognised as the major predisposing factor, anything that disrupts the intestinal flora of the gut, including another gastrointestinal infection, can lead to CDI. The time from antibiotic exposure to onset of symptoms can range from one day up to eight weeks. Elderly patients are most susceptible to CDI. Although it is most often acquired in hospitals, community-acquired CDI can occur.

C. difficile produces two exotoxins, A and B, which are cytotoxic for a number of cell types; they cause haemorrhage, and induce a local inflammatory response and destruction of the intestinal mucosa. The pseudomembrane—consisting of necrotic tissue, mucus, and neutrophils and monocytes—forms over the mucosa (see Figure 18.4). Perforation of the colon, abscess formation and vascular thrombi are possible late complications.

The emergence of a hypervirulent strain of *C. difficile*, associated with high morbidity and mortality, is of great concern. This strain, called ribotype 027, was first identified in Quebec, Canada in 2005, and has since been reported

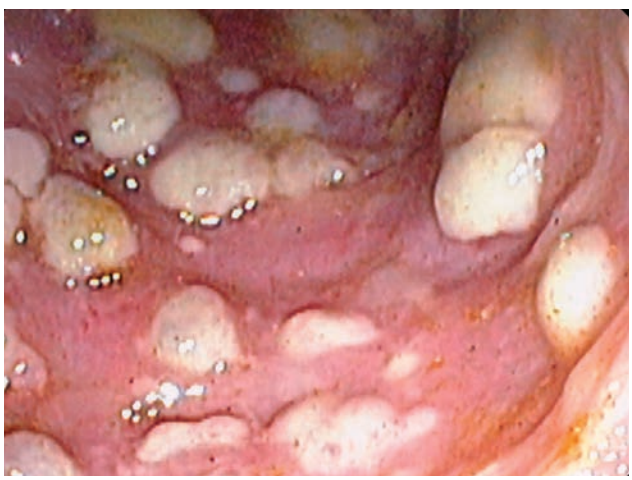

FIGURE 18.4

Pseudomembranous colitis

Photo taken through a colonoscope shows lesions in the wall of the colon.

Source: David M. Martin, MD/Science Photo Library.

in hospital outbreaks in Canada, the US and Europe and in some clusters of infection in residential aged-care facilities.

Contact precautions including traditional soap and water handwashing (rather than an alcohol handrub) are recommended in this situation. Environmental cleaning with a hypochlorite solution is necessary.

Vibrio parahaemolyticus

Vibrio parahaemolyticus is a Gram-negative, halophilic (salt-loving) organism commonly found in seafood such as crabs and fish, and oysters, which are usually eaten raw. In Japan and South-East Asia, where seafoods are often consumed uncooked, this bacterium is responsible for a large number of cases of food-borne illness. The mechanism of pathogenesis is thought to be due to two toxins which have enterotoxic and cytotoxic effects. A number of other vibrios cause diarrhoeal disease, including *V. vulnificus*, *V. fluvalis* and *V. hollisae*.

Yersinia

Yersinia enterocolitica is a Gram-negative rod that is a member of the family Enterobacteriaceae. The organism is found in a large variety of animals, especially pigs and cows. As a result, many cases of yersiniosis are traced to undercooked pork or other meats and dairy products. *Yersinia* is also sometimes found in other domestic animals—transmission to humans from pet dogs has been reported. Faecal–oral spread between humans also occurs. Most infections occur in young children. In Australia, 200–400 cases of yersiniosis are reported each year.

The pathogenesis of *Y. enterocolitica* stems mainly from its ability to invade the mucosa of the ileum and replicate in the Peyer's patches. It causes tissue destruction and produces an inflammatory diarrhoea. After an incubation period of 4–7 days, diarrhoea, low-grade fever and abdominal pain usually develop. Mucosal ulceration and necrotic lesions in the small intestine may occur in severe cases. Another species, *Y. pseudotuberculosis*, is also associated with diarrhoeal disease in humans.

CASE HISTORY 18.4

Clostridium difficile ribotype 027

The first case of *Clostridium difficile* ribotype 027 infection acquired in Australia was considered to have occurred in January 2010. An 83-year-old man developed a urinary tract infection and severe sepsis two days after an aortic valve replacement. Initially he was given ticarcillin-clavulanate. Vancomycin was then added when coagulase negative *Staphylococcus* was isolated from blood cultures. Five days after surgery he developed watery diarrhoea, and *C. difficile* was isolated from his stools. Despite commencement of therapy with metronidazole his diarrhoea persisted, and the metronidazole was replaced after nine days with another course of vancomycin. *C. difficile* ribotype 027 was confirmed as the causative agent from repeat stool samples. His condition continued to worsen and different regimes of vancomycin and metronidazole were instituted over the following weeks. The infection was ultimately cleared five weeks after the surgery.

Source: Adapted from M. Richards et al. 2011, Severe infection with *Clostridium difficile* PCR ribotype 027 acquired in Melbourne, Australia. *Medical Journal of Australia* 194: 369–71.

Questions

1. What was the most likely predisposing factor for this man's *C. difficile* infection?
2. Why is *C. difficile* difficult to eradicate from the hospital environment?
3. What infection control measures should be introduced in a case such as this?

Clostridium perfringens

Clostridium perfringens is associated with diarrhoeal disease when the bacteria are ingested in contaminated food, followed by *in vivo* secretion of an enterotoxin. This organism produces two different food-borne illnesses. Type A organisms cause a self-limiting diarrhoeal illness, which occurs in developed countries, and type C organisms cause a potentially life-threatening syndrome known as *enteritis necroticans* in underdeveloped tropical areas.

Type A clostridial food poisoning is typically associated with meat and poultry products in which there is a long delay between cooking and serving of the food. Clostridial spores, which are widely present in human and animal faeces and uncooked meats and vegetables, can survive cooking temperatures and can germinate and multiply when meat products are allowed to stand at 15–60°C for several hours or more. The food must be heavily contaminated for infection to occur. This food-borne illness is usually associated with situations where bulk food preparation occurs, such as in institutions and fast-food outlets.

Enteritis necroticans is usually due to the consumption of ß-toxin-producing type C strains of *C. perfringens* in undercooked pork. It was traditionally associated with ritual pig-feasting by the natives of highland New Guinea, where it is known as 'pig-bel'. This syndrome has also been reported in countries such as Thailand, Nepal and China.

C. perfringens also causes gas gangrene in wounds and soft tissue (see Chapter 16).

Other bacterial causes

A variety of other bacteria, including *Aeromonas* spp. and *Plesiomonas* spp., are potential causes of diarrhoeal illness. These organisms are most often associated with seafood or contaminated water.

Viral causes of acute diarrhoea

Viruses are responsible for more cases of diarrhoeal illnesses in developed countries than any other types of organisms, and they particularly affect children under the age of 5 years. Worldwide, rotaviruses are the most important in this age

group, but noroviruses, other caliciviruses and enteric adenoviruses also cause disease in this and other age groups. Enteric viruses survive well in the environment and are somewhat resistant to sewage treatment processes and post-treatment chlorination.

Rotavirus

Rotaviruses (Figure 18.5), first identified in 1973 by the Melbourne scientists Ruth Bishop and Ian Holmes, are a major cause of gastroenteritis in infants. Rotavirus gastroenteritis is a significant cause of death in young children, mainly in underdeveloped countries. Most infections occur in children under 2 years of age and are potentially life-threatening due to severe dehydration. Intravenous fluids may be urgently required. Older children and adults are less susceptible, presumably because of immunity developed early in life.

Very large numbers of virus particles (>10^{12} particles per gram) are shed in faeces of an infected person. Cross-infection readily occurs since the infective dose in a child is thought to be only 10–100 particles. The virus infects enterocytes at the villous tips in the small intestine, causing lysis of the cells and destruction of the villous tip epithelium. Transport mechanisms in the intestine are disrupted and the loss of water and electrolytes results in a severe watery diarrhoea lasting 4–7 days.

Safe and effective rotavirus vaccines have recently become available in many countries. Rotavirus immunisation was added to the Australian Immunisation Program from 1 July 2007. In the pre-vaccine era Australia, rotavirus was thought to be responsible for as many as 10 000 children hospitalisations each year. However, there are early indications that there has been a significant reduction in the incidence since 2007. Outbreaks may still occur in hospital nurseries in babies under vaccination age.

Norovirus

Noroviruses (formerly known as Norwalk and Norwalk-like viruses) are a diverse group of viruses belonging to the Caliciviridae family. Noroviruses are important food-borne pathogens that can cause outbreaks of gastroenteritis—sometimes called 'gastric flu'. Infection is most common in older children and adults, usually associated with contaminated food, or in institutions such as nursing homes or hospitals by person-to-person contact or in food. A contaminated healthcare environment may also act as a source, since the virus survives well on surfaces and is resistant to some disinfectants. The foods most commonly implicated are poorly cooked or raw shellfish. For example, Sydney rock oysters have been implicated in a number of outbreaks of gastroenteritis, resulting in the introduction of compulsory purification of oysters in tanks of clean water. Contaminated drinking water and natural waters used for swimming have also been implicated. A number of outbreaks on cruise ships have occurred in the last decade.

Symptoms of diarrhoea, vomiting and sometimes fever typically develop abruptly, sometimes within eight hours after exposure. Symptoms usually last for 1–2 days, but may persist for much longer in young children and the elderly. Norovirus is excreted in the faeces of infected people and shedding may continue in asymptomatic people for more than a year. Noroviruses may also be transmitted via droplets aerosolised after a vomiting episode. The infectious dose is thought to be low, possibly less than 100 viruses.

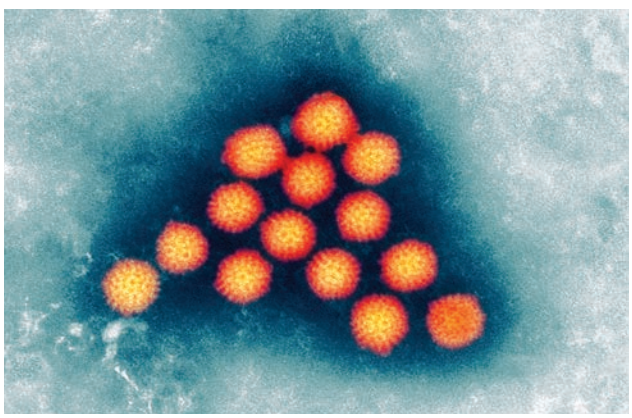

FIGURE 18.5

Rotavirus—wheel-shaped viruses that cause diarrhoea

Source: Hazel Appleton, Centre for Infections/Health Protection Agency/Science Photo Library.

Other viruses

Enteric adenoviruses (serotypes Ad40 and Ad41) cause gastroenteritis and are thought to be major causes of acute diarrhoea in paediatric patients. Caliciviruses have been found to cause diarrhoea in children in daycare centres, typically affecting children aged 3 months to 6 years. Prolonged excretion of these viruses by symptomatic and asymptomatic individuals can make control extremely difficult. Astroviruses mainly affect children under 4 years of age and are a common cause of diarrhoea in hospitalised children. The symptoms are similar to those of rotavirus, but less severe. Aichi virus, a relatively newly identified virus, has recently been linked to food-associated diarrhoea.

Protozoal causes of acute diarrhoea

The three main protozoal pathogens in diarrhoeal disease are *Giardia intestinalis*, *Entamoeba histolytica* and *Cryptosporidium parvum*. In most cases, infection depends on contact with faecally contaminated material, infection flourishing where low standards of hygiene and sanitation, poverty and overcrowding exist.

Giardia intestinalis

The first intestinal parasite to be seen under a microscope was *Giardia intestinalis* (also called *G. lamblia* and *G. duodenalis*) when, in 1681, Anton van Leeuwenhoek peered through the simple microscope he had just made and saw the moving 'animalcules' in a preparation of his own faeces. *G. intestinalis* is a flagellated protozoan (Figure 18.7) now recognised to have a worldwide distribution, with up to 20 per cent of the world population thought to be chronically infected.

CASE HISTORY 18.5

Norovirus

In June 2002 the Australian Capital Territory Health Protection Service commenced investigation of an outbreak of gastroenteritis in two aged-care facilities and one hospital. A particular norovirus genotype (type II) was detected in some staff and residents/patients in each of the institutions. The outbreak lasted just over a month, in which 281 cases were identified. The attack rate in staff in each institution ranged from 43.0 to 48.6 per cent, and in residents from 51.3 to 66.1 per cent. As can be seen from Figure 18.6, transfer of residents had occurred between the institutions during the period of the outbreak. Secondary transmission of the virus appeared to occur from staff members of institutions A and B to their family members.

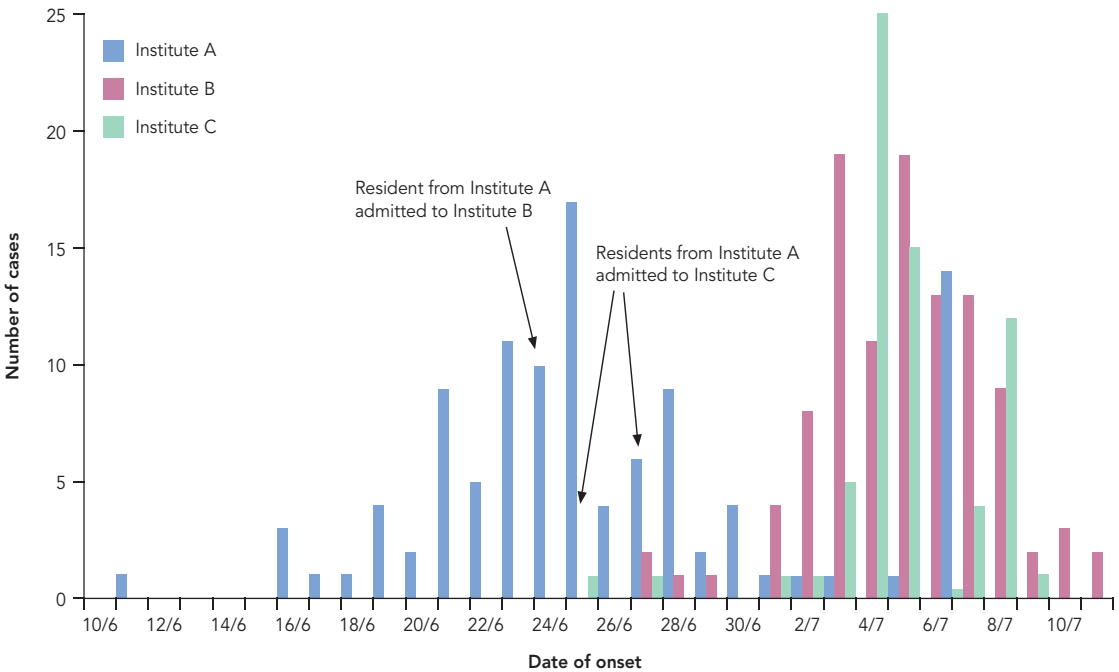

FIGURE 18.6

Number of cases of gastrointestinal illness by date of onset in three institutions, Australian Capital Territory, 2002

Source: Adapted from M. Miller et al. 2002, Norwalk-like virus outbreak in Canberra: Implications for infection control in aged care facilities. *Communicable Diseases Intelligence* 26(4): 555–61.

Questions

1. What factor suggests that the outbreaks in the three institutions were linked?
2. How could the outbreak have spread between institutions?
3. What is the likely means of transmission of infection in the institutions in this outbreak?
4. What does the secondary transmission of infection to family members of some healthcare workers indicate?

In developing countries, infections are associated with overcrowding, poor sanitary conditions and poor water quality. In endemic areas, people often become reinfected within months of treatment. In developed countries, where prevalence rates are in the range of 2–7 per cent, faecal–oral transmission is most common and children are most likely to be infected. *Giardia* is readily spread in daycare centres, from child to child, and from children to other family members. In Australia, high rates of infection have been reported in some remote Aboriginal communities. A high prevalence of the parasite has also been found in domestic cats and dogs, but whether they act as a reservoir for human infection remains uncertain. Direct transmission of *Giardia* among homosexual men is known to occur.

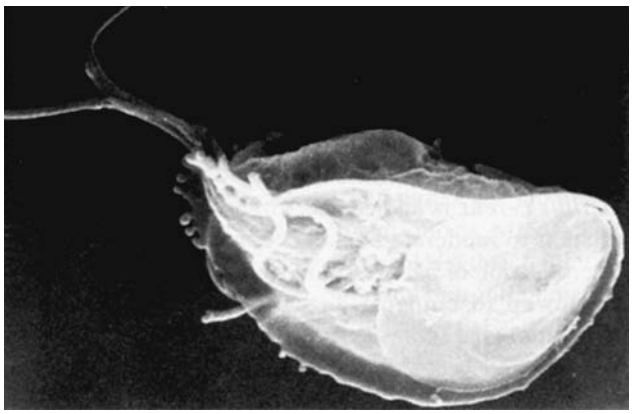

FIGURE 18.7

Giardia intestinalis

A flagellated protozoan that causes diarrhoea in humans.

Source: Professor Andrew Thompson, Murdoch University.

Giardia has a simple life cycle with two forms: the flagellate trophozoite and the resistant cyst. The trophozoites live in the upper portion of the small intestine, attaching to the mucosa and rapidly multiplying there. Cyst formation occurs at regular intervals and they are passed out in stools. These resistant structures are the infective form and can survive for months in the environment when conditions are suitable. They are also somewhat resistant to chlorine, so can survive some water purification procedures.

Infection occurs when cysts are ingested, with as few as 10–25 being able to establish infection. Once in the small intestine they develop into the trophozoite form. The mechanisms by which *Giardia* causes diarrhoea and intestinal malabsorption have not been fully identified. Attachment of the parasite to the mucosa and resultant physical damage is thought to be part of the disease process, but the role of other factors such as cytopathic substances is unclear.

Many cases of giardiasis are asymptomatic but when illness occurs it is characterised by diarrhoea, nausea, a foul flatulence and fatigue. The incubation period is about 1–3 weeks. It is usually a self-limiting disease (resolving within a week) but may persist for months. Chronic infection is typically manifested as periodic episodes of these symptoms between periods without symptoms. In children in developing countries or from disadvantaged groups, the major concern is the possibility of worsening malnutrition and retardation of growth and development.

Entamoeba histolytica

Entamoeba histolytica is an enteric protozoan with a worldwide distribution. It is thought to infect as much as 10 per cent of the world's population. It is endemic in many areas, including Central and South America, India, Egypt and Africa, and in northern parts of Australia, where it predominantly affects Indigenous people. Infection is transmitted by faecal contamination of food or drink by infected food-handlers or as a result of inadequate sanitation. Travellers to endemic regions are at risk of infection. Homosexual men are also recognised as a high-risk group.

Trophozoites of *E. histolytica* live on the mucosal surface of the large intestine (see Figure 18.8). They replicate there, periodically forming resistant cysts that are excreted in faeces. The cysts can survive in the external environment and then act as the infective stages. Most infected people are asymptomatic cyst passers or have only mild to moderate diarrhoeal symptoms. Production of small, localised ulcers in the colon causes a mild diarrhoea, whereas the formation of deep, confluent ulcers leads to the classic inflammatory diarrhoea called **amoebic dysentery**. The trophozoites of some strains can cause invasive disease, ulcerating the bowel wall and spreading via the bloodstream to remote sites. Amoebic liver abscess is the most common manifestation of invasive disease, but other organs can also be involved, including the lungs, kidneys and brain.

FIGURE 18.8

Coloured SEM of *Entamoeba histolytica* in the colon

Source: Eye of Science/Science Photo Library.

Cryptosporidium parvum

Cryptosporidium parvum was first recognised as a cause of diarrhoea in humans in 1976. The advent of the AIDS epidemic focused attention on the capacity of *Cryptosporidium* to produce disease and its widespread distribution. Prevalence rates of 1–3 per cent of patients with diarrhoea are reported in developed countries, and 5–10 per cent in Africa and Asia.

Cryptosporidiosis became nationally notifiable in Australia in 2001. Annually, around 2500 cases are reported, most occurring in children under the age of 4 years. Transmission is possible by a variety of routes, including faecally contaminated food, water and hands, and from pets. A number of outbreaks in daycare centres have been reported, often with daycare workers and family contacts also being affected. A massive outbreak, involving over 400 000 people, occurred in Milwaukee, in the United States, in 1993 due to contamination of the public water supply. The organism is also recognised as an important cause of traveller's diarrhoea and of outbreaks associated with swimming pools.

An important feature of *C. parvum* is its thick-walled oocyst, which is resistant to many disinfectants including hypochlorite (hence its association with swimming pool outbreaks). Another feature is its low infective dose (as low as 30 oocysts) and hence its ease of transmission.

In the small intestine the organism invades the epithelial cells where it undergoes sexual reproduction, eventually forming oocysts that are excreted in faeces (see Figure 18.9). A watery diarrhoea occurs, on average seven days after ingestion of oocysts, and lasts 1–3 weeks. There may also be fever, abdominal pain, malaise and vomiting, although many infections are asymptomatic. In young children the diarrhoea may persist for more than three weeks, and in immunodeficient hosts the disease may be severe and chronic, and sometimes fatal. Infected people can shed millions of oocysts in one gram of faeces.

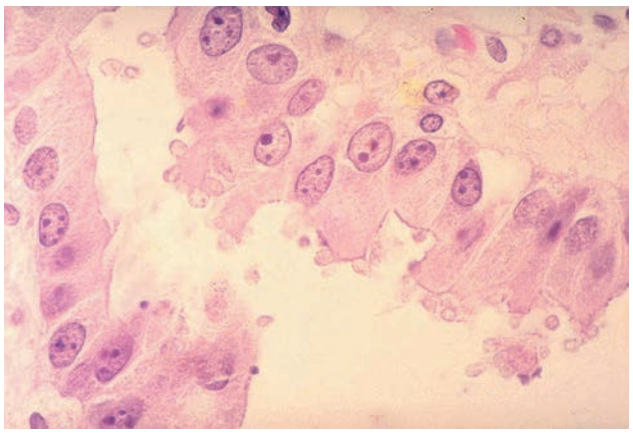

FIGURE 18.9

Cryptosporidium parvum

This section shows numerous Cryptosporidium *at the luminal surface of epithelial cells.*

Source: CDC/Dr Edwin P. Ewing, Jr.

Other intestinal protozoa

Cystoisospora belli is another protozoan that, as a result of the AIDS epidemic, has been identified as an important cause of diarrhoeal disease. It is endemic in South America, Africa and South-East Asia, and is transmitted by faecally contaminated food or water. Sporozoites invade the mucosa of the small intestine, destroying the microvilli. It can cause diarrhoea in travellers, but its highest prevalence is in immunocompromised people. Clinically, it is similar to cryptosporidial infection.

The protozoan *Cyclospora cayetanensis* causes diarrhoea in people in developing countries, and in travellers and people with AIDS. If untreated, symptoms can last for months.

Dientamoeba fragilis is a protozoan that has been shown to be a common cause of chronic diarrhoea. Prevalence in developed countries is around 1–4 per cent, but much higher rates have been reported in people in crowded living conditions and in conditions of poor hygiene.

Food intoxications

These illnesses are characterised by the rapid onset of nausea, vomiting and abdominal pain, usually within 6–8 hours of ingestion of contaminated food. Fever and diarrhoea can occur, but are less common. The major causes are the enterotoxins (see Chapter 10) of *Staphylococcus aureus*, and *Bacillus cereus* preformed in food. Thus, they are food intoxications, rather than infections. The fact that the toxins are preformed in the food is the reason why the incubation period is so short in comparison to infections. In these intoxications the organisms do not have to multiply in the intestine to produce disease; instead, the toxin must simply be absorbed.

Clostridium botulinum secretes a toxin that is absorbed in the intestine and enters the bloodstream, eventually reaching and acting on peripheral nerve synapses, causing a flaccid paralysis. Since the botulinum toxin blocks nerve transmission rather than causing gastrointestinal disease, botulism is discussed in Chapter 20.

Staphylococcus aureus intoxication

Foods rich in protein, salt and sugar (e.g. ham, creamy cakes, salads with mayonnaise), which have been inadequately refrigerated, favour the growth of staphylococci. If an enterotoxin-producing strain is involved, consumption of the food can result in gastrointestinal symptoms. Foods usually become contaminated by resident staphylococci in the nose or on the skin of food-handlers. Skin lesions colonised by staphylococci are a particularly dangerous source. Symptoms occur rapidly, typically 1–6 hours after consumption of the contaminated food.

There are at least seven distinct enterotoxins produced by strains of *S. aureus*, with enterotoxin A the most commonly implicated. Staphylococcal enterotoxins stimulate an inflammatory response in the intestine that results in diarrhoea and vomiting.

Bacillus cereus

Bacillus cereus is an aerobic, Gram-positive rod that is resistant to moderate cooking temperatures because of the production of heat-resistant spores. If the cooked food is allowed to cool for an extended period at room temperature, the spores germinate and the vegetative cells then produce a heat-stable enterotoxin in the food. Ingestion of preformed toxin in the food causes an acute onset of nausea, vomiting and abdominal pain without diarrhoea, within 1–6 hours. This type of food intoxication is frequently associated with rice dishes, especially fried rice. The practice in restaurants of cooking a large amount of rice and allowing it to cool slowly over several hours, then flash frying it just before serving, is believed to be the major reason for the disease's association with this food in particular.

Laboratory diagnosis

Generally, it is not possible to distinguish between the different causes of diarrhoeal disease solely on clinical grounds. Information on the patient's recent food consumption and/or travel history is often helpful, but a precise diagnosis

can only be achieved by laboratory investigations. For most patients with mild to moderate diarrhoea, the illness will be self-limiting and will require no evaluation other than a clinical one. However, for severe or persistent illness, or in the investigation of an outbreak, a definitive diagnosis is necessary. A number of laboratory tests can be used to provide such a diagnosis.

Stool culture is the standard method for the diagnosis of most bacterial causes of diarrhoea. Fresh stool specimens should be sent immediately to the laboratory in a sterile, wide-mouth container with a firmly fitting screw lid. If transport is delayed, refrigeration is recommended. Special media are required for cultivation of some bacteria, such as vibrios and *Y. enterocolitica*, so the request form accompanying the specimen should provide adequate information about the patient's history and food consumption and an indication that such organisms are suspected. Testing for the Shiga toxin in a faecal sample may be performed when enterohaemorrhagic *E. coli* or haemolytic uraemic syndrome is suspected.

CASE HISTORY 18.6

Staphylococcal gastroenteritis

Six guests who had been to a noon wedding attended Casualty at the local hospital between 3 and 5 pm the same afternoon. All were complaining of diarrhoea, vomiting and abdominal cramps. Public health authorities were notified and the other 60 guests and the owner of the wedding reception venue were subsequently contacted.

Overall, 19 of the guests experienced gastrointestinal symptoms, all within 2–5 hours of the lunch. Faecal specimens were collected from each of the 10 people who ultimately attended the hospital. Enterotoxin-producing *Staphylococcus aureus* was cultured from two of these specimens. No other pathogenic bacteria were isolated from any of these samples.

An examination of the kitchen of the wedding reception house revealed a physically clean kitchen. None of the food served at the wedding remained for testing. The only food consumed by all the ill guests was cold chicken pieces, and it was assumed that this was the source of the staphylococcal food poisoning.

Questions

1. What information supports the diagnosis of staphylococcal food poisoning?
2. What are the possible sources of contamination of the chicken?
3. What treatment would you expect the affected people to receive?
4. What is the risk of transmission of the infection to healthcare workers in the hospital?

For suspected *C. difficile* infections, anaerobic culture on special selective media and the detection of toxins A and B in stool filtrate by enzyme immunoassay have been the standard diagnostic procedures for some time. Commercial real-time PCR tests for *C. difficile* toxin genes have recently become available, and are likely to be more commonly used in the future. In staphylococcal or *Bacillus* food intoxications, the organisms are often not present in faeces and are not able to be cultured, but culture of vomitus may be more productive. In all cases of bacterial food-borne illness, culture of the suspect food may be attempted, particularly in the investigation of an outbreak.

Culture of enteric viruses is not routine in diagnostic clinical laboratories. Laboratory confirmation of viral gastroenteritis has generally been based on the demonstration of viral particles or antigens in stools by serologic or electron microscopic means. However, these tests are not always available in routine laboratories and are not highly sensitive. Improved diagnostic methods, such as enzyme immunoassays and latex agglutination for faecal antigens and polymerase chain reaction (PCR), have been developed for the two major viral causes.

Diagnosis of protozoal infections usually involves the microscopic identification of cysts or trophozoites in stool specimens. Collection of three stool specimens on alternate days is recommended to improve the likelihood of organism detection. Highly sensitive and reliable ELISA and fluorescein-labelled antibody tests have recently been developed for detecting antigens or oocysts of some protozoa (e.g. *Giardia*, *Cryptosporidium*) in stools. A highly sensitive and specific stool antigen test for *E. histolytica* has been developed.

Management of patients with diarrhoeal disease

The major problem of infectious diarrhoea, irrespective of the cause, is loss of fluid and dehydration. Therefore, for any patient with acute diarrhoea, fluid and electrolyte replacement is essential and can be lifesaving in severe cases. In mild disease, oral intake of fluids is usually adequate; but in cases of moderate to severe fluid loss, especially in young infants, intravenous hydration may be necessary.

Most patients with acute diarrhoea do not require specific antimicrobial therapy. In fact, antibiotic therapy should be avoided if possible, because in some cases it may prolong the carriage and excretion of an organism (e.g. *Salmonella*) by the patient, increasing the risk of transmission to others. Antibiotics may even increase the risk of severe complications, notably haemolytic uraemic syndrome in people infected with enterohaemorrhagic *E. coli*. Also, the risk of increasing microbial resistance to antibiotics is not warranted in self-limiting diseases. There is no effective antimicrobial treatment for viral causes, and antibiotics are not warranted in food intoxications because disease is caused by toxins, not multiplication of the bacterium in the intestine.

There are some situations, however, where antibiotic therapy is justified for acute diarrhoea. For example, in *Shigella* infections, an antimicrobial agent such as ampicillin or cotrimoxazole may be administered to shorten the duration of symptoms and prevent the spread of infection.

Antibiotic-associated diarrhoea or colitis caused by *C. difficile* may be resolved by discontinuation of the antibiotic, but severe cases may require treatment with vancomycin or metronidazole. Relapse of disease often occurs with the termination of treatment, requiring a second course of the drug. Enteropathogenic *E. coli* can cause severe and protracted diarrhoea, especially in hospital nursery and paediatric ward outbreaks. When the organism is hospital-acquired, it may be highly resistant to antibiotics, making antibiotic sensitivity testing of the isolate necessary.

Although cholera is potentially fatal, most cases can be adequately treated with oral rehydration and electrolyte replacement. In severe cases, or to prevent transmission, furazolidone or amoxycillin are generally used.

Treatment of protozoal infections is often with metronidazole, because of its broad activity. For confirmed *Giardia* infection, tinidazole or metronidazole is often used. Treatment of symptomatic *E. histolytica* infections is with metronidazole plus diloxanide furoate (an amoebicide). In cases of disseminated infection that do not respond to therapy, surgical drainage is usually required. Cryptosporidial infections are generally self-limiting, which is fortunate because there is currently no effective treatment for them.

A number of non-specific drugs may be used to reduce the symptoms of acute diarrhoea. Mostly, these are adsorbents (e.g. kaolin) to reduce the liquidity of stools, or drugs to inhibit intestinal motility and enhance fluid absorption (e.g. loperamide), thereby reducing the number of stools passed.

Prevention

Prevention of diarrhoeal disease essentially involves avoidance of the organisms. In the case of food-borne illness and traveller's diarrhoea, this means proper food processing, proper cooking of foods, the non-consumption of high-risk foods (especially raw foods) and beverages, and the sterilisation of drinking water where appropriate. In daycare centres, strict handwashing practice, good general hygiene, sanitary food preparation, use of disposable gloves when contact with faecal matter is possible, and exclusion of symptomatic children are important measures (see Chapter 14).

In hospitals and other institutions, people with severe diarrhoea are a potential source of infection for other patients. Standard Precautions and strict handwashing after contact with these patients is essential. While the use of disposable gloves is strongly recommended, this *does not* obviate the need for strict handwashing practices.

Currently, there is substantial interest in the use of probiotics to prevent or treat certain infectious diseases, including diarrhoeal diseases. **Probiotics** are formulations of microorganisms that, when ingested or applied to the body in some other way, may be beneficial to one's health. The formulations usually comprise live preparations of one or a mixture of microbes, such as certain strains of *Lactobacillus*, *Bifidobacterium* or *E. coli*. They are available in a wide variety of forms, ranging from capsules and powders with specific microorganism compositions to natural foods such as yoghurts, milks or juices. There is some evidence that probiotics can help to prevent or treat various diarrhoeal conditions, such as antibiotic-associated diarrhoea and some other types of acute diarrhoea. Researchers are investigating many issues related to their use, including the optimal compositions and doses, the potential physiological and microbiological benefits, and the possible adverse effects.

OTHER GASTROINTESTINAL DISEASES

Helicobacter pylori infection

The initial suggestions made in the early 1980s by Nobel laureates Barry Marshall and Robin Warren, in Western Australia, that the spiral-shaped bacterium, *Helicobacter pylori* (Figure 18.10), might be responsible for duodenal ulcer were treated with great scepticism. However, *H. pylori* is now recognised as a major cause of gastritis (inflammation of the stomach mucosa) and duodenal inflammation, which can lead to gastric and duodenal ulcers, respectively (see Figure 18.11). It has also been established as a major contributing factor in gastric cancer and in gastric mucosa-associated lymphoid tissue (MALT) lymphoma. Gastric cancer is a leading cause of cancer-related death worldwide.

In developed countries, as much as 50 per cent of the population (especially those over 40 years) may be infected with *H. pylori*. The prevalence of infection is higher in lower socioeconomic groups and especially high in developing countries (up to 70–90 per cent). Approximately 20 per cent of people infected with *H. pylori* develop symptomatic disease. The organism is probably spread by person-to-person contact (oral–oral and faecal–oral), and is mainly

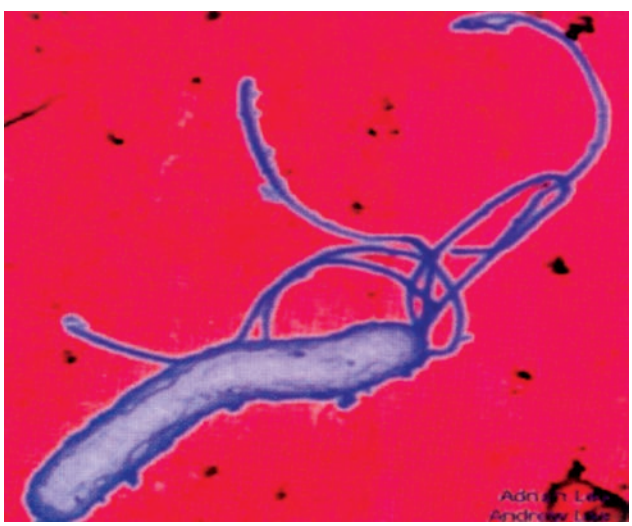

FIGURE 18.10

Electron micrograph of *Helicobacter pylori*

Source: Stephen Neville, Department of Microbiology & Infectious Diseases, Sydney South West Pathology Service, Liverpool Hospital campus.

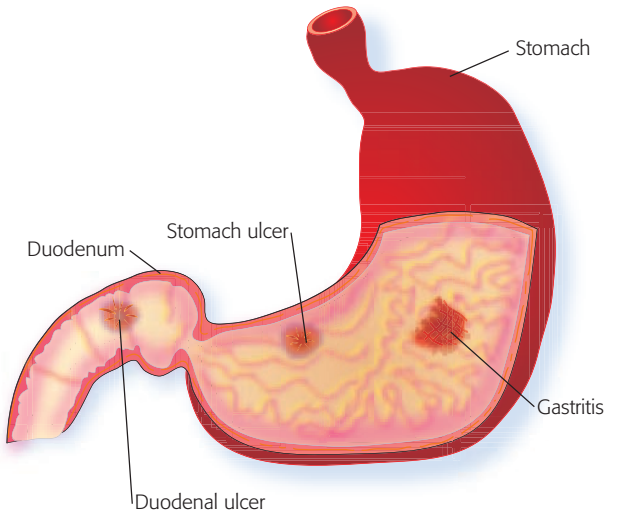

FIGURE 18.11

***Helicobacter pylori* diseases of the gastrointestinal tract**

acquired in childhood. Iatrogenic transmission via contaminated endoscopes has been reported.

Studies have indicated that motility and adhesins are probably important in the organism's ability to colonise the gastric epithelium. It has recently been established that the organism can form a biofilm that helps it to stick to the gastric epithelium. The organism produces large amounts of the enzyme urease, which is important in its pathogenesis. By breaking down urea in the stomach, this enzyme generates a 'cloud' of ammonia there; this neutralises the acid and thus protects the organism from the gastric acidity.

There are differences in virulence between different strains of *H. pylori* such that some are able to cause gastric disease where others are not. Host factors, particularly the inflammatory response, are also involved in the production of disease. Only in some people is the inflammation induced by the bacterium severe enough to produce symptoms. Severe and prolonged inflammation of the gastric mucosa appears to be a major requirement for ulceration and carcinogenesis. While *H. pylori* appears to play an important role in the initiation of gastric cancer, other factors, such as diet, also seem to be important.

The diagnosis of *H. pylori* infection is usually confirmed in one of two ways: by a urea breath test or by endoscopic biopsy. The non-invasive urea breath test has high sensitivity and involves the detection of labelled carbon dioxide in the breath of an infected person after they have ingested an isotopically labelled solution of urea. A novel, rapid faecal antigen test has recently been developed. There are a number of treatment regimes for *H. pylori* infection. A common approach is combination therapy with clarithromycin, amoxicillin and tinidazole (or metronidazole) and a proton pump inhibitor (to reduce gastric acidity). Drug resistance in *H. pylori* is an increasing problem, especially to clarithromycin and fluoroquinolones. An alternative regime of metronidazole, tetracycline, bismuth salt and proton pump inhibitor is commonly used. Treatment is not recommended for asymptomatic *H. pylori* infection. Considerable work is being undertaken to develop a vaccine.

Enteric fevers: typhoid and paratyphoid

Causative agents and transmission

Salmonella typhi and *S. paratyphi*, types A, B and C, all cause enteric fevers (typhoid and paratyphoid fever), although *S. typhi* is responsible for the vast majority of infections. These organisms are restricted to humans and are usually spread via faecally contaminated food or water. Infections are most common in developing countries (especially in Africa and South-East Asia) with poor standards of water supply and sewage disposal. Typhoid is also common in Indonesia and Papua New Guinea.

There are an estimated 20 million infections worldwide each year, resulting in approximately 200 000 deaths. In Australia, fewer than 100 new cases of enteric fever are reported each year and most of these have been acquired overseas, the majority in India.

Pathogenesis

After ingestion, many of the salmonellae are killed by the acidity of the stomach; however, if the infecting dose is high enough, some bacteria enter the small intestine. There they penetrate the intestinal mucosa, where they are ingested by macrophages. An important attribute of these organisms is their ability to survive and multiply inside macrophages. They are transported by these cells to the mesenteric lymph nodes and eventually they reach the thoracic duct and then the bloodstream. Once in the blood they can seed many organs, especially the spleen, bones and liver. After further multiplication there is reinvasion of the blood, which marks the end of the incubation period and the beginning of clinical illness.

This second phase of bacteraemia leads to invasion of other organs, such as the kidney and gall bladder. The organism grows actively in the bile and enters the intestine for a second time. Most of the symptoms caused by *S. typhi* can be attributed to the inflammation induced by its cell wall lipopolysaccharide.

Clinical features

After an incubation period of 10–14 days, the early symptoms of fever, headache and respiratory symptoms (typically a dry cough) appear. Mild abdominal discomfort with either diarrhoea or constipation occurs. Without treatment, the fever increases in a stepwise fashion and the patient may eventually lapse into a stupor. Other common symptoms are rose spots (pink macular spots that blanch on pressure), bradycardia and splenomegaly. Without treatment, an uncomplicated infection lasts 4–6 weeks. A serious complication is secondary invasion of the intestine from the gall bladder. In a small proportion of patients this can lead to inflammation and then perforation or haemorrhage of the intestine. Death occurs in approximately 10–15 per cent of untreated cases with this complication. If appropriate treatment is initiated

within the first few days of the illness, the patient begins to recover after about two days.

Many patients continue to excrete *S. typhi* in the faeces for several weeks after recovery. In up to 4 per cent of infected people, the organism persists in the gall bladder and kidneys, and bacteria can be shed, asymptomatically, in faeces and urine for years and sometimes permanently. Such chronic carriers are a major public health problem. In developed countries, chronic carriers can become a major hazard if they are employed in food handling. Mary Mallon ('Typhoid Mary'), a cook in New York City in the early 1900s, is the classic example. As a carrier, she caused many outbreaks of the disease and was virtually 'imprisoned' for life on North Brother Island, in New York's East River, for refusing to stop working as a cook.

Laboratory diagnosis

The diagnosis of enteric fever relies on the isolation of *S. typhi* or *S. paratyphi* from the patient. Blood cultures are the standard method and generally become positive at the onset of symptoms and remain positive over the next two weeks (see Figure 18.12). Faeces and urine are also sometimes cultured, especially 2–4 weeks after onset of symptoms, when seeding of the kidneys and secondary infection of the intestine occurs. Asymptomatic chronic carriers are diagnosed by positive cultures from faeces and urine. Serum antibodies to the organisms can be detected by an agglutination test (the Widal test), but results can be unreliable and difficult to interpret, and the test is no longer recommended. Enzyme-linked immunoassays (ELISA) and polymerase chain reaction (PCR) tests have recently been developed for diagnosis of enteric fever, but these have had variable success.

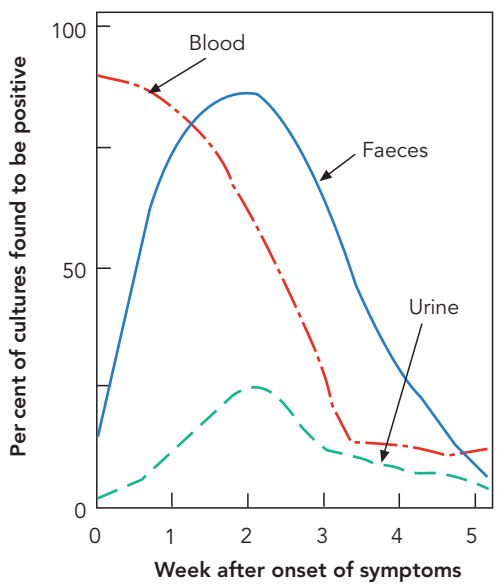

FIGURE 18.12

The isolation of typhoid bacteria from different specimens over the course of the disease

Treatment and prevention

Antibiotic resistance in *S. typhi* is an increasing problem, so treatment should be based on antibiotic susceptibility testing. For empirical treatment before sensitivity test results are available, fluoroquinolones (e.g. ciprofloxacin) are generally recommended; however, resistant strains are increasingly being found. Third-generation cephalosporins (e.g. ceftriaxone) have been used in regions with high fluoroquinolone resistance rates, but sporadic resistance against these drugs has also been observed, and is expected to worsen. Up to 10 per cent of patients treated with antibiotics experience a relapse after initial recovery. A relapse is generally milder and of shorter duration. A small percentage of treated cases may become chronic carriers. For chronic carriers, long-term antibiotic therapy may be effective but, if not, cholecystectomy (removal of the gall bladder—the site of carriage) may be warranted if the person is a risk to the community.

In developing countries, prevention of infection depends on good personal hygiene, adequate sewage disposal and a clean water supply. In the developed world, outbreaks of enteric fever are rare. Typhoid carriers are a particular concern and should be treated or excluded from employment that involves food handling. Vaccines against *S. typhi* are available, and are recommended for travellers to countries where typhoid is endemic, for close contacts of known carriers and for laboratory workers in high-risk situations.

Listeriosis

Listeriosis is a food-borne disease caused by the Gram-positive coccobacillus *Listeria monocytogenes*. The organism is found in the intestine of a wide variety of animals and is excreted in their faeces. It is therefore widely distributed in soil, water, sewage and animal feed. It is predominantly a food-borne pathogen, and foods usually become contaminated during processing or by contact with soil. Raw foods, such as fruit and vegetables, and uncooked foods of animal origin, such as meat and soft cheeses, are the usual sources of infection. The organism is able to multiply in foods kept at refrigerator temperatures. Person-to-person transmission is rare.

The incidence in Australia is around 60 cases per year. It is usually a mild or often symptomless disease in healthy adults. Mild disease is generally characterised by flu-like symptoms of sore throat, fever, chills, myalgia, and sometimes nausea or diarrhoea. This non-invasive form of the infection is spontaneously cleared in most people in about seven days. More serious infections, usually bacteraemia and meningitis, can occur in the immunocompromised and the elderly. It is also an important cause of infection in pregnant women. Bacteraemic infection in a pregnant woman is not usually severe, but the organism can spread to the foetus *in utero* or be transmitted at birth, resulting in severe foetal or neonatal disease. Intrauterine infection can result in abortion, premature labour or intrauterine death. Perinatal infection of the neonate is most often manifested as

a meningitis. Pregnant women are advised to avoid foods of animal origin that are eaten uncooked, such as soft cheeses, pate or cold delicatessen meats.

Definitive diagnosis of invasive listeriosis is by culture of blood, cerebrospinal fluid or amniotic fluid. Ampicillin is the usual choice of antibiotic for severe infections. Case History 18.7 describes an outbreak of listeriosis in the United States in 2011.

HELMINTH INFECTIONS OF THE GASTROINTESTINAL TRACT

The types and characteristics of helminths (worms) are described in Chapter 6. Briefly, there are three main groups:

- nematodes (roundworms, hookworms and threadworms)
- cestodes (tapeworms)
- trematodes (flukes).

Parasitic helminths are a major cause of morbidity, particularly in the tropics and subtropics, and where there is poor sanitation. It has been estimated that more than half of the world's population could be infected with an intestinal helminth.

In Australia, helminth infections occur mostly in the northern (tropical) parts of the country and are predominantly due to nematodes. Intestinal nematodes are found particularly in northern Aboriginal communities with poor sanitation and inadequate medical facilities. Some of the more serious helminth infections, such as schistosomiasis, river blindness and filariasis (elephantiasis), which are endemic in other parts of the world, do not occur in Australia. The common helminth infections in Australia are listed in Table 6.3, page 127.

The real incidence of helminth infections in Australia is difficult to determine. The most common is undoubtedly the pinworm (or threadworm) *Enterobius vermicularis*, which is found throughout the country.

Helminths in Australia are acquired in two distinct ways. *Ancylostoma duodenale* and *Necator americanus* (the hookworms) and *Strongyloides stercoralis* infect humans by penetration of the skin by infective larvae, whereas all the others are acquired by ingestion of the infective eggs of the helminths. All helminths specifically inhabit the bowel except for *E. granulosis* (liver and lungs) and *S. stercoralis*, which can cause disseminated infection.

In nematode infections, the severity of disease is largely a function of the number of worms in the body. An increasing worm load generally requires repeated exposure to the infectious larvae or eggs, since all except *Strongyloides* do not multiply in the host. Thus, travellers and immigrants from non-endemic areas are likely to have mild, if any, symptoms, whereas residents of endemic areas are more likely to suffer more severe disease.

CASE HISTORY 18.7

Listeria outbreak

Between 31 July and 21 October 2011 a multi-state outbreak of listeriosis occurred in the United States involving 139 reported cases. The outbreak was responsible for the hospitalisation of 132 people, 29 deaths, and a miscarriage by a woman who contracted the infection. The majority of ill people were over 60 years old.

Investigations by health authorities found that the source of the outbreak was cantaloupes (rockmelons) from a particular producer in Colorado. On 14 September the producer recalled all whole and cut cantaloupes of the type implicated in the outbreak.

Questions

1. How could the cantaloupes have become contaminated in this outbreak?
2. What does this outbreak demonstrate about which people are susceptible to severe infection?
3. What treatment would you expect the affected people to receive?
4. What is the risk of transmission of the infection to healthcare workers in the hospital?

Enterobius vermicularis

Enterobius vermicularis is prevalent in temperate as well as tropical regions, and humans are the only natural host. The worm can infest individuals of any age group, but the prevalence is greatest in children aged 5–9 years. Tens of thousands of Australians are believed to harbour this worm, but it does not cause serious illness. Adult pinworms (approximately 1 cm in length) pass out of the anus at night, depositing eggs in the perianal area. Many people are asymptomatic; however, when symptoms do occur, anal pruritis (itching), especially at night, is the classic sign. Transmission usually occurs directly from fingers contaminated by scratching or via contaminated fomites, such as toys and clothing. The worms mature entirely in the intestine after hatching from ingested eggs. Infestation usually lasts a maximum of six weeks.

The eggs of *Enterobius* are rarely present in faeces but can be identified by applying adhesive tape to the perianal skin when the patient wakes, followed by microscopic examination of the tape. Infections are effectively treated with mebendazole or pyrantel, but re-infestation is common.

Ascaris lumbricoides

Adult *Ascaris* worms are 15–35 cm long and 0.5 cm thick, hence the common name of giant roundworm (Figure 18.13). The females lay an enormous number of eggs into the intestine (about 200 000 in a day) and these are passed in faeces. The eggs can remain infective in soil for many weeks or months, depending on the conditions. The infection is usually transmitted by the faecal–oral passage of eggs via contaminated hands.

FIGURE 18.13

Ascaris lumbricoides, the giant roundworm

Female worms can lay as many as 200 000 eggs in a day in the gut of an infected person.

Source: Stephen Neville, Department of Microbiology & Infectious Diseases, Sydney South West Pathology Service, Liverpool Hospital campus.

After ingestion, the eggs hatch in the intestine, releasing the larvae. The larvae penetrate the gut wall and are carried via the blood through the liver to the lungs. From there they work their way up the bronchi and trachea and are swallowed, and once again enter the intestine. The adult worms live freely in the gut lumen, feeding on intestinal contents. Most people with a small worm load are asymptomatic or experience only mild abdominal discomfort. They often only become aware of their infection when they pass a worm in their stools. When symptomatic, early symptoms of non-productive cough, dyspnoea and wheezing within two weeks of infestation result from the migration of larvae through the lungs. Six to eight weeks after infestation gastrointestinal symptoms may occur, related to the effects of high parasite load in the intestine.

In some cases, the migration of the larvae through the lungs can cause severe respiratory distress (pneumonitis). With large worm loads, especially in children, intestinal obstruction may occur, which is potentially fatal.

Diagnosis is based on the microscopic examination of faeces. The eggs of *Ascaris* are characteristic and readily recognised in a fresh stool specimen. Ideally, the specimen should be transported to the laboratory so that it can be examined within 30 minutes of collection. Three or more specimens may have to be collected in some cases, because of low numbers or intermittent shedding. A variety of anti-helminthic drugs are available for treatment of intestinal nematodes (e.g. mebendazole, pyrantel).

Hookworms

The hookworms *Ancylostoma duodenale* and *Necator americanus* are serious causes of long-term morbidity in developing countries, because of the anaemia and hypoproteinaemia that result from heavy infection. *A. duodenale* is very common in some Aboriginal communities and probably contributes to the iron deficiency and anaemia seen in many of these people. Adult **hookworms** (approximately 1 cm in length) attach to the intestinal mucosa by specialised mouth structures (Figure 18.14). They rupture capillaries and suck blood, and lay eggs that are passed in faeces into the environment. The eggs mature and hatch in soil, and infection of a new host takes place when larvae come into contact with the unprotected skin of a person (sometimes swallowed in the case of *Ancylostoma*). They penetrate the skin, migrate via the bloodstream to the lungs, climb the trachea and are swallowed, maturing into adult worms in the small intestine six weeks after initial infection.

Most people with light worm loads are asymptomatic. Heavy infections may cause abdominal pain, flatulence, a bloody diarrhoea, and iron deficiency anaemia in children or in anyone with existing malnutrition. One of the main concerns of heavy infection is chronic blood loss. Untreated, severe infection in children can result in physical and mental retardation. Hookworm infections are diagnosed by microscopic visualisation of eggs in stool specimens. Treatment is with antihelminthic drugs such as albendazole, mebendazole or pyrantel.

Strongyloides stercoralis

Two species of *Strongyloides* cause human infection. *S. stercoralis* is the most common, and *S. fuelleborni* is found sporadically in limited regions of the world. *Strongyloides* infection occurs when larvae in faecally contaminated soil or fomites penetrate the skin or mucous membranes. They then enter the bloodstream and break into the alveoli in the lungs. Like the hookworms, they ascend the bronchi and trachea, are swallowed and reach the small intestine where maturation is completed. The tiny adult worms (2–3 mm in length) dwell just beneath the intestinal mucosa, where they lay their eggs. These eggs hatch in the intestine and are passed in the faeces.

This worm is unlike any other intestinal nematode in its ability to multiply in the host. In some cases, the internally

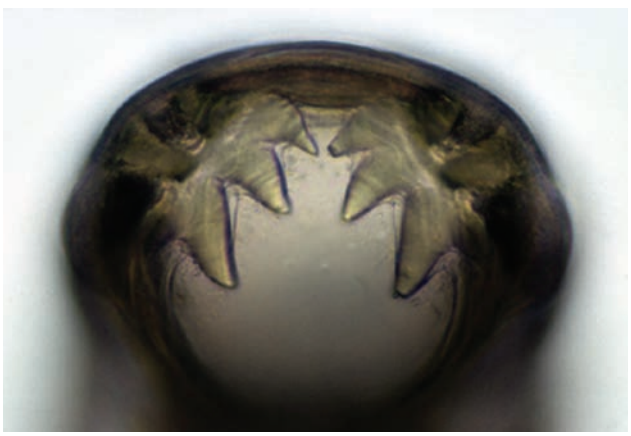

FIGURE 18.14

Ancylostoma spp.

This worm attaches to the intestinal mucosa by specialised mouth structures.

generated larvae can then penetrate the intestinal mucosa or the perianal skin and migrate via the lungs back to the small intestine, in what is essentially an 'auto-infection cycle'. This cycle, which can result in a hyperinfection with a high mortality rate, is most likely to occur in the immunocompromised, or in patients with malnutrition or other debilitating disease. *Strongyloides* worms may persist and replicate in a host for decades. Larvae may also infect other organs, including the urinary tract, liver and brain.

People with low worm burdens are often asymptomatic. With greater numbers of worms, there may be non-specific gastrointestinal symptoms of abdominal pain, intermittent diarrhoea and nausea. Heavy intestinal infection causes a persistent and profuse diarrhoea, with dehydration and electrolyte imbalance. Invasion of other body sites by large numbers of auto-infective larvae can be fatal. Asymptomatic immigrants from endemic areas such as South-East Asia, especially those with unexplained eosinophilia, are often found to be infected with *Strongyloides*. It is also found in some Aboriginal communities.

Strongyloides infection is difficult to diagnose, but it can be done by finding larvae in fresh stools. Many stool specimens (at least three on consecutive days) may have to be examined before larvae are found. Serological tests for *Strongyloides* are available, but do not distinguish between past and current infection. All people infested with *Strongyloides* should be treated. Infections are usually treated with ivermectin or thiabendazole but eradication of the worm can be difficult, especially in the immunosuppressed. Repeated, short courses of treatment may be necessary to keep the worm load at a low level.

Hydatid disease

Hydatid disease is caused by the tapeworm *Echinococcus granulosus*. The parasite's definitive host is the dog, but it can be transmitted to other animals, particularly sheep. Other dogs can then become infected when fed on the viscera of infected sheep. Humans act as accidental hosts when the eggs of the parasite are ingested, usually as a result of contact with the fur or tongue of an infected dog. Young children of sheep-farming families are at greatest risk. Recent evidence indicates that the parasite is also found in certain wildlife species, such as dingoes, wallabies, kangaroos and foxes; these animals could therefore be additional sources of infection. In Australia, there are around 50 notifications of hydatid infection each year, with an average of three deaths per year.

After ingestion of eggs, the larvae emerge and migrate through the gut and into the bloodstream, and eventually localise in any organ. In most patients a single organ is involved—usually the liver, but sometimes the lungs or brain. In these organs they form fluid-filled sacs, called cysts (see Figure 10.10 in Chapter 10, page 237), which contain thousands of developing worms and can reach the size of a grapefruit. Worms within these sacs are protected from host defence mechanisms.

Symptoms, which can take months or years to develop, result from the local pressure of the cyst on the organ, and are highly variable. Bursting of the cyst can lead to seeding of other organs with the worms and, in some people, a life-threatening anaphylactic reaction to hydatid antigens.

Diagnosis of hydatid disease is by radiologic demonstration of a cyst, supported by positive serology. Treatment may be achieved with prolonged, high-dose albendazole, but surgical removal of the cysts is often necessary.

Taenia saginata

The beef tapeworm attaches by its head to the intestinal mucosa. Most patients are asymptomatic or experience only mild abdominal discomfort. The most obvious sign of infection is the passing of white, motile proglottids, or long segments of a worm. Infections are diagnosed in a similar way to *Ascaris* infections. Treatment is usually with praziquantel.

Trichuris trichiura

Trichuris trichuria (whipworm) causes an intestinal infestation that is thought to affect about a quarter of the world's population. Adult worms are 3–4 cm in length. The worms remain within the large bowel, attaching to the epithelial layer. As with all intestinal worms, children are the members of the community most heavily infected with this parasite, acquiring eggs from contaminated soil or vegetation. Most infections are asymptomatic, but moderate to heavy worm loads, especially in children, can cause abdominal pain and diarrhoea, occasionally leading to prolapse of the rectum. Impaired nutrition and retarded growth of children may result. Infections are diagnosed and treated in a similar way to *Ascaris* infections.

Hymenolepis nana

Hymenolepis nana is a tapeworm (2–4 cm long) that causes an asymptomatic infection in most patients. In heavy infections it can cause diarrhoea, abdominal pain and loss of appetite. There are thought to be many millions of people worldwide infected with this parasite, predominantly children in warm, dry regions in developing countries. Infections are diagnosed and treated in a similar way to *Ascaris* infections.

HEPATITIS

The term **hepatitis** refers to injury and inflammation of the liver. Viruses are major causes of hepatitis but other microorganisms, alcohol, drugs and various other disorders (e.g. biliary obstruction) may also be responsible (see Table 18.5). A number of important viruses, including cytomegalovirus, Epstein-Barr virus (infectious mononucleosis) and rubella virus, can infect the liver and cause hepatitis as part of a systemic infection. These are dealt with in Chapter 19. Here we focus on the viruses that infect mainly the liver. These are the hepatitis viruses A, B, C, D and E. Each of these belongs to a different virus family and they have little in common except the target organ they affect. The characteristics of the different hepatitis viruses are outlined in Table 18.6.

Viral hepatitis is a major cause of morbidity and mortality throughout the world. Hepatitis A virus (HAV) and hepatitis E virus (HEV) are spread mainly by faecal–oral means; the other three—hepatitis B virus (HBV), hepatitis C virus (HCV) and hepatitis D virus (HDV)—are spread principally by exposure to blood, although transmission via other body fluids, especially genital secretions for HBV, also occurs.

Hepatitis viruses produce acute inflammation of the liver. This may result in a clinical illness with variable features (see Table 18.7), including gastrointestinal symptoms such as anorexia, nausea and vomiting, fever, malaise and jaundice (yellowing of the skin and whites of the eyes) (see Figure 18.15). In more serious cases, liver function can fail. Marked increases in serum concentrations of the liver enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), are characteristic of acute viral hepatitis. Generally, the different types of hepatitis cannot be distinguished by their signs and symptoms. The patient's history and risk factors may indicate the likely type(s), but specific laboratory tests are required for a definitive diagnosis.

At present there is no completely effective treatment for acute viral hepatitis, although some antiviral drugs have been used with some success in chronic hepatitis B and C. Vaccines are currently available only for hepatitis A and B.

TABLE 18.5 Causes of hepatitis

VIRUSES
Hepatitis viruses A, B, C, D, E
Cytomegalovirus
Epstein-Barr virus (infectious mononucleosis)
Herpes simplex virus
Rubella virus
Yellow fever virus
OTHER INFECTIONS
Syphilis
Tuberculosis
Toxoplasmosis
Q fever
Amoebiasis
NON-INFECTIOUS CAUSES
Biliary obstruction
Primary biliary cirrhosis
Drug toxicity
Drug hypersensitivity
Alcohol abuse

Hepatitis A

Hepatitis A virus is a small RNA virus, belonging to the family of picornaviruses. The virus was first isolated in cell culture in 1979 and is still the only hepatitis virus that has

TABLE 18.7 Common clinical features of viral hepatitis

- Jaundice
- Anorexia, nausea, vomiting
- Diarrhoea or constipation
- Abdominal pain
- Fever, malaise
- Myalgia, arthralgia
- Fatigue, weakness
- Anaemia
- Increased serum alanine aminotransferase (ALT)
- Increased serum aspartate aminotransferase (AST)

TABLE 18.6 Characteristics of hepatitis viruses

	HEPATITIS A	HEPATITIS B	HEPATITIS C	HEPATITIS D	HEPATITIS E
Family	Picornaviridae	Hepadnaviridae	Flaviviridae	(unclassified)	Caliciviridae
Genome	ssRNA	dsDNA	ssRNA	ssRNA	ssRNA
Mode of transmission	Faecal–oral	Blood; sexual; mother to child	Blood; sexual; mother to child	Blood; sexual	Faecal–oral
Incubation period	2–6 weeks	1–6 months	2–26 weeks	2–9 weeks	2–9 weeks
Acute mortality rate	0.2%	0.2%	0.2%	2–20%	0.2%
Infectious period	2 weeks before onset to 1 week after symptoms subside	Many weeks before onset to life in carriers	1 or more weeks before onset to life in carriers	Not known, but maximal just before onset	Unclear, but up to 2 weeks after onset
Chronicity	None	5–10%	50–70%	2–20%	None
Vaccine	Yes	Yes	No	No	No

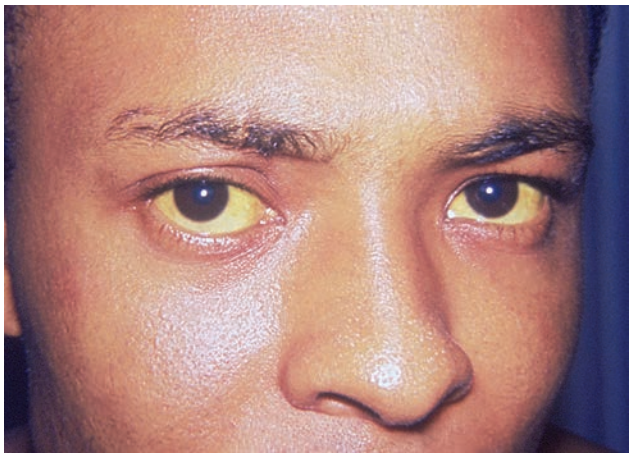

FIGURE 18.15

Jaundice caused by hepatitis A infection

Source: CDC/Dr Thomas F. Sellers/Emory University.

been propagated *in vitro*. Humans appear to be the only reservoir of the virus.

HAV has a worldwide distribution, with highest prevalence in Africa, Asia and South America. In countries where HAV is endemic, most infections occur in young children. In Australia there are usually around 200 to 300 notifications per year, although there were over 550 notifications in 2009. This increase was largely attributable to an outbreak related to consumption of contaminated semi-dried tomatoes (see Case History 18.8, page 466). There has been a marked decline in notifications of hepatitis A since 1999, coinciding with the introduction of targeted vaccination programs for Indigenous children (see Figure 18.16).

Transmission

Transmission of HAV is primarily by the faecal–oral route. Peak infectivity from an infected person is in the two weeks before the onset of illness and for at least a week later, when the concentration of virus in faeces is highest. It is spread mainly from person to person by contact (hands) or by contamination of food or water. Once transferred to fomites from unwashed hands, the virus can persist on surfaces for several days.

Contamination of food or water can lead to sudden, explosive epidemics of hepatitis A. Poor personal hygiene, poor sanitation and close personal contact facilitate transmission of the virus. It may also be transmitted via shellfish from polluted waters, especially if eaten raw.

HAV infection often occurs in institutional settings and has been reported in healthcare workers caring for infected patients. In Australia there have been several reports of hepatitis in nursing staff, particularly in paediatric wards in hospitals in northern and central parts of the country. Failure to wear gloves when contact with faeces occurred, or omitting to wash hands thoroughly before eating, have been suggested as major faults in these cases. Lack of good sanitation and hygiene can lead to outbreaks in schools, camps and daycare centres.

Outbreaks of hepatitis A among injecting drug users have been reported. Such outbreaks are most likely due to faecal contamination of drugs or injecting materials, or poor hygiene and close contact among abusers. In recent years,

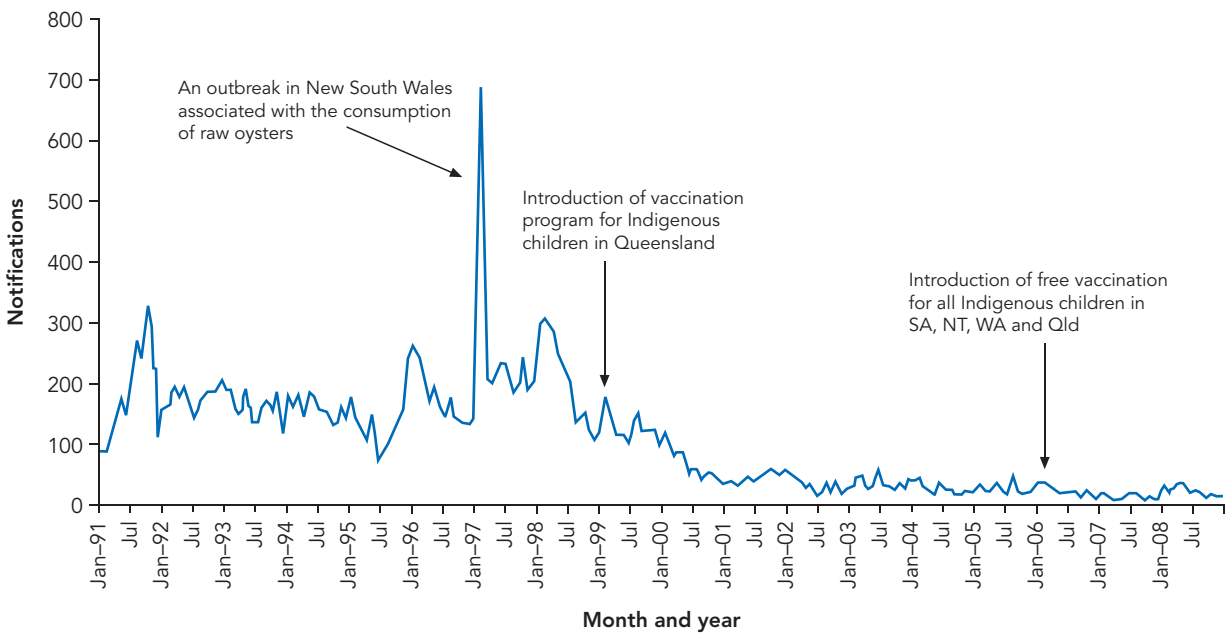

FIGURE 18.16

Notifications of hepatitis A in Australia, 1991–2008

Source: *Communicable Diseases Intelligence* 2010, 34(3): 183.

there has been an increased incidence of hepatitis A among male homosexuals, possibly related to oral–anal sexual practices.

Pathogenesis and clinical features

After infection, the virus is believed to replicate initially in intestinal cells. This is followed by a brief period of viraemia, and then it spreads to the liver. The incubation period ranges from two to six weeks, with an average of about 25 days. During this period liver cells are damaged, probably by immune mechanisms rather than direct viral action.

Hepatitis A varies considerably in its presentation. The illness is usually not apparent in children less than 4 years old, but it becomes progressively more severe with increasing age. When symptomatic, it is generally a mild to moderate disease with typical manifestations of fever, anorexia, nausea, vomiting, fatigue, liver pain and sometimes jaundice, due to impaired liver function. Complete recovery occurs in most cases in 4–6 weeks; there is no carrier state and chronic infection does not generally occur. However, shedding of the virus may continue for up to six months in asymptomatic neonates. Rarely, HAV causes a fulminant hepatitis with potential mortality. Infection induces long-term immunity.

Laboratory diagnosis

HAV is not routinely cultured and the other hepatitis viruses have not yet been cultured, so diagnosis of hepatitis in general is based on serological tests. The serologic tests for hepatitis virus infections are summarised in Table 18.8.

Currently, the standard method for diagnosing hepatitis A is by detection of anti-hepatitis A antibodies in the patient's serum. Anti-HAV IgM is usually detectable 25–30 days after infection—that is, usually at the time of onset of symptoms. This test remains positive for several months after infection, so it indicates current or recent infection. Anti-HAV IgG appears soon after the IgM and persists for many years, so it is an indicator of immunity from past infection, or vaccination if IgM is absent. Serum concentrations of liver enzymes, particularly ALT and AST, are usually markedly increased during infection.

Treatment and prevention

As for many viral infections, there is no specific cure for hepatitis A. Symptomatic treatment (e.g. anti-emetics, hydration fluids) is all that is available. Prevention relies on good hygiene handwashing and cleaning procedures, and the prophylactic use of gammaglobulin after suspected exposure. Normal human immunoglobulin (NIGH) is a pooled serum preparation from normal blood donors that contains antibodies to hepatitis A virus and other common diseases. NIGH must be given within 14 days of exposure to be effective. It is also recommended for close contacts of cases used to contain outbreaks of hepatitis A. Effective inactivated HAV vaccines are available. Vaccination is recommended for high-risk groups including:

- travellers to, and all expatriates living in, moderately to highly endemic areas (including all developing countries)
- Aboriginal and Torres Strait Islander children residing in the Northern Territory, Queensland, South Australia or Western Australia
- people whose occupation may put them at risk of acquiring hepatitis A
- people whose lifestyle may put them at risk of acquiring hepatitis A, including men who have sex with men, and injecting drug users
- people with intellectual disabilities
- people chronically infected with either hepatitis B or hepatitis C viruses
- people with chronic liver disease.

School-based outbreaks are often attributed to inadequate toilet facilities. Improvement of facilities and thorough handwashing are the most important interventions for preventing infections in schools.

CASE HISTORY 18.8

Hepatitis A

Between March 2009 and March 2010 there was a marked increase in reported cases of hepatitis A, especially in Victoria, though the outbreak also involved other states and the ACT. Many of the cases recalled eating semi-dried tomatoes during the period when they were likely to have been exposed, and a significant association between consumption of semi-dried tomatoes and illness with hepatitis A was subsequently demonstrated.

Investigations revealed a complicated supply chain with multiple suppliers for multiple brands, but there appeared to be a link to imported frozen tomatoes which were used to make the final product locally. During the outbreak, health authorities mandated the pasteurisation or chlorine washing of tomatoes used in the manufacture of semi-dried tomatoes, and advised consumers to avoid eating loose semi-dried tomatoes unless thoroughly cooked.

Source: Adapted from OzFoodNet Working Group 2010, Monitoring the incidence and causes of diseases potentially transmitted by food in Australia: Annual report of the OzFoodNet Network, 2009. *Communicable Diseases Intelligence* 34(4): 396–426.

Questions

1. What is the most likely way that the tomatoes became contaminated with hepatitis A virus?
2. Would it be possible for an infected person to transmit the infection to an attending healthcare worker?
3. What would be the purpose of pasteurising the tomatoes during manufacture as recommended by the health authorities?
4. Why is NIGH usually ineffective if given more than two weeks after exposure to the hepatitis A virus?

TABLE 18.8 Tests for the diagnosis of hepatitis

	HEPATITIS A	HEPATITIS B	HEPATITIS C	HEPATITIS D	HEPATITIS E
Antigens detected	HAV Ag	HBsAg, HBeAg	None available	HDV Ag	None available
Antibodies detected	Anti-HAV IgM Anti-HAV IgG	Anti-HBs Anti-HBc Anti-HBc IgM Anti-Hbe	Anti-HCV	Anti-HDV IgM Anti-HDV IgG	Anti-HEV IgM Anti-HEV IgG
Molecular techniques		HBV DNA	HCV RNA	HDV RNA	

Hepatitis B

Hepatitis B is caused by a hepadna (hepatitis DNA) virus. The HBV has an outer coat containing an antigen called the *hepatitis B surface antigen* (HBsAg). This was formerly called the Australia antigen because it was originally identified in the serum of an Australian Aborigine. The inner component is called the hepatitis core antigen (HBcAg) and inside this is the viral DNA (HBV-DNA) and the viral enzyme, DNA polymerase. Another antigen is the hepatitis Be antigen (HBeAg), which is a secreted product of the core gene. The whole virus particle is called a *Dane particle*, named after the scientist who first saw it by electron microscopy in 1970. HBV has not been isolated or propagated in cell culture.

Hepatitis B has a worldwide distribution. There are thought to be 2 billion people infected throughout the world, with approximately 350 million suffering with chronic hepatitis B. The virus is responsible for approximately 1.5 million deaths each year. High rates of chronic infection (up to 25 per cent) are found in some populations, such as in some Asian and African countries, in some Pacific islands and in some Australian Aboriginal communities. Because acute HBV infection is often asymptomatic, it is not always clear when a person actually becomes infected. In Australia, around 300 new cases of hepatitis B and around 6000 cases for which the time of infection is not specified are reported annually.

There are currently eight known genotypes of HBV, designated A to H. The significance of the different genotypes is not clear, but they may influence the course and severity of the disease, the risk of progression to hepatocellular cancer (see below) and responsiveness to therapy.

Transmission

Hepatitis B virus can be found in the blood and body fluids of acutely infected people or carriers. Blood and blood products have the highest concentrations of HBV and are the most common sources of transmission, but other body fluids, especially semen, vaginal secretions, breast milk and serous fluids, can also transmit the virus. Since the number of infectious particles in blood can be extremely high (up to a million infectious doses per microlitre), very small amounts of blood can be infectious. The virus is fairly stable and can survive for days on surfaces or in dried blood. The virus is infrequently found in saliva, urine and faeces, and in lower concentrations. It is therefore only rarely transmitted via these body substances but it is important to recognise them as potential sources of infection.

Most cases in developed countries now occur in homosexual and bisexual males, injecting drug users, prison inmates, and people working in the sex industry. Babies born to mothers with hepatitis B have a high risk of infection. Perinatal transmission occurs as a result of the infant's exposure to infected blood or genital secretions during delivery or via breast feeding. Close personal contact (e.g. sharing of a toothbrush or razor) with an infected person has a low but important risk. Healthcare workers may be exposed to HBV via needlestick injuries, or when contaminated blood comes into contact with mucous membranes (e.g. the splashing of blood into the eyes) or with skin cuts or abrasions. Doctors, nurses and dental workers can be at high risk if appropriate preventative measures are not followed. It is unlikely that HBV is spread by mosquitoes or other biting insects, and if so, very infrequently.

Pathogenesis and clinical features

Replication of the hepatitis B virus in the liver results in cell damage, but the virus does not directly kill hepatocytes. Much of the pathology is due to immune mechanisms, including destruction of infected liver cells by cytotoxic T lymphocytes. The virus replicates prolifically in the liver, but assembly of components to form complete virions is very inefficient. As a result, large numbers of virus particles are released into the bloodstream, but only a small fraction of these are complete viruses (see Figure 18.17). Large amounts of antigenic viral components are released and these can be detected in the blood in diagnostic laboratory tests.

The incubation period of hepatitis B is 4–26 weeks (average 75 days). Acute infection is symptomatic in approximately 50 per cent of adults, but is usually asymptomatic in young children. Acute hepatitis B is clinically indistinguishable from other forms of hepatitis. Symptoms may include fever, jaundice, malaise, anorexia, nausea, abdominal pain, myalgia, arthralgia, skin rash and dark-coloured urine. A large proportion of cases are anicteric (without jaundice). As in most cases of hepatitis, serum levels of various enzymes are usually raised, especially alanine aminotransferase.

In otherwise healthy adults, 90 per cent will spontaneously recover within six months of the onset of the illness.

CASE HISTORY 18.9

Hepatitis B

A 34-year-old pregnant woman at 26 weeks gestation was admitted to hospital with severe acute hepatitis. Laboratory tests were positive for HBs antigen, HBe antigen and HBc IgM antibody, confirming acute hepatitis B infection. Lamivudine 100 mg daily was initiated. The patient had negative hepatitis B (HBV) serology prior to the pregnancy. At seven weeks' gestation she had miscarried two of her three foetuses, and required a transfusion of three units of blood.

One week after admission, the patient delivered a baby girl. The woman's liver function tests gradually improved, and three months later hepatitis B was undetectable. The baby was given hepatitis B immunoglobulin and hepatitis B vaccine at birth.

The Australian Red Cross Blood Service (ARCBS) recalled the three implicated blood donors for additional testing. One donor was found to have hepatitis B infection. All blood donors are screened by testing for HbsAg. However, due to an average of 38 days between infection and the individual having detectable levels of HBsAg (the window period), there is a very small risk of a blood product being infected with HBV. During 2010 the ARCBS introduced HBV nucleic acid testing (HBV NAT), which improves the detection of HBV by reducing the window period from 38 days to 24 days. It is expected that this will decrease the risk of HBV transmission from a unit of donated blood from approximately 1 in 739 000 to below 1 in 1 000 000.

Source: Adapted from A. P. Rode et al. 2011, Acute hepatitis B infection following blood transfusion. *Internal Medicine Journal* 41(6): 509–10.

Questions

1. Explain how the test results confirmed that the woman had hepatitis B?
2. Why was the baby given both hepatitis B immunoglobulin and hepatitis B vaccine?
3. How would you explain the meaning of a 'window period' to a patient?
4. What infection control measures should hospital staff observe when attending this patient?

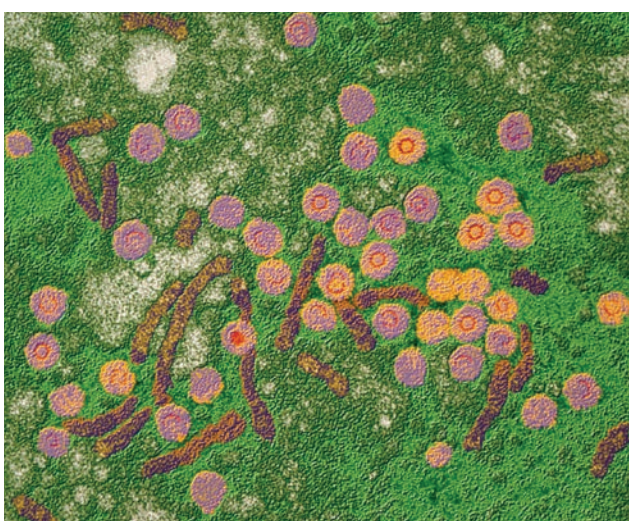

FIGURE 18.17

Hepatitis B virus

The complete virion (called a Dane particle—the larger, roughly circular particle) and different forms of HBsAg as seen in the serum of infected people.

Source: Eye of Science/Science Photo Library.

Fulminant hepatitis occurs in less than 1 per cent of patients. This is a clinical syndrome in which there is severe impairment or necrosis of liver cells, liver failure and often death. Acute hepatitis B has a mortality rate of around 0.2 per cent.

In 5–10 per cent of infected individuals, complete elimination of the virus does not occur and they develop chronic hepatitis, becoming chronic carriers of the virus. The risk of chronicity decreases with age. Vertical transmission (from mother to baby) is very serious, because it is associated with an extremely high rate of chronicity of around 90 per cent. Children infected at the age of 1–5 years have a risk of chronicity of about 30 per cent, and for children older than five years and adults the risk of chronicity is about 2 per cent. Around 25 per cent of people chronically infected develop chronic active hepatitis and are symptomatic. The remainder are asymptomatic chronic carriers. Some people with chronic hepatitis B (active or asymptomatic) will eventually develop liver cirrhosis, and people with cirrhosis and HBV infection may then develop hepatocellular carcinoma. Hepatitis B is thought to be responsible for as much as 80 per cent of all cases of liver cancer in the world, resulting in 1–2 million deaths per year.

The possible outcomes of hepatitis B infection are shown in Figure 18.18.

Laboratory diagnosis

Diagnosis of hepatitis B is by testing of serum for evidence of various viral antigens and antibodies to those antigens (see Figure 18.19). HBsAg appears in the blood during the incubation period and implies ongoing infection,

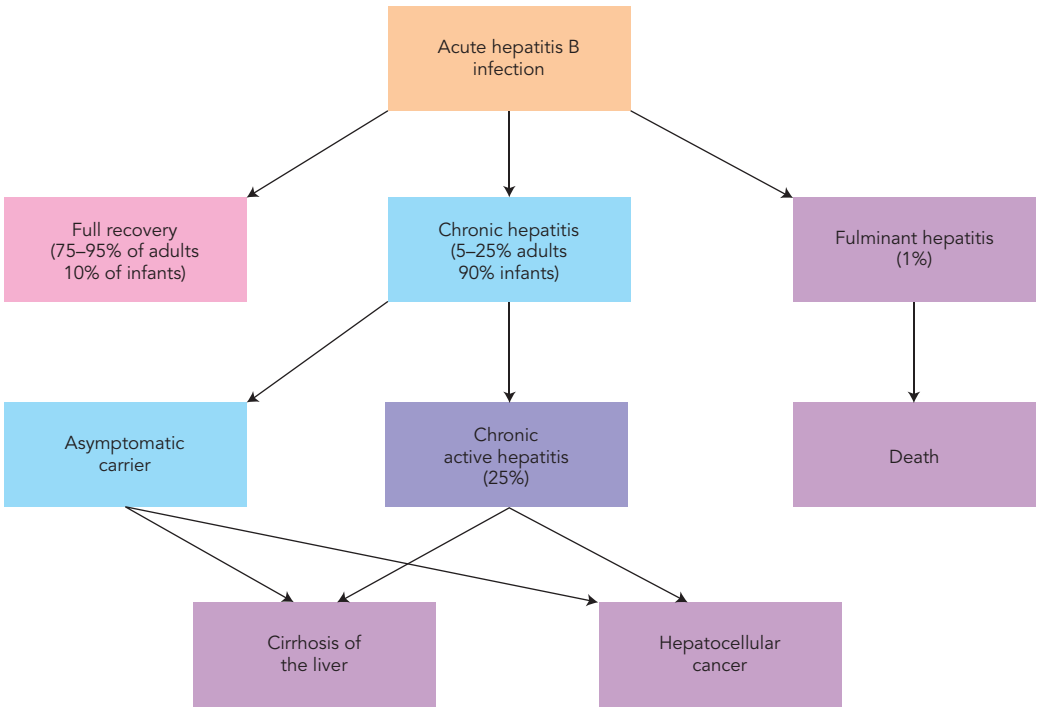

FIGURE 18.18

Possible outcomes of hepatitis B infection

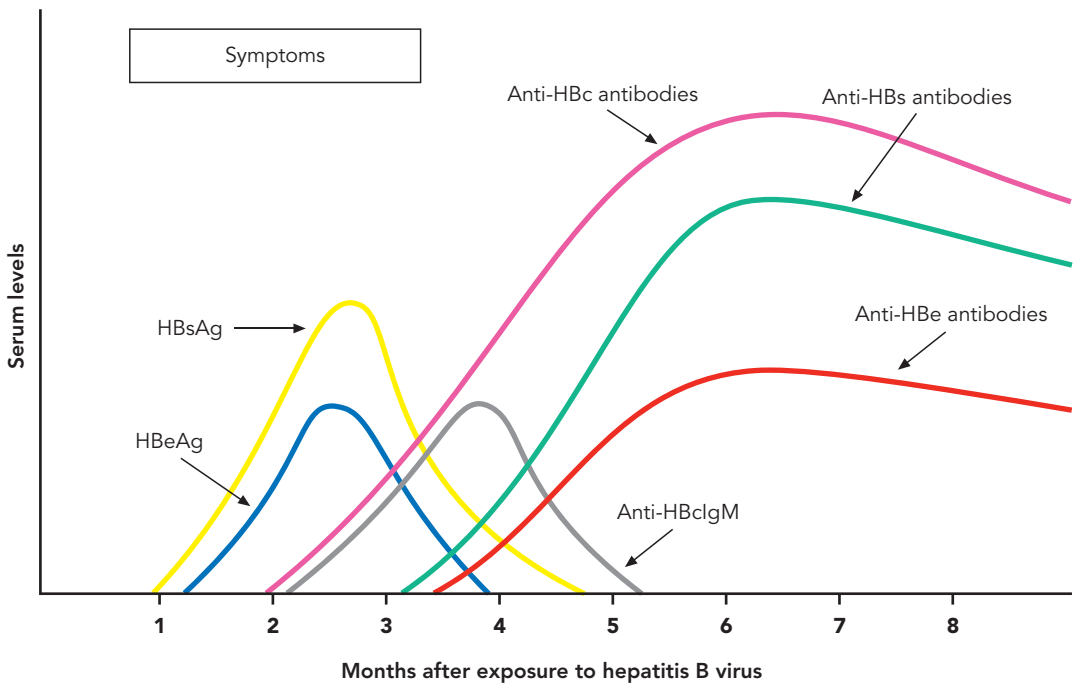

FIGURE 18.19

The typical course of acute hepatitis B infection and the associated serological events

either acute or chronic. HBsAg levels generally fall and finally disappear during recovery, but HBsAg remains in the blood of carriers. Persistence of HBsAg in the blood for more than six months indicates **chronic hepatitis B** infection. As HBsAg disappears, anti-HBs antibody becomes detectable. Anti-HBs antibodies are present for years in the serum of people who have fully recovered and are not carriers.

HBeAg is found in serum early during acute infection and in chronic active HBV infections. Detection of HBeAg indicates that there are large amounts of virus in the blood and thus high infectivity. The appearance of anti-HBe antibody and disappearance of HBeAg signals a cessation of viral replication and resolution of disease.

HBV-DNA can be detected by PCR in serum during acute and chronic infection. It is considered to be the best evidence of viral replication and infectivity in patients and is often positive in patients with low-grade viraemia who are negative for HBeAg. The loss of HBV-DNA from serum indicates resolution of disease.

During the illness, antibodies to the core antigen, HBcAg, appear. These antibodies represent an immunologic response to infection but are not indicative of disease resolution.

The interpretation of the tests for hepatitis B is summarised in Table 18.9.

Treatment and prevention

There is no fully effective therapy for hepatitis B. Hydration and anti-nauseants are the basic forms of symptomatic treatment. Alpha (α)-interferon, which appears to have both antiviral and immunomodulatory effects, has been used for the treatment of chronic hepatitis B with some success. In approximately one-third of patients with chronic HBV infection, treatment with α-interferon results in a suppression of HBV replication and clinical improvement, but discontinuation of the drug results in a relapse in 5–10 per cent of these people.

Possible side effects associated with α-interferon use range from fever, chills, fatigue and muscle aches to vascular collapse, coma, cyanosis, congestive heart disease, bone marrow depression and severe depression.

The drugs lamivudine, adefovir dipivoxil and entecavir directly suppress HBV replication but rarely succeed in permanently curing infection, usually due to the development of resistance.

Both passive and active immunisation are available for prevention of hepatitis B. Hepatitis B immunoglobulin (HBIG) is used to provide immediate post-exposure protection to unimmunised people following needlestick injury or high-risk sexual encounter, or to neonates born to HBsAg positive mothers. HBIG is prepared from plasma donated through routine blood bank collection and selected on the basis of containing high antibody titres to HBsAg. It should be administered within 72 hours of exposure.

The best way to prevent hepatitis B infection is with the HBV vaccine. This is a safe, effective vaccine consisting of genetically engineered HBsAg. Three doses of vaccine generally give good protection (efficacy around 95 per cent). Vaccination of all babies at birth is included in the Australian childhood immunisation schedule, with boosters at 2, 4, and 6 or 12 months. It is essential that the vaccination schedule be completed for full protection. Non-immune adults in high-risk groups should also be immunised. This includes household contacts, sexual partners of people with hepatitis B, injecting drug users, haemodialysis patients, people with chronic liver disease or hepatitis C, healthcare workers, inmates and staff of long-term correctional facilities, and residents and staff in institutions for the intellectually impaired.

Administration of both HBIG and HBV vaccine to neonates within 12 hours of birth is recommended if the mother is HBsAg positive, to prevent transmission of infection to the baby.

People with acute or chronic hepatitis B should be warned about the risk of infecting close contacts who are not immune. They should be made aware that some practices, such as the sharing of toothbrushes or razors, carry a risk but that ordinary domestic, social and work contact carry negligible risk. Healthcare workers should follow Standard Precautions to prevent cross-infection. Sensitive screening methods for detecting HBsAg are now employed by blood banks in Australia.

Hepatitis C

The existence of a third hepatitis virus became apparent in 1975, when new diagnostic tests for hepatitis A and hepatitis B revealed that many cases of transfusion-associated hepatitis were not due to either HAV or HBV. The genome of the hepatitis C virus (HCV) was cloned in 1989 and viral proteins produced. Soon after, antibody assays were developed and HCV was established as the cause of 80–95 per cent of cases of transfusion-associated non-A,

TABLE 18.9 Interpretation of tests for hepatitis B

	NO INFECTION PAST OR PRESENT	ACUTE INFECTION	CHRONIC INFECTION	PAST, RESOLVED INFECTION	PREVIOUS IMMUNISATION
HBsAg	–	+	+	–	–
Anti-HBc	–	+	+	+	–
Anti-HBc IgM	–	+	–	–	–
HBeAg	–	+	+/–	–	–
Anti-HBeAg	–	–	+/–	+/–	–
Anti-HBsAg	–	–	+/–	+	+
HBV DNA	–	+	+	–	–

non-B hepatitis. Australian blood banks began screening blood for anti-HCV antibody in early 1990. The virus itself has not yet been isolated. It is an RNA virus that is related to the flaviviruses. Six different genotypes (designated 1 to 6) have been identified. Figure 18.20 is an electron micrograph of the hepatitis C virus.

HCV has a worldwide distribution, with a very high prevalence in some countries in Africa (up to 18 per cent of the population), South-East Asia, and the Eastern Mediterranean and Egypt in particular. The WHO estimates that 170 million people worldwide are infected with HCV, and that there are about 3 million new infections each year. In Australia, around 400 new cases and 11 000 unspecified cases are reported each year. It is difficult to determine rates of HCV infection accurately because many cases are asymptomatic. In 2009 it was estimated that around 165 000 people in Australia were chronically infected with the virus and had early liver disease. The highest number of infections occurs in people aged 20–40 years, with a preponderance in males.

Transmission

Transmission of HCV is predominantly via contaminated blood. Since 1990, blood banks have been able to screen blood donors for HCV, so injecting drug use is now the greatest risk factor in developed countries, such as Australia, where screening is routine. However, blood transfusions and unsafe medical and surgical procedures are still significant modes of transmission in some developing countries. The prevalence of HCV among injecting drug users has been reported to be as high as 80 per cent. A high proportion of prison inmates are seropositive, largely related to injecting drug use. Unsafe tattooing or body piercing also represents a risk. Occupational acquisition of infection after needlestick injury has also been reported.

Vertical transmission of HCV from mother to baby during pregnancy rarely occurs, with the risk being greatest when the mother has a high viral load. It is thought that sexual transmission and perinatal transmission of HCV can occur, but are uncommon modes of infection. There appear to be other, as yet unknown, modes of transmission, because many cases occur in people with no known risk factor.

Pathogenesis and clinical features

The incubation period of hepatitis C appears to be quite variable, ranging from 14–180 days (average 50 days). Within 1–3 weeks of infection, HCV-RNA can be detected in blood. Most cases of HCV infection are asymptomatic. When symptomatic, the disease is usually mild and non-specific, with jaundice occurring in a minority of cases and liver enzymes only minimally to moderately elevated. The liver

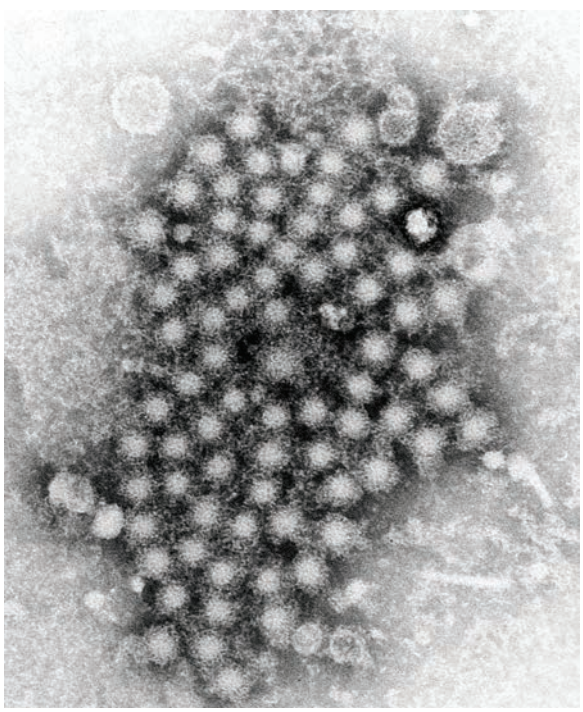

FIGURE 18.20

Electron micrograph of hepatitis C virus

Source: CDC/E.H. Cook Jr.

CASE HISTORY 18.10

Hepatitis C

In 2010–11 the Victorian Department of Health investigated a cluster of hepatitis C cases linked to an anaesthetist at a private medical centre in Croydon, Victoria. After contacting and testing more than 4000 patients who had undergone a procedure involving the anaesthetist, 49 women were identified as being infected with a hepatitis C virus that was linked to the anaesthetist by genetic sequencing. All of these women had procedures at the clinic between January 2008 and December 2009. Tests on 19 other women indicated past infection, but HCV DNA could not be detected in their blood, and therefore could not be definitively linked to this outbreak.

The anaesthetist, who had a history of drug abuse, was suspended from practice and a police investigation was commenced.

Questions

1. Is transmission of HCV in this case likely to be consistent with the normal modes of transmission of HCV? Explain.
2. Is the doctor's drug abuse a possible contributing factor?
3. Is it possible that any of the other clinic staff were infected by the doctor?
4. If most cases are asymptomatic, why is hepatitis C considered a serious disease?

disease appears to be at least partly due to the host immune response in which T cells, attempting to kill virus-infected hepatocytes, cause damage and induce ongoing inflammation. Clinically, it is indistinguishable from other forms of acute viral hepatitis.

Some 20–30 per cent of infected people clear the virus spontaneously. Despite the mild nature of acute infection, around 70–80 per cent of cases progress to chronic infection. This is a much higher rate than occurs with hepatitis B infection. As with chronic hepatitis B infections, the majority of chronic infections are asymptomatic. In symptomatic cases, there are often only mild symptoms of fatigue, weakness and a tender liver. But whether asymptomatic or not, the virus causes relentless damage to hepatocytes and 10–20 per cent of chronic cases develop serious liver damage with symptoms ranging from disabling lethargy to cirrhosis and liver failure within 20–30 years. Between 1 and 5 per cent of chronic cases develop liver cancer.

Laboratory diagnosis

Laboratory diagnosis of hepatitis C is based on the detection of antibodies in the serum of the patient. Unfortunately, the time from infection to seroconversion can be anywhere between two and nine months. This long 'window period' means that many cases of potentially infectious HCV may be missed by antibody tests. Direct demonstration of the virus itself is achieved by detection of viral RNA in serum, using the polymerase chain reaction (PCR) technique. PCR tests may be positive when antibody tests are negative, and a quantitative PCR test can provide information about the viral load in the bloodstream of the patient. The HCV RNA level in blood can be monitored to assess response to treatment.

When a diagnosis of hepatitis C is made, the patient is often advised to have a liver biopsy to determine the extent of their liver disease.

Treatment and prevention

Supportive care, as for other types of hepatitis, should be provided. Since acute HCV infection is asymptomatic and often undetected, specific treatment is rarely undertaken. Specific treatment for patients with chronic infection is recommended if progression to cirrhosis is likely. The current treatment regime is a combination of pegylated α-interferon and ribavirin, which achieves a sustained virological response (i.e. PCR-negative six months after completion of treatment) in around 50 per cent of cases. Treatment must be maintained for 24–48 weeks, depending on the genotype involved. Side effects of this treatment can be severe, so regular clinical and laboratory monitoring are warranted. Improved therapeutic outcomes have recently been reported in trials that have added a protease inhibitor (e.g. telaprevir) to the current regime.

There is no vaccine currently available for hepatitis C.

Hepatitis D

In 1977 a new hepatitis virus, now known as the **hepatitis D virus**, was discovered in hepatitis B carriers. This virus has a very small RNA genome and is a defective virus—that is, it requires a coexisting, active hepatitis B infection to be able to complete its own replicative cycle. When it buds from the surface of an HBV-infected liver cell it is coated with the hepatitis B surface antigen, HBsAg, and it is this hepatitis B coat that enables it to attach to, and infect, other hepatocytes. Thus, a person cannot contract an HDV infection unless simultaneously infected with HBV (i.e. a co-infection) or unless already infected with HBV (i.e. an HDV superinfection). Approximately 15 million people are infected with HDV worldwide. There are three known genotypes of HDV.

HDV is found in many parts of the world, but its highest prevalence is in Central and South-East Asia, the Middle East, Africa and South America. Hepatitis D is uncommon in Australia, with around 30 notifications annually. It is estimated that 5 per cent of HBV carriers worldwide are also infected with HDV, meaning that a total of 15 million people are infected with both viruses.

Blood and blood products are the major sources of HDV. Injecting drug users or people who have received multiple blood transfusions are at highest risk. Perinatal transmission has been reported, but is rare. The incubation period is in the range of 2–9 weeks, being shorter in HBV carriers superinfected with HDV than when co-infection with both viruses occurs. When co-infection occurs, clinical recovery and clearance of both viruses is the usual outcome. Chronic infection with HBV and HDV occurs in less than 5 per cent of patients. When HDV superinfects a chronic carrier of HBV, the infection is usually severe and can lead to cirrhosis and hepatic failure, with high mortality. Chronic hepatitis develops in 70–80 per cent of superinfections. Most patients with chronic HBV/HDV hepatitis develop hepatic cirrhosis, and almost 25 per cent of these die of liver failure.

Laboratory diagnosis is based on the detection of HDV-RNA by reverse transcriptase PCR. Serological tests are available for HDV antigen (HDAg) or for antibody to HDAg. Anti-HDV IgM levels are suggestive of acute infection. There is no fully effective treatment, other than physiologic support in rehydration and anti-nauseants. Limited success has been achieved with α-interferon, but this treatment has a low response rate and a high relapse rate. There is no HDV vaccine, but vaccination against hepatitis B prevents hepatitis D infection.

Hepatitis E

Hepatitis E is caused by a small RNA virus that is a member of the calicivirus family. The virus is shed in faeces and spread by the faecal–oral route.

HEV appears to have a restricted distribution in Central and South-East Asia, Africa, the Middle East and Central America. It is best known for causing water-borne outbreaks, but is also responsible for a large proportion of sporadic hepatitis in countries where it is endemic. It occasionally causes hepatitis in travellers to endemic areas. There are around 30 notifications of HEV in Australia annually, mainly in travellers returning from endemic areas.

The incubation period lasts 2–9 weeks, with a mean of about 40 days. Clinically, HEV infection is similar to HAV infection. Hepatitis E is generally a mild to moderate disease, but initial illness may be severe and fatal when associated with the malnutrition or other medical problems that are typical of its Third World victims. It can also cause severe infection in pregnant women, in whom the mortality rate from severe liver disease can reach as high as 20 per cent if infected in the third trimester. Overall, the case fatality rate is 1–4 per cent. The virus is eliminated from the body on recovery and does not cause chronic infection or long-term sequelae.

Serological tests for antibodies to HEV are used to diagnose hepatitis E infection. HEV infection should be suspected in a patient who has travelled to an endemic region and exhibits signs of hepatitis, but who has negative test results for other hepatitis viruses. The risk of infection when travelling in endemic countries may be minimised by practising rigorous hygiene, drinking only appropriately treated water and care in food consumption. No effective therapy is currently available.

Other hepatitis viruses

Up to 10 per cent of transfusion-associated hepatitis and up to 5 per cent of community-acquired infections cannot be attributed to a known virus. Other hepatitis viruses have thus been suggested. The hepatitis G virus may be responsible for some of these infections. This virus can be detected in blood using PCR methodology, but its role in causing liver disease has not been clearly established.

SUMMARY

- The gastrointestinal tract (GIT) is a portal of entry into the body for numerous microorganisms.
- Food poisoning (food-borne illness) is a general term for any gastrointestinal disease related to the consumption of food.
- Gastrointestinal pathogens are acquired mainly by the faecal–oral route.

ACUTE DIARRHOEAL DISEASES

- The major symptom of gastrointestinal infection is acute diarrhoea.
- Most acute diarrhoeal infections are self-limiting.
- Infectious diarrhoea can be divided into non-inflammatory diarrhoea and inflammatory diarrhoea.
- The common bacterial causes of acute diarrhoea include:
  - *Campylobacter* spp.
  - *Salmonella* serovars
  - *Escherichia coli*
  - *Vibrio cholerae* (cholera)
  - *Shigella* species
  - *Clostridium difficile* (also pseudomembranous colitis)
  - *Vibrio parahaemolyticus*
  - *Yersinia enterocolitica*
  - *Clostridium perfringens*
- The most common viral causes of acute diarrhoea are rotavirus and norovirus.
- The most common protozoal causes of acute diarrhoea are *Giardia intestinalis*, *Entamoeba histolytica* and *Cryptosporidium parvum*.
- Food intoxication is a disease caused by the presence of preformed toxins in food.
- The major causes of food intoxications are the enterotoxins of *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium botulinum*.
- For any patient with acute diarrhoea, fluid and electrolyte replacement is essential.

OTHER GASTROINTESTINAL DISEASES

- *Helicobacter pylori* is a major cause of gastritis, a major factor in the development of duodenal and gastric ulcers, and has a role in gastric cancer.
- *Salmonella typhi* and *S. paratyphi*, types A, B and C, cause typhoid and paratyphoid fevers.

HELMINTH INFECTIONS OF THE GASTROINTESTINAL TRACT

- Helminth infections are acquired either by penetration of the skin by larvae, or by ingestion of the eggs of helminths.
- The severity of the disease is largely a function of the number of worms in the body.
- The major helminth infections in humans include *Enterobius vermicularis*, *Ascaris lumbricoides*, hookworms (*Ancylostoma duodenale*, *Necator americanus*), *Strongyloides stercoralis*, *Echinococcus granulosus*.

HEPATITIS

- The term 'hepatitis' refers to injury and inflammation of the liver.
- Viruses are major causes of hepatitis, but other microorganisms, alcohol, drugs and various other disorders may also be responsible.

Hepatitis A

- Transmission of hepatitis A virus (HAV) is primarily by the faecal–oral route.
- The incubation period is 2–6 weeks.
- Complete recovery occurs in most cases within 4–6 weeks; there is no carrier state or chronic infection.
- An effective HAV vaccine is available and recommended for high-risk groups.

Hepatitis B

- Blood and blood products are the most common means of hepatitis B virus (HBV) transmission, but semen, vaginal secretions and serous fluids can also transmit the virus.

- Most healthy adults recover spontaneously within six months.
- Around 5–10 per cent of infected individuals develop chronic hepatitis and are chronic carriers of the virus.
- Chronic hepatitis is associated with an increased likelihood of hepatic cirrhosis and hepatocellular carcinoma.
- The HBV vaccine is a part of the standard childhood immunisation schedule and is also recommended for adults in high-risk groups.

Hepatitis C

- Transmission of hepatitis C (HCV) is mainly via contaminated blood.
- Around 80 per cent of cases of HCV progress to chronic infection.
- Most chronic infections are asymptomatic, but more than 20 per cent of patients eventually develop hepatic cirrhosis and/or hepatocellular carcinoma.

Hepatitis D

- A person can only contract an HDV infection when simultaneously infected with HBV, or when already infected with HBV.
- Blood and blood products are the major sources of HDV.
- There is no HDV vaccine, but vaccination against hepatitis B prevents hepatitis D infection.

Hepatitis E

- Hepatitis E virus (HEV) is shed in faeces and spread by the faecal–oral route.
- Clinically, HEV infection is similar to HAV infection.
- HEV can cause severe infection with high mortality in pregnant women.

STUDY QUESTIONS

1. What is the physiological basis of diarrhoea?
2. What microorganisms are commonly associated with outbreaks of diarrhoeal disease in daycare centres?
3. Describe the differences between inflammatory and non-inflammatory diarrhoea.
4. How are *Salmonella* diarrhoeal infections usually acquired?
5. Explain how enterohaemorrhagic *E. coli* cause haemolytic uraemic syndrome.
6. Why is cholera a potentially fatal disease?
7. Why is cholera vaccine not recommended for routine use in travellers?
8. Why is shigellosis so readily transmitted from person to person?
9. What is the basis of antibiotic-associated colitis?
10. What characteristics of *Clostridium difficile* make it difficult to eradicate from the hospital environment?
11. Why is norovirus infection so readily spread?
12. What is amoebic dysentery?
13. Why are pregnant women advised to avoid eating soft cheeses and delicatessen meats?
14. What characteristics of *Cryptosporidium parvum* enable it to survive so well in water?
15. What is a food intoxication?
16. How are diarrhoeal diseases usually diagnosed?
17. What are the major aspects of treatment of a patient with diarrhoea?
18. What important diseases are thought to be caused by *Helicobacter pylori*?
19. How is *Helicobacter pylori* infection diagnosed?
20. What important virulence factor is possessed by *Salmonella typhi*?
21. Explain how *Salmonella typhi* causes the symptoms of typhoid fever.
22. What specimens are required for the laboratory diagnosis of typhoid fever?
23. What are the typical symptoms of *Enterobius vermicularis* infection, and how is the infection diagnosed?
24. Describe the life cycle of *Ascaris lumbricoides*.
25. Describe the life cycle of hookworms.
26. What is the cause of hydatid disease? Describe its main features.
27. Define 'hepatitis' and list the possible causes.
28. Draw up a table to show the cause, common modes of transmission, important diagnostic tests, treatment and prevention of each of the five major types of viral hepatitis.
29. In which types of viral hepatitis is chronic infection possible, and what are the major problems associated with chronic viral hepatitis?
30. Explain the meaning of the terms 'HDV co-infection' and 'HDV superinfection'.

FURTHER READING

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CHAPTER 19

Cardiovascular and multisystem infections

CHAPTER FOCUS

- What microorganisms commonly cause cardiovascular and multisystem infections in Australia and other countries?
- How are cardiovascular and multisystem diseases usually transmitted?
- What are the important clinical aspects of the common cardiovascular and multisystem infections?

INTRODUCTION

Blood that circulates through the body of a healthy person is normally sterile. When large numbers of microorganisms enter the bloodstream from a wound or other focus of infection, they are readily disseminated throughout the body. They may then reach other body organs, where they may cause serious and often life-threatening diseases. Infections of the bloodstream and/or multiple organs are called **systemic infections**. A wide variety of microbes can cause such infections. Bacteria and viruses are most common, but protozoa are responsible for some serious systemic infections, the most notable being malaria. A number of fungi can cause systemic infections, but usually only in an immunocompromised person. Helminths also cause some important systemic infections, such as filariasis (elephantiasis) and schistosomiasis.

The terms used to signify microorganisms in the bloodstream often have a prefix that reflects part of the name of the microorganism, or the type of microorganism, plus the suffix *-aemia*. Thus, **viraemia** denotes the presence of viruses in the blood. Similarly, **fungaemia**, **parasitaemia** and **meningococcaemia** mean the presence of fungi, parasites (protozoa or helminths) and meningococci, respectively, in the bloodstream.

However, there is often some confusion over the usage and meaning of the terms 'bacteraemia' and 'septicaemia' because they are frequently used interchangeably. **Bacteraemia** literally means the *presence* of bacteria in the bloodstream. It may be manifested by the whole range of conditions from asymptomatic to seriously ill. Transient bacteraemia is in fact a fairly common occurrence in even healthy people, since low numbers of bacteria often spill into the bloodstream when minor trauma to skin (e.g. cuts, abrasions) or mucous membranes (e.g. teeth cleaning, chewing) occurs. However, the army of phagocytes located in the bloodstream and associated organs (see Chapter 9) usually remove small numbers of microbes very quickly from the blood. **Septicaemia** is a serious clinical syndrome resulting from the persistent or repeated presence of bacteria in the bloodstream; it thus represents a bacteraemia with severe clinical manifestations. A similar term is **sepsis**, which is used, by some, to describe the clinical syndrome related to the inflammatory response of the body to the presence of bacteria or their toxins in the bloodstream. Often, however, the terms 'septicaemia' and 'sepsis' are used interchangeably.

SYSTEMIC BACTERIAL INFECTIONS

Septicaemia

The bloodstream is a relatively inhospitable place for microorganisms. First, it is constantly moving, and thus it is difficult for microbes to colonise a surface and multiply. Second, it contains a variety of antimicrobial defence mechanisms, in particular the phagocytic leucocytes, antibodies and complement (see Chapter 9). Third, the blood recirculates through the spleen and liver, where sinusoids are lined with more phagocytic cells and where the blood flow slows, enhancing their phagocytic activity. Thus, microbes are usually quickly cleared from the bloodstream.

However, some pathogenic organisms are able to persist in the blood and actually be transported by it. In the majority of such cases the organisms enter the bloodstream from a primary focus of infection in organs such as the lungs, gastrointestinal tract or kidneys, or from an infected wound. Intravascular catheters (see Figure 19.1), especially central venous catheters, are important causes of bloodstream infections. These devices are readily colonised by microorganisms, which can lead to infection of surrounding tissue and the bloodstream. The use of intravenous catheters is very common, not only in hospitalised patients, but increasingly in patients receiving intravenous therapy at home.

For microorganisms to cause septicaemia, *at least one* of the following conditions must exist:

- The organisms have to be released, either continuously or intermittently, in large enough numbers to overwhelm the defence systems.
- There is some anatomical defect that facilitates the colonisation of a site (e.g. a damaged heart valve).
- The organisms have some protective mechanism that helps them to evade the defences of the blood (e.g. an antiphagocytic capsule or a coagulase enzyme).
- There is some impairment in the body's defences (e.g. hypogammaglobulinaemia resulting from cancer therapy; or neutropenia due to chronic infection).

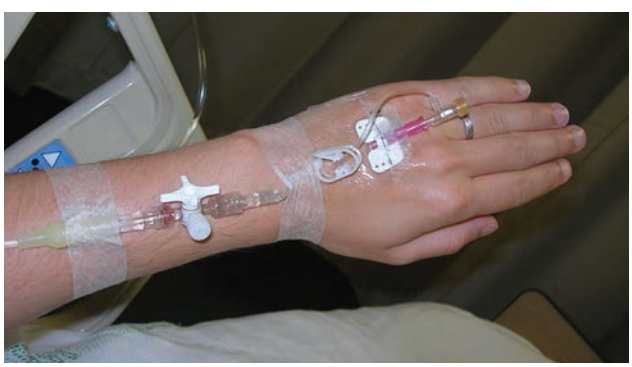


FIGURE 19.1

Intravascular catheter for administration of fluids to a patient

Source: Michael Berry/Wikipedia.

While bacterial multiplication in the blood may occur, it is not a requirement for septicaemia to develop. The common causes of septicaemia are shown in Table 19.1.

Clinical features

The clinical manifestations of septicaemia are extremely variable. Usually, the patient has a high, spiking fever, often with alternating shaking chills and sweats. These symptoms are often intermittent, interspersed with periods of improvement. Shock, manifested by low blood pressure and vascular collapse, is also common. **Endotoxic (septic) shock** is a severe, life-threatening form of septicaemia caused by the endotoxin (the lipid-A component of lipopolysaccharide) in the cell wall of Gram-negative bacteria.

Diagnosis

The diagnosis of septicaemia is classically made by the demonstration of bacteria in the blood, usually by blood culture (see Chapter 15). Indirectly, the diagnosis may sometimes be made by the demonstration of a focus of infection somewhere in the body. The bacteria causing septicaemia may be difficult to culture from a sample of blood, for a number of reasons.

1. The organisms, even when causing severe infection, may be present in the bloodstream only in low numbers. In addition, the amount of blood collected for culture (around 10 mL) is a small sample size of the total blood volume of 5–6 litres.
2. The organisms may be only transiently present in the bloodstream, particularly if they are shed intermittently from a primary focus of infection. In these circumstances, after each shower of organisms into the bloodstream bacterial numbers will gradually be reduced by the body's defence mechanisms. Thus, they may be present in large numbers for only a short time after release.
3. Some septicaemia-causing bacteria are difficult to grow or are very slow-growing *in vitro*.

To maximise the chance of isolating the causative agent, three samples of blood for culture are usually taken from a patient over a 24–48-hour period. The best time to take a sample is when the patient is experiencing a febrile episode, as this generally represents a showering of bacteria into the bloodstream from the focus of infection. It is preferable that blood for culture be collected before commencement of antibiotic therapy (see Chapter 15). Culture of a catheter tip (see Chapter 15) may be helpful if it is thought to be a possible source of the infection.

TABLE 19.1 Common causes of septicaemia

HOSPITAL-ACQUIRED SEPTICAEMIA	COMMUNITY-ACQUIRED SEPTICAEMIA
Staphylococcus aureus	*Escherichia coli*
Coagulase negative staphylococci	*Staphylococcus aureus*
Escherichia coli	*Streptococcus pneumoniae*
Pseudomonas aeruginosa	*Klebsiella pneumoniae*
Enterobacter spp.	Group B streptococcus
Enterococci	*Pseudomonas aeruginosa*

CASE HISTORY 19.1

Septicaemia

A 49-year-old man had a Hickman catheter surgically inserted for total parenteral nutrition. Six days after surgery it was noticed that the catheter entry site had become inflamed. He had a temperature of 39.8°C, a high respiratory rate and rapid pulse. Blood cultures were collected and two out of three sets grew *Staphylococcus epidermidis.*

Questions

1. Does this patient have septicaemia? If yes, what is the likely cause?
2. What is the likely source of the *Staphylococcus epidermidis*?
3. Why are multiple samples of blood usually collected from a patient for the diagnosis of septicaemia?
4. What infection control precautions should be observed by a health professional when collecting blood from this patient?

Culture-based methods for isolation and identification of pathogens from blood are relatively slow, even with recent advances in faster culture methods. A number of alternate techniques hold great promise for faster diagnoses of bloodstream infections. For example, matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALD-TOF MS) has been used to achieve identification of organisms grown in blood cultures to within 1–2 hours. And PCR technology is also being used to develop rapid diagnostic methods for septicaemia. These methods are described in detail in Chapter 15.

Treatment

Treatment involves the administration of an appropriate antimicrobial drug. Because of the potential severity of the disease, antimicrobial therapy is usually commenced at the first clinical suspicion. A broad spectrum agent is used and then modified, if necessary, once the identity of the causative agent and its antimicrobial sensitivity are known. The treatment of septicaemia can be very difficult because of the involvement of organisms resistant to multiple antibiotics in both hospital-acquired (e.g. MRSA, VRE) and community-acquired (e.g. MRSA) infections. Location and treatment of the primary focus of infection are also necessary; if it is an abscess it may require surgical drainage. The patient may also require symptomatic treatment for systemic complications, such as hypotension.

Toxic shock syndrome

Toxic shock syndrome is mainly caused by certain strains of *Staphylococcus aureus* that produce a particular toxin called toxic shock syndrome toxin 1 (TSST-1). The disease came to prominence when outbreaks occurred in women, associated with their use of super-absorbent tampons. The tampons were sometimes left in place for too long and caused abrasion of the vaginal wall, providing appropriate conditions for infection. It is now recognised that toxic shock syndrome can result from any infection caused by strains of *S. aureus* that produce TSST-1 or enterotoxin A, B or C. Also, some strains of group A streptococcus (*Streptococcus pyogenes*) that produce pyrogenic exotoxins can cause a toxic shock syndrome. These staphylococcal and streptococcal toxins cause widespread tissue injury and shock because they act as superantigens (see Chapter 10).

Toxic shock syndrome is a potentially life-threatening condition, even with treatment, characterised by high fever, hypotension and a red, sunburn-like rash. In one or two days the skin starts to peel off in sheets, particularly on the palms and soles of the feet. Other parts of the body are usually involved, such as the gastrointestinal system (vomiting, diarrhoea), muscles (myalgia), central nervous system (disorientation) and mucous membranes (vaginal or conjunctival hyperaemia). Patients should be examined for a focus of infection, and vigorous antibiotic therapy is warranted. Death, when it occurs, is usually due to shock.

Rheumatic fever

Incidence

Rheumatic fever is a non-suppurative (not pus-forming) complication of *Streptococcus pyogenes* sore throat. It is a non-suppurative disease because it is not a direct infection. It affects only a small percentage of people (< 3 per cent), following a streptococcal sore throat or, less commonly, skin infection. Its highest incidence occurs in 5–15 year-olds. Rheumatic fever is still a major cause of cardiovascular disease in developing countries where poverty, overcrowding and poor hygiene are important factors. In Australia and other developed countries, the incidence of rheumatic fever has generally been declining for several decades, except in socioeconomically deprived communities. In some Aboriginal communities the incidence is estimated to be 200–500 per 100 000 population, which is among the highest documented rates in the world. Similarly, the disease is much more common in New Zealand's Indigenous population than in non-Maori people.

Clinical features and pathogenesis

Onset of rheumatic fever usually occurs 2–4 weeks after a streptococcal sore throat. Without treatment, the susceptible person can suffer recurrence of rheumatic fever after each episode of streptococcal sore throat, with a latent period of 2–4 weeks each time. The major diagnostic criteria of rheumatic fever are variable combinations of:

- carditis, manifested usually by sinus tachycardia (fast heart rate), a heart murmur, chest pain or extra heart sounds
- acute migratory polyarthritis, mainly of large joints
- chorea (sudden, involuntary, irregular movements)—in some children, rare in adults
- subcutaneous nodules near joints
- erythema marginatum (a distinctive macular rash)—in some children, rare in adults.

An acute attack of rheumatic fever lasts up to three months. If untreated, rheumatic fever can result in scarring and deformation of the valves, which is known as **rheumatic heart disease**. Valve damage and disruption to normal blood flow around the valve can predispose it to colonisation by bacteria and, hence, endocarditis. This is discussed in the next section.

CASE HISTORY 19.2

Acute rheumatic fever

An 11-year-old Indigenous girl presented to an emergency department with a painful, swollen right foot and fever (38.5°C). After admission she was found to have a raised ESR (erythrocyte sedimentation rate), and raised ASOT and anti-DNAse B titres, and therapy with intravenous antibiotics was commenced. The next day she had a tender right knee, which quickly improved, and she was discharged after three days.

Eighteen months later she again went to the emergency department with a swollen and painful knee (with no history of injury) and fever (38.6°C). She was not admitted, but oral antibiotic therapy was prescribed for possible osteomyelitis.

She was admitted to hospital again at 13 years of age, again with a painful left knee. The initial diagnosis was reactive arthritis or septic arthritis. After further examination and tests, a diagnosis of acute rheumatic fever was made.

Source: Adapted from J.N. Hanna and M.F. Clark 2010, Acute rheumatic fever in Indigenous people in North Queensland: Some good news at last? *Medical Journal of Australia* 192(10): 581–84.

Questions

1. What signs and symptoms of this girl are characteristic of rheumatic fever?
2. What is/are the likely factor(s) that led to the development of rheumatic fever in this girl?
3. What possible effect(s) would the antibiotics have had on her condition?
4. Once the diagnosis of acute rheumatic fever was made, how would you expect the girl's condition to be managed in the future?

The development of rheumatic fever is due to cross-reactivity between antigens of the streptococci and heart tissue antigens. Thus, antibodies produced against the streptococcus (while it is infecting the throat) cross-react with heart tissue, causing inflammation and damage. Certain strains of *S. pyogenes* have M-proteins that have an antigenic similarity to cardiac myosin and sarcolemma membrane proteins.

Genetic factors also seem to be important, since people with certain MHC types (see Chapter 9) have a much greater risk of rheumatic fever than people who lack those types.

Diagnosis

Diagnosis of rheumatic fever is made by correlation of clinical findings (especially the major criteria above) with certain laboratory findings indicating recent streptococcal infection, such as a positive throat culture, or high or rising antibody titres. Multiple serological tests may be performed, including an anti-streptolysin-O titre (ASOT) and anti-DNAse tests.

Treatment and prevention

Antibiotics (e.g. penicillin or erythromycin) are given to remove the remaining streptococci, and aspirin or other anti-inflammatory drugs are used to reduce pain, fever and inflammation. Prompt antibiotic treatment of *S. pyogenes* throat infections in people known to be susceptible to rheumatic fever can prevent further attacks. Continuous, prophylactic use of antibiotics may be prescribed for people known to suffer severe attacks of rheumatic fever.

Infective endocarditis

The wall of the heart consists of three layers. The innermost layer is called the endocardium. It is a layer of epithelium covering the heart muscle and the valves, and is in direct contact with the blood. **Infective endocarditis** is an inflammation of the endocardium, initiated by an infectious agent. It often presents as a fever of unknown origin (FUO) and is a fatal disease if untreated. It may occur as an acute, rapidly progressing disease or have a chronic onset and development. In more than 60 per cent of patients there is a pre-existing heart condition such as congenital heart disease, mitral valve prolapse, valvular damage from rheumatic fever, or a prosthetic heart valve.

Causative organisms and pathogenesis

Infective endocarditis can be caused by a wide variety of bacteria, but streptococci and staphylococci are the most common. Although in most cases a pre-existing heart condition is present, in some cases there is no apparent defect. On abnormal or damaged valves, oral streptococci (e.g. *Streptococcus sanguis*, *S. milleri* and *S. oralis*) and staphylococci (especially *Staphylococcus aureus*) are the most common causes. The oral bacteria can enter the bloodstream through minor abrasions such as those caused by chewing, teeth cleaning or flossing, or following dental procedures or dental surgery. They can then colonise a heart valve. Similarly, staphylococci can enter the bloodstream following minor skin injury (e.g. cuts and abrasions) and then colonise a valve.

On prosthetic valves, staphylococci (especially *Staphylococcus epidermidis* and *S. aureus*) are a major cause of early endocarditis—that is, occurring less than two months after implant surgery. These organisms are probably introduced during surgery. Late valvular endocarditis (more than two months after implant) can be caused by a wide range of organisms, including staphylococci, oral streptococci and enterococci. Up to 3 per cent of patients with a prosthetic valve implant develop an endocarditis within a year.

Intravenous drug users may develop an endocarditis caused by microorganisms they inject into themselves in unsterile procedures. In these cases, *Staphylococcus aureus* is most common, followed by oral streptococci and Gram-negative bacteria (e.g. *Pseudomonas*). Fungi, such as *Candida*, *Aspergillus* and *Mucor*, are also found occasionally as the cause of endocarditis in IV drug users. Polymicrobial infections (infection caused by more than one organism) may also occur. Iatrogenic endocarditis is an important type. Predominant causes are staphylococci and enterococci, associated with IV catheters, haemodialysis and other medico-surgical procedures. Table 19.2 summarises the causative agents of endocarditis.

Infective endocarditis is often associated with prior endocardial damage. On damaged or abnormal valves, collagen is exposed and this stimulates the deposition of fibrin and platelets. The valve abnormality and these fibrin-platelet clumps cause a disturbance to the normal smooth flow of blood through the valve, resulting in turbulence. If bacteraemia occurs in conditions of turbulent flow, the bacteria have the opportunity to attach to valve surfaces, particularly to the deposited fibrin, via specific adhesins or sticky polysaccharides secreted from the cell.

TABLE 19.2 Common causative agents of endocarditis in different groups of patients. For each category the organisms are listed in decreasing order of frequency

Abnormal/damaged valve	Oral streptococci *Staphylococcus aureus*
Prosthetic valve (< 2 months after implant)	Coagulase negative staphylococci *S. aureus* Gram-negative (enteric) bacteria Oral streptococci Fungi (mainly *Candida albicans*)
Prosthetic valve (> 2 months after implant)	Oral streptococci Coagulase negative staphylococci *S. aureus* Enterococci
IV drug abuser	*S. aureus* Oral streptococci Gram-negative (enteric) bacteria

Having attached, the bacteria are able to multiply and attract monocytes which release cytokines. The bacteria and cytokines cause further platelet and fibrin deposition. This growing clump of bacteria, fibrin and platelets is called a **vegetation** (see Figure 19.2). The clumps of fibrin and platelets provide some shelter for the bacteria against body defences and antibiotics in the blood. These lesions can develop anywhere on the endocardium, but usually occur on heart valves or surrounding structures.

Vegetations vary in size and can grow to several centimetres. With increasing growth of the vegetations, there will be increasing destruction to underlying tissues and increasing valvular dysfunction, with the possibility of cardiac distress and congestive heart failure. Also, septic emboli (pieces of vegetations containing bacteria) may eventually break off and be carried to other organs, where they can infect and/or occlude blood vessels, causing inflammation and necrosis.

Clinical features

The signs and symptoms of infective endocarditis are extremely variable because they usually reflect one or more of the following:

- damage and deformation of heart valves
- embolisation of vegetations with necrosis in remote tissues
- deposition of antigen-antibody complexes in blood vessels in the skin, the glomeruli or joints.

Typical clinical manifestations are fever, chills and heart murmur, often with anorexia, weight loss, malaise, chills, nausea, vomiting and night sweats. However, because of the variability of symptoms, diagnosis of the condition can be difficult. Splinter haemorrhages in the nail bed, skin lesions, and signs of glomerulonephritis or synovitis may also occur. Death is usually due to congestive heart failure secondary to valvular dysfunction.

Diagnosis, treatment and prevention

Untreated endocarditis is usually fatal, so an accurate diagnosis is important. Blood culture is the key to the laboratory diagnosis. The isolation of the causative agent is necessary so that antibiotic sensitivity results can be obtained and appropriate therapy provided. If possible, blood cultures should be collected before commencement of antibiotic therapy. Preferably three, and up to five, specimens of blood should be collected for culture within a 24–48-hour period (see Chapter 15). Bacteria may be released from vegetations at a fairly constant rate, resulting in a continuous bacteraemia. In some cases of endocarditis blood cultures are negative, which may be due to:

- commencement of antibiotic therapy before collection of blood for culture
- infection by slow-growing, fastidious or unusual organisms
- non-infectious causes.

An echocardiogram provides supporting evidence of an abnormality of a valve or associated structure.

Prolonged, high-dose antibiotic therapy (4–6 weeks) is often necessary for infectious endocarditis because the organisms within vegetations are afforded a degree of protection from antibiotics. Even with treatment, infective endocarditis has a mortality rate as high as 40 per cent, depending on the causative agent and the nature of the underlying valvular abnormality. Empirical therapy, before culture results are available, usually includes a combination of drugs such as penicillin, flucloxacillin and gentamicin, or vancomycin plus gentamicin if the infection was acquired in hospital. This would then be modified once culture and sensitivity results become available.

Prevention is based on the use of prophylactic antibiotics in people with certain high-risk cardiac conditions or undergoing certain surgical or dental procedures. New Australian guidelines were published by Therapeutic Guidelines Ltd in 2010, and can be accessed at <www.tg.com.au>.

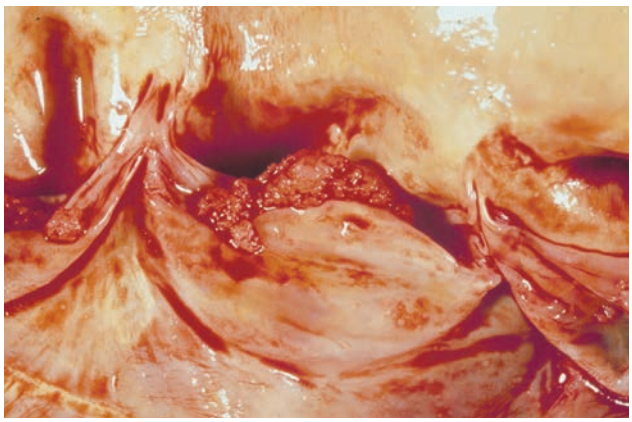


FIGURE 19.2

Infective endocarditis

The pathological feature of this disease is the development of vegetations (clumps of fibrin, platelets and bacteria) on the heart valve.

Source: Dr David Nevell, Anatomical Pathology PaLMS, Royal North Shore Hospital.

Osteomyelitis

Cause and pathogenesis

Osteomyelitis is an inflammation of bone caused by infectious microorganisms. Although bone is normally resistant to infection, trauma, surgery, or the presence of foreign bodies may increase its susceptibility. Trauma is the most common predisposing factor. The infection may be categorised according to the mode of entry of the microbe.

1. *Haematogenous osteomyelitis* is caused by organisms that are carried by the blood to the bone tissue, generally from a site of infection elsewhere in the body. Intravenous drug abusers may also develop haematogenous osteomyelitis from the use of contaminated injecting equipment.
2. *Exogenous osteomyelitis* is caused by organisms introduced directly from outside the body—for example, through a compound fracture (fracture in contact with an open wound), penetrating wound or surgery. Often the organism first infects adjacent soft tissue before spreading to the bone.

Haematogenous osteomyelitis most commonly affects the growing ends of long bones (e.g. the tibia and femur) and thus most often afflicts children and adolescents. The vulnerability of these bones is due to the anatomy of their vascular supply. A slow blood flow in the large-diameter vessels in these bones facilitates the colonisation and growth of bacteria. The most common cause is *Staphylococcus aureus*, but other organisms such as Group B streptococci, *Haemophilus influenzae* and *Mycobacterium tuberculosis* may also cause this infection. Haematogenous osteomyelitis is rare in adults, and most often affects the spine.

Staphylococcus aureus is also the most common cause of exogenous osteomyelitis, but there can be other causes, depending on the circumstances of the trauma and the area of the body involved.

Clinical features, diagnosis and treatment

Haematogenous osteomyelitis in a child is usually manifested by a sudden onset of high fever, chills, nausea and progressive pain over the infected bone. In adults a more insidious and vague onset of fever, malaise and anorexia is usual. In exogenous infections, signs and symptoms of soft tissue infection usually predominate.

A variety of imaging methods, such as plain radiographs, MRI (magnetic resonance imaging) and ultrasonography, are used to provide confirmation of osteomyelitis and assessment of degree of tissue damage. Blood culture or bone biopsy (if blood culture is negative) may identify the causative organism and its antibiotic sensitivity. However, the causative organism is isolated in only around 60 per cent of cases.

Treatment requires immediate intravenous antibiotics, usually starting with a broad spectrum drug, and then adjustment when culture and sensitivity results become available. Antibiotic therapy is usually maintained for 4–6 weeks. Chronic infection can develop when bone necrosis occurs. Necrotic bone tissue presumably shields the bacteria from body defences and acts as a continuous source of infection. In this situation, surgical intervention for debridement and drainage as well as prolonged antibiotics may be necessary.

Lyme disease

Causative agent and transmission

Lyme disease was first identified as a bacterial infection in 1975 in the United States, in a cluster of 51 people with a distinctive skin rash in the village of Old Lyme in Connecticut. It is caused by *Borrelia burgdorferi*, a spirochaete that is 20–30 μm long and approximately 0.25 μm wide (see Figure 19.3). It is a very common vector-borne disease in North America, with around 20 000 cases per year being reported. It is also common in many areas of Europe and has been identified in several other countries, including Japan and China. It is found mainly in forested areas of these parts of the world. The incidence of Lyme disease in Australia is unknown, but there have been sporadic reports of Lyme disease-like illness in eastern coastal areas since 1982.

In the US the natural cycle of infection takes place in white-footed field mice and deer. It is transmitted in these animals and to humans by ticks of the genus *Ixodes*. The species of tick that carry the bacterium in other parts of the world have not been found in Australia, and *B. burgdorferi* has been difficult to find in Australian ticks that bite humans. While clinical and serological information suggests that the disease may occur in this country, there is no definitive evidence that it does.

CASE HISTORY 19.3

Osteomyelitis

Thomas, a 13-year-old boy, injured his lower left arm in a rugby match at school. The injury occurred when he fell heavily on the arm while being tackled. The next day his arm became extremely painful and was hot and swollen. He was taken to the family doctor, who recorded his temperature at 39.4°C. The doctor also noticed several small boils on his chest, some of which had been broken.

Questions

1. What microorganism is the most likely cause of Thomas's osteomyelitis, and what is its likely source?
2. Is this likely to be a haematogenous or exogenous osteomyelitis?
3. Why is haematogenous osteomyelitis most common in adolescents?

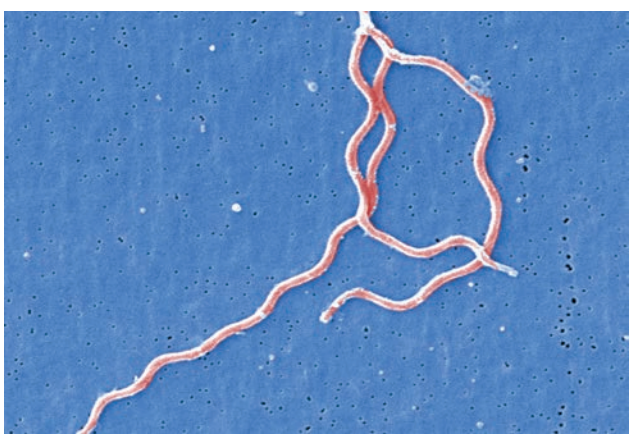


FIGURE 19.3

Colourised scanning electron micrograph of *Borrelia burgdorferi*, the causative agent of Lyme disease

The micrograph shows three cells of the bacterium.

Source: CDC/Claudia Molins.

Pathogenesis and clinical features

The spirochaete is injected into the skin of humans while the tick is feeding and then travels via the blood or lymph to virtually anywhere in the body. It seems to favour synovial tissue, skin and the nervous system. The course of Lyme disease is variable, ranging from no symptoms or only localised skin manifestations to severe and chronic disease. After a variable incubation period, usually about a week, the full disease progresses in three stages.

Stage 1 is found in 50–80 per cent of infected people and is characterised by **erythema migrans**. This is a unique skin lesion which begins as a red macule, which then expands to form an erythematous annular lesion, often with a clear centre, giving it a 'bulls-eye' appearance (see Figure 19.4). The skin lesion may be accompanied by local lymphadenopathy and minor symptoms of fever, headaches, tiredness and joint pains. This stage spontaneously resolves in several weeks.

Stage 2 follows within days to weeks and represents disseminated disease. Manifestations may include secondary annular skin lesions in other sites, neurologic disorders (e.g. lymphocytic meningitis, mental deterioration), cardiac disorders (e.g. myocarditis) and recurrent attacks of arthritis. Patients may also suffer debilitating malaise and fatigue.

Stage 3 occurs after a latent period of several months or more and represents chronic disease of the skin, nervous system and joints. Arthritis is the most common late sign. There may also be neurological problems, such as short-term memory loss. This stage may persist for years. Stages 2 and 3 develop in 10–15 per cent of untreated patients.

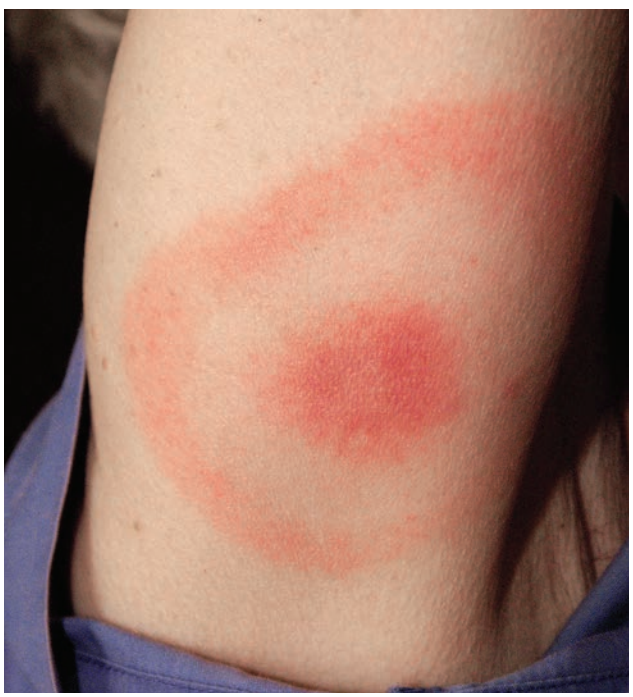


FIGURE 19.4

The characteristic 'bulls-eye' rash of Lyme disease

Source: CDC/James Gathany.

Diagnosis

Lyme disease is difficult to diagnose. It is mostly based on the clinical features (especially erythema migrans) and a history of tick bite in an endemic area. However, the clinical picture is often atypical or non-specific and exposure history is vague. Laboratory tests may be helpful but have significant limitations. Antibody tests are the most common diagnostic methods, but false positive and negative results are a problem. Culture of biopsy material from skin lesions provides a definitive diagnosis, but it is slow (requiring up to eight weeks) and has low sensitivity. PCR (polymerase chain reaction) testing is superior to culture, but is currently not widely available in clinical laboratories.

Treatment and prevention

Early-stage Lyme disease is usually effectively treated with doxycycline, amoxycillin or ceftriaxone. Ceftriaxone is usually preferred in more established infections. Chronic arthritis and other late manifestations may not respond to antibiotic therapy because of their immunological basis. Prevention is based mainly on avoidance of tick bites. An effective vaccine has been licensed in the United States.

Rickettsial infections

Causative organisms and incidence

Rickettsiae are named after Howard Ricketts, who first identified them as the causes of typhus and Rocky Mountain spotted fever in the US. The **rickettsiae** are Gram-negative, rod-shaped bacteria. They have the unusual characteristic for bacteria of being strictly intracellular parasites—that is, like viruses, they can multiply only inside host cells. Another feature of these organisms is that they multiply in arthropods such as ticks, lice, fleas or mites.

By far the most common rickettsial infection in Australia is Q fever, caused by *Coxiella burnetii*. It differs from other rickettsial infections in that it is most often transmitted to humans by aerosol, rather than by arthropods. It can be transmitted by faeces, milk or hides of infected sheep and cattle. Thus, farmers, meat-workers and animal transporters are most commonly affected. The primary site of infection is the lung; hence, Q fever is discussed more fully in Chapter 17.

After Q fever, scrub typhus and Queensland tick typhus, caused by *Orientia tsutsugamushi* and *Rickettsia australis* respectively, are the most common in Australia, although fewer than ten cases of both types of typhus occur per year. Worldwide, there are many species that can cause human infection (see Table 19.3). Generally, infections caused by *Rickettsia* are not very common but are important because they can be fatal.

Pathogenesis and clinical features

The classic rickettsial diseases occur following the bite of an arthropod vector. The rickettsiae disseminate through the bloodstream, localising in small blood vessels in many organs, including the skin, brain and heart. They invade and damage the endothelial cells of these vessels, causing them to leak.

TABLE 19.3 Features of selected rickettsial infections

DISEASE	CAUSATIVE ORGANISM	DISTRIBUTION	VECTOR
Spotted fevers			
■ Rocky Mountain spotted fever	*Rickettsia rickettsii*	Western hemisphere	Ticks
■ Rickettsialpox	*R. akari*	Russia, Korea, Turkey	Mites
■ Queensland tick typhus	*R. australis*	Australia	Ticks
Typhus fevers			
■ Epidemic typhus	*R. prowazekii*	South America, Africa	Lice
■ Murine typhus	*R. typhi*	Worldwide	Fleas
Scrub typhus	*Orientia tsutsugamushi*	Asia, Australia, Pacific Islands	Mites
Cat-scratch disease	*Bartonella henselae*	Worldwide	Cat flea
Q fever	*Coxiella burnetii*	Worldwide	—

The resultant haemorrhages and surrounding tissue necrosis produce the typical clinical features of rickettsial infections: skin rash (except in Q fever), severe headache and fever. Other possible symptoms include a local lymphadenopathy in the region of the arthropod bite, myalgia, and liver and spleen enlargement. The disease can be mild (e.g. Queensland tick typhus) to fatal (e.g. epidemic typhus). Death usually results from shock and cardiorespiratory failure.

Laboratory diagnosis and treatment

Serology on paired serum samples is currently the preferred method for diagnosis. PCR tests show promise for faster diagnosis and at an earlier stage of the disease. Rickettsiae are hazardous organisms, so careful handling of patient blood should be observed. Prompt treatment, within the first week of illness, with an appropriate antibiotic is effective. Doxycycline is the drug of choice, but azithromycin and chloramphenicol are also effective. Supportive therapy for thrombocytopenia, hypotension, coagulation defects and other disorders may be necessary.

Leptospirosis

Leptospirosis is a zoonosis caused by *Leptospira interrogans*. This highly coiled spirochaete, 5–15 μm long, has the ends of the cell bent into a hook, giving the cell the appearance of a question mark—hence the name *interrogans* (see Figure 19.5). Leptospirosis is a notifiable disease in Australia, where the annual incidence is 100–200 cases, with the majority in Queensland.

The bacteria infect dogs, cats, cattle, sheep, pigs and many wild mammals, especially rats, causing a chronic kidney infection. Infected animals excrete large numbers of bacteria in urine. Humans usually become infected through skin abrasions or mucous membrane contact with water, soil, vegetation or food contaminated with animal urine. Australian cases are often associated with occupational exposure, particularly among abattoir workers, farmers and stock-transporters. The bacteria invade the bloodstream and then are carried throughout the body. Many infections are asymptomatic, but in some a febrile, flu-like illness occurs after an incubation period of 1–4 weeks. In most of the cases an uneventful recovery takes place within several weeks.

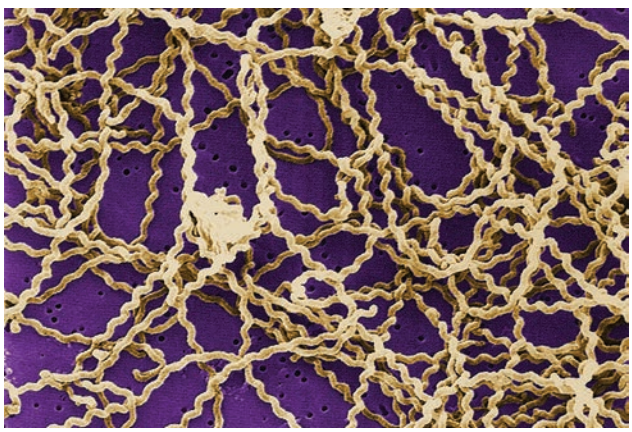


FIGURE 19.5

Leptospira interrogans

An electron micrograph of this hook-shaped spirochaete which causes urinary tract infections in animals and systemic infections in humans.

Source: CDC/Rob Weyant.

A severe form, called Weil's disease, represents infection of the liver and kidneys, and is characterised by hepatitis, jaundice, uraemia and bacteriuria. Pulmonary haemorrhage may also occur. Mortality can be as high as 20 per cent in this form of the disease.

Bacteria may be isolated from urine, blood and CSF, with urine culture being the most reliable method. Serological methods are generally the main ways by which the infection is diagnosed, but are slow, requiring the demonstration of a rising antibody titre in serum samples collected two weeks apart.

Penicillin or doxycycline are usually effective, especially if given within the first week of illness. Patients with severe disease require hospitalisation and supportive care. An animal vaccine is available, but there is not yet one for humans.

CASE HISTORY 19.4

Leptospirosis

Two weeks after a holiday in Vanuatu, a 24-year-old man presented to an emergency department with chest pain and chest wall tenderness. He had previously been in good health. In Vanuatu he had cut his foot on coral, and after that had swum in a freshwater river.

He was admitted to hospital with an initial diagnosis of musculoskeletal chest pain of unknown origin. Over the next few days his condition deteriorated. He initially developed an erythematous rash, vomiting, diarrhoea and a mild headache, and subsequently acute renal failure, jaundice, respiratory failure and myocarditis. During hospitalisation he was given a number of antibiotics and supportive therapy. On day 8 he suffered a cardiac arrest from which he could not be resuscitated.

Source: Adapted from F.M. O'Leary et al. 2004, Fatal leptospirosis presenting as musculoskeletal chest pain. *Medical Journal of Australia* 180: 29.

Questions

1. How do humans usually acquire leptospirosis?
2. What is the treatment for leptospirosis?
3. Would there be any risk of cross-infection for a healthcare worker caring for this person?

CASE HISTORY 19.5

Anthrax in heroin users

Between December 2009 and July 2010 an outbreak of anthrax occurred among heroin users in Scotland. There were 47 confirmed cases, 13 of whom died. Linked sporadic cases also occurred in England and Germany. The symptoms tended to reflect the way in which the heroin was taken—injected, smoked or snorted. Typical clinical presentations included swelling and redness or an abscess or ulcer at the injection site, septicaemia or severe headache. Some patients had symptoms typical of inhalational anthrax.

Health authorities in Scotland considered that the heroin had been contaminated in or near its source country. One theory forwarded was that the heroin may have been contaminated during storage in an anthrax-contaminated goat skin; goat skins apparently being a common method for smuggling of the drug. Authorities were concerned about the ongoing risk associated with the possible stockpiling of the contaminated heroin.

Questions

1. How do humans usually acquire anthrax?
2. Why is anthrax able to survive for so long in contaminated products?
3. Why were the affected people in this outbreak not immune to anthrax?

Anthrax

Anthrax is a zoonosis that is acquired primarily from herbivores, especially sheep, goats and cattle. It is caused by *Bacillus anthracis*, a large, spore-forming, Gram-positive rod. Its spores are extremely resistant to adverse environmental conditions, able to survive for decades in soil. Human infection mainly comes from direct contact with infected animals (skins or carcasses), inhalation of spores present in aerosolised animal products (e.g. milk) or ingestion of infected meat. Anthrax is generally not a major problem in developed countries, but the cases of anthrax in the United States in 2001, resulting from the deliberate release of anthrax spores in letters, illustrates the ease with which the disease is transmitted. Its ease of transmission is a major reason why some countries (including the US, Russia and Iraq) have, in the past, developed anthrax as a biological warfare agent. Between 1998 and 2009, only three human cases of cutaneous anthrax have been reported in Australia. Outbreaks of anthrax still occur in livestock (cattle and sheep) in Australia, despite the availability of an animal vaccine.

There are three classical forms of anthrax: cutaneous, pulmonary and intestinal. In cutaneous anthrax a cut or skin abrasion is the usual site of entry, where the spores germinate and the bacteria multiply and release a number of toxins. In 1–2 days a papular lesion develops, surrounded by a ring of vesicles. Eventually it ulcerates and becomes black and necrotic (Figure 19.6). In a minority of cases, septicaemia and severe toxaemia can occur, with generalised toxic effects. This form of anthrax has a mortality rate of 10–20 per cent if untreated. Pulmonary anthrax ('woolsorter's disease') is the more serious form of anthrax, but is rare in developed countries. Inhalation of spores leads to multiplication in the

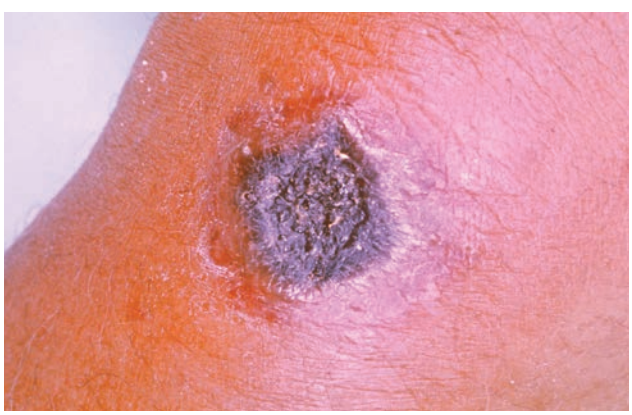


FIGURE 19.6

The ulcer of cutaneous anthrax

Source: CDC/James H. Steele.

lungs, pulmonary oedema and haemorrhage, and septicaemia. The mortality rate in this form exceeds 40 per cent, even with treatment. Intestinal anthrax is very rare and usually occurs after ingestion of meat contaminated with spores.

Anthrax is best diagnosed by culture of organisms from skin lesions, pleural fluid or blood, or microscopic demonstration of Gram-positive, spore-forming rods in these specimens. Treatment with antibiotics (e.g. benzylpenicillin or ciprofloxacin) does not alter the course of the infection but, if given early, reduces the risk of systemic symptoms in cutaneous anthrax. Animal vaccination greatly reduces the incidence of human disease. Infected animals are killed and the carcasses cremated or deep buried and covered with anhydrous calcium oxide (quicklime). A human vaccine is available in Australia for use in high-risk persons, such as people working with potentially infected animals or their products. Prophylaxis for exposed, non-vaccinated people is with antibiotics such as doxycycline or ciprofloxacin.

Brucellosis

Cause and incidence

Human **brucellosis** was first described in 1860 among British soldiers in Malta, where it became known as **Malta fever**. It is also sometimes called **undulant fever** because of the rising and falling nature of the fever. The disease occurs worldwide and is caused by three species of the genus *Brucella*: *B. melitensis*, *B. abortus* and *B. suis*. These small, Gram-negative coccobacilli are primarily animal pathogens but cause disease in humans after contact with infected animals or their products.

Brucella localise in the reproductive organs of host animals, causing abortions and sterility. They are shed in large numbers in the animal's urine, milk and other fluids. *Brucella melitensis* is found mainly in sheep and goats, and is endemic in Mediterranean countries, the Middle East, South America, and parts of Europe, Asia and Africa. *Brucella abortus* is acquired mainly from cows. It has a worldwide distribution except in certain developed countries which have succeeded in eradicating the organism. Australia was declared free of bovine brucellosis in 1989. *Brucella suis* infects pigs and occasionally other animals such as horses, cows and dogs. It occurs mainly in South America and South-East Asia.

Around 40–50 cases of brucellosis are notified per year in Australia. Most *B. suis* infections occur in Queensland among wild boar hunters. Any case due to other *Brucella* species is assumed to have been acquired overseas.

Transmission

Brucellosis is highly communicable. Transmission from animals to humans occurs by direct contact with infected tissues or animal products, or via dust or aerosols. The organisms enter the body via abrasions in the skin, via the conjunctivae or the gastrointestinal or respiratory tract. Infection may occur in people consuming unpasteurised milk products (e.g. 'village cheeses') and in farmers, abattoir workers and hunters. Pig hunters are often heavily exposed to blood and body fluids during butchering of the animals.

Pathogenesis and clinical features

Inside the human host, *Brucella* enter the lymphatic system and then the blood, causing an acute bacteraemia. They are able to multiply within phagocytes in the spleen, liver, bone marrow and other lymphoid tissues. Granulomatous inflammatory reactions and necrosis of tissues may occur, with enlargement of affected organs. Often the infection is subclinical, but in clinical cases the onset of symptoms is gradual and begins one or more weeks after contact. Typical presenting symptoms are night drenching sweats, chills and fever, disabling lethargy, malaise, headache, myalgia and arthralgia. Anorexia and substantial weight loss may occur. Complications such as splenic abscesses, endocarditis, septic arthritis, osteomyelitis and meningitis are possible, especially if diagnosis and treatment are delayed.

Diagnosis

Serology is currently the most common method of diagnosis; a high titre is used to confirm infection in conjunction with typical clinical manifestations. Culture of the causative organism from blood or bone marrow is also possible. Blood cultures have low sensitivity, whereas bone marrow culture identifies up to 90 per cent of cases. PCR testing for brucellosis has recently been developed, with promising results.

Treatment and prevention

Combination antimicrobial therapy is recommended because of the high rate of relapse when a single drug is used. Doxycycline or ciprofloxacin in combination with rifampicin or streptomycin are the regimes recommended by the World Health Organization. Rifampicin and trimethoprim-sulfamethoxazole are recommended for young children. A prolonged course of therapy for at least six weeks is necessary. At present there is no effective human vaccine. Prevention is based on (1) pasteurisation of dairy products, (2) animal vaccination, (3) education of workers at risk of occupational exposure, and (4) the use of protective clothing by at-risk workers. There is a high risk of laboratory-acquired infection by aerosol transmission from an infected specimen. Appropriate infection control procedures must therefore be used when collecting and processing a specimen from a person suspected of having brucellosis.

Plague

Cause and incidence

Few diseases have ravaged the human population throughout time in the way plague has done. Some authorities believe that the first historic mention of the disease is in the Book of Samuel, which describes an epidemic of 'emerods' (buboes?) among the Philistines. Several great pandemics of plague have been recorded. The Black Death, as it was known in the Middle Ages, killed an estimated one-quarter of the population of Europe in the 14th century. The Great Plague of 1665–66 was restricted largely to London but

caused more than 75 000 deaths. Even in the 20th century, the disease was responsible for 10 million deaths in India alone, in a pandemic affecting central Asia. Nowadays, most cases are restricted to some parts of Asia (e.g. Myanmar, Vietnam, China), Africa (e.g. Madagascar, Zaire, Uganda), the Americas, and countries of the former Soviet Union. In Australia, as in other developed countries, the disease is rare. The last case recorded in this country was in 1923.

The causative agent of **plague** is *Yersinia pestis* (see Figure 19.7), a small, Gram-negative rod belonging to the family Enterobacteriaceae. The organism infects wild rodents, especially rats, and is transmitted from animal to animal and animal to human by flea bites. In epidemics, massive numbers of rats die of the disease, and the fleas, deprived of their natural hosts, feed voraciously on humans instead.

Pathogenesis and clinical features

Yersinia pestis possesses a number of virulence factors including an antiphagocytic capsule, a coagulase, endotoxin and various other protein toxins. The organism multiplies rapidly and is able to survive within macrophages. It spreads via the lymphatics to regional lymph nodes. Within 1–7 days of the flea bite a haemorrhagic inflammation causes these nodes to form very large and tender **buboes** (enlarged lymph nodes)—hence the term **bubonic plague**. They are most frequently found in the armpit or groin. If the organisms move into the circulatory system, infection, haemorrhage and necrosis occur in many parts of the body, including the skin, lungs, liver, spleen and CNS. The haemorrhages and resultant cyanosis turn the skin black—hence the name 'Black Death'. Around 50 per cent of untreated cases of bubonic plague die.

Pneumonic plague occurs when the organism invades the lungs. About two days after exposure symptoms including fever, chills, headache and myalgia develop, followed by cough, dyspnoea and chest pain. Unlike the bubonic form of the disease, it can be transmitted from person to person via droplets expelled during coughing (see Figure 19.8). The mortality rate is close to 100 per cent if treatment is delayed more than 24 hours after the onset of symptoms.

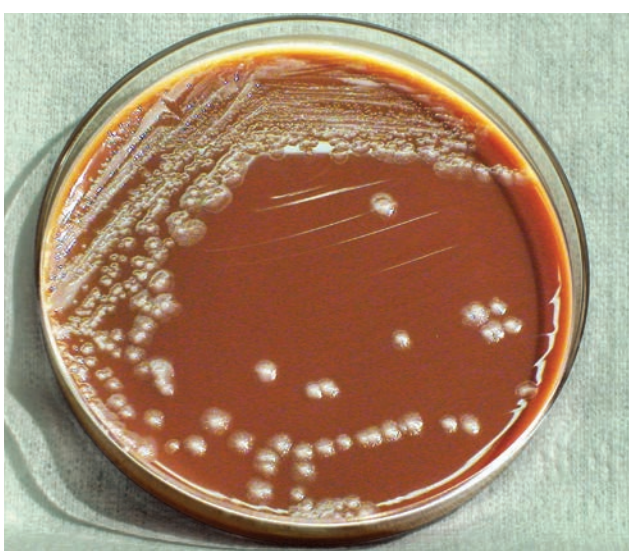


FIGURE 19.7

Culture of *Yersinia pestis* on chocolate blood agar

Diagnosis, treatment and prevention

Culture of *Y. pestis* from blood, sputum, cerebrospinal fluid, or fluid aspirated from buboes is the standard diagnostic procedure. However, *Y. pestis* is slow growing, so culture takes time. Other possible approaches include direct microscopic examination of stained smears, serology and, more recently, PCR. Antibiotics used for the treatment of plague include streptomycin, gentamicin or doxycycline. Prevention is based on the following:

- rodent and flea control
- strict isolation of infected patients
- quarantine and fumigation of infected ships
- vaccination of workers in endemic areas (boosters are required every six months).

Standard Precautions are required when caring for patients with bubonic plague. Droplet precautions are necessary for patients with pneumonic plague.

SYSTEMIC VIRAL INFECTIONS

Mumps

Mumps is caused by a single-stranded RNA virus of the family Paramyxoviridae. Mumps remains endemic in many countries throughout the world, particularly in developing countries with inadequate or non-existent vaccination programs. Infected humans are the sole source of mumps virus. Transmission of the virus is via the respiratory route in air-borne droplets or by direct contact with saliva. After an incubation period of 2–3 weeks a variety of clinical outcomes may be seen, ranging from asymptomatic infection to widespread systemic involvement. A large proportion of patients experience mild upper respiratory symptoms, fever, headache, malaise, myalgia and anorexia. The characteristic swelling of the parotid glands (parotitis) occurs in the majority, but not all cases. Orchitis (inflammation of one or both testicles) occurs in up to 20 per cent of post-pubertal males with mumps. Patients are most infectious several days before the onset of symptoms and for about a week after the onset of parotitis. Encephalitis may occur in up to 1 in 200 cases, with a case-fatality rate of around 1 per cent. Mumps infection during the first trimester of pregnancy may result in spontaneous abortion, but maternal infection is not linked with congenital malformations.

Before vaccination, mumps was primarily a disease of childhood. However, the highest incidence in developed countries now occurs in older adolescents and young adults. Currently, the average annual notification rate of mumps in Australia is around 300 cases. Despite the availability of vaccines in developed countries, large outbreaks, involving thousands of people, still occur; examples include the US in 2006 and 2009, Canada in 2007, and the UK in 2009. In Australia, mumps vaccine is a component of the MMR vaccine, which is part of the standard immunisation schedule,

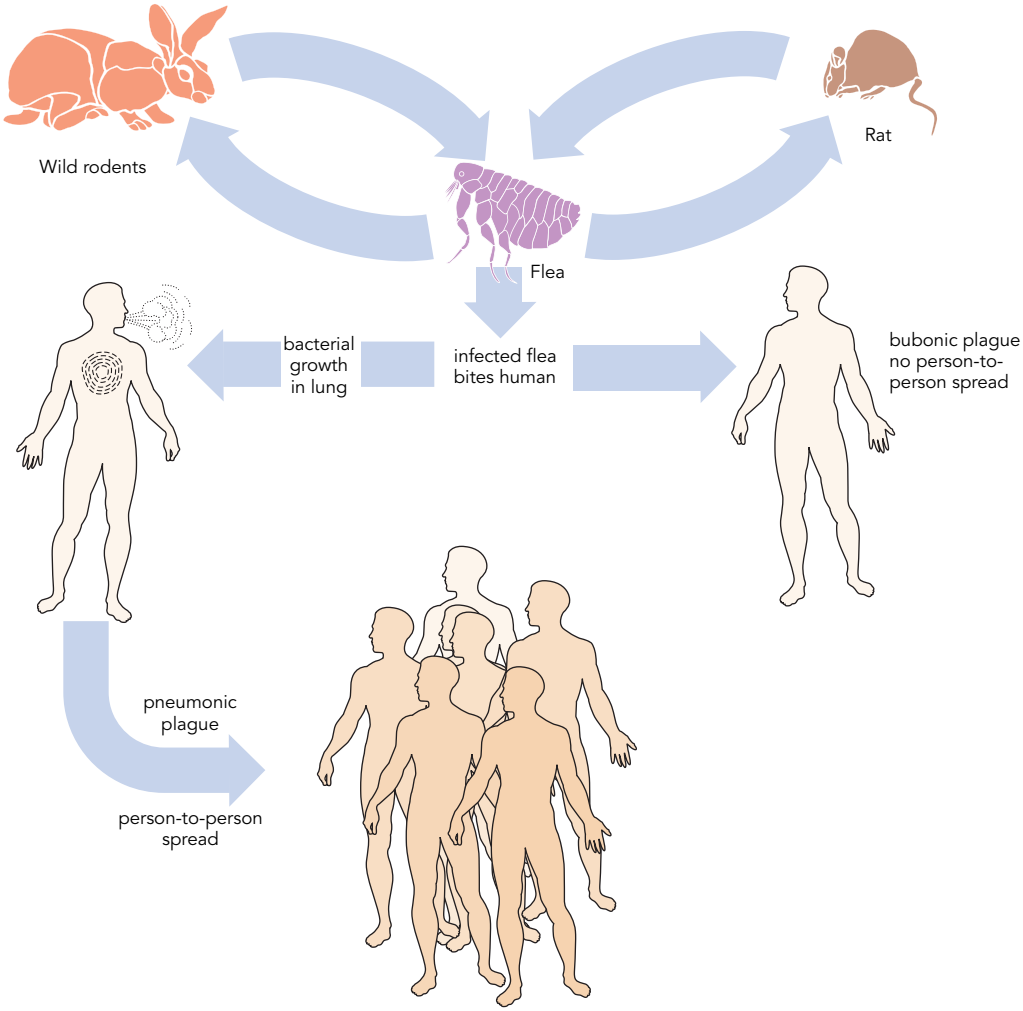


FIGURE 19.8

The epidemiology of plague

administered at 12 and 18 months of age. Outbreaks in highly vaccinated populations (as above) are likely due to a combination of inadequate uptake, incomplete vaccination (i.e. one dose instead of two), incomplete efficacy of the vaccine and waning immunity. Adverse reactions to mumps vaccine are rare and largely inconsequential.

Diagnosis of mumps is usually based on clinical findings. If required, laboratory diagnosis is based on isolation of the virus, PCR or serology. There is no specific treatment for mumps.

Human immunodeficiency virus infection and AIDS

In 1981 a new syndrome, subsequently called the **acquired immunodeficiency syndrome (AIDS)**, was first recognised among homosexual men in the United States. It was characterised by the appearance of rare and fatal infections and rare types of cancer. In 1983 the causative agent was isolated from blood lymphocytes and became known as the human immunodeficiency virus (HIV). The course of the disease varies from person to person, but it is estimated that, of untreated people, up to 30 per cent will develop AIDS within five years, and almost all will eventually develop AIDS and die from it. A very small percentage of people control their infection naturally and recover without treatment. With appropriate treatment, however, the course of the disease is significantly altered.

Causative agents

Two serotypes of HIV have been identified: HIV-1 and HIV-2. By far the most common cause of AIDS throughout the world is HIV-1. The modes of transmission and the clinical features of HIV-1 and HIV-2 infection are similar. In this book, HIV will be used to refer to both HIV-1 and HIV-2 unless stated otherwise.

The structure of HIV is shown in Figure 19.9. It is a roughly spherical particle comprising an outer envelope of a bilayer of lipid molecules. The envelope is studded with numerous 'spikes', which are two types of glycoproteins—gp120 and gp41. Beneath the envelope is a layer of protein called p18, which in turn surrounds the capsid (p24 antigen).

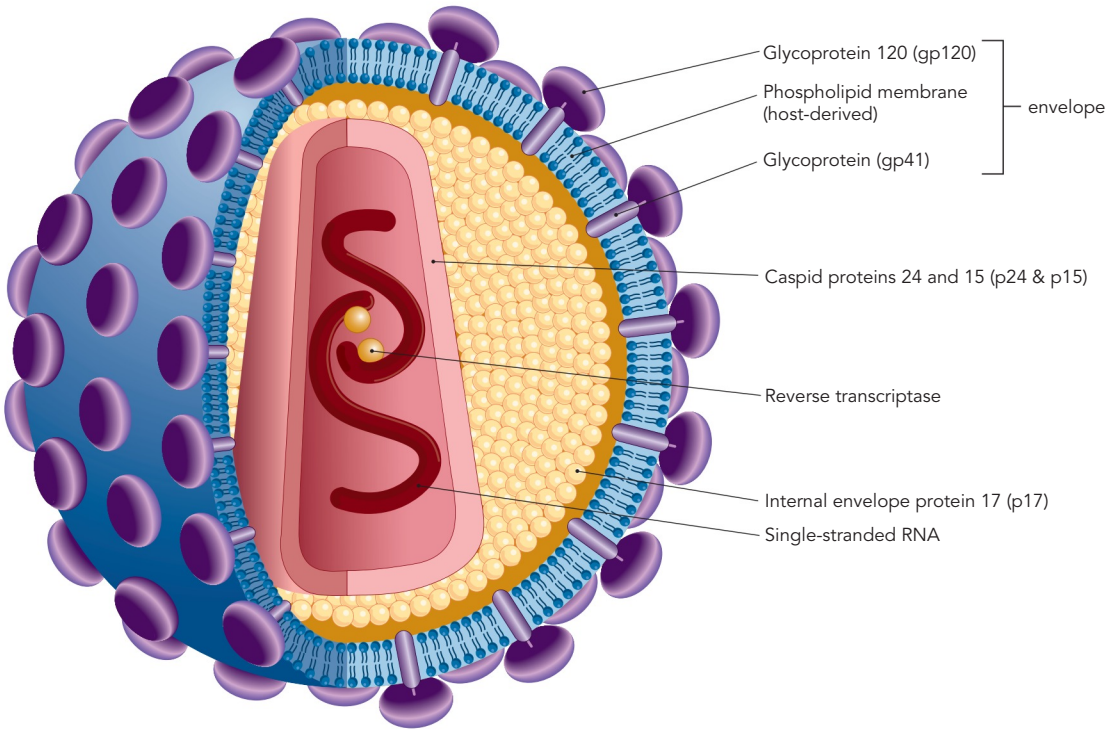


FIGURE 19.9

The structure of the human immunodeficiency virus (HIV), the causative agent of AIDS

Inside the capsid there are two single-stranded molecules of RNA. Attached to the RNA are molecules of the enzyme **reverse transcriptase**, which transcribes the viral RNA into DNA once the virus is inside its host cell. HIV has two other critical enzymes: an **integrase**, which integrates the viral DNA into the host cell DNA; and a **protease**, which cleaves newly produced viral proteins for a proper assembly of new viruses.

Incidence

The Joint United Nations Programme on HIV/AIDS (UNAIDS) estimated that in 2008 there were around 33 million people infected with HIV worldwide. Of these, 2.7 million were newly infected during that year, around a quarter of them children. Since the epidemic began, there have been over 35 million deaths. The vast majority (90 per cent) of people currently infected live in Africa or in the developing countries of Asia. Currently in Australia there are an estimated 22 000 people with HIV infection. About 150–300 new cases occur annually. There have been around 7000 deaths due to AIDS in Australia.

Transmission

It has been established that HIV is transmitted in the following ways:

- through unprotected sexual intercourse (vaginal or anal)
- through oral sex
- through blood or blood products (e.g. transfusion, sharing of needles)
- from mother to child.

Characteristically, in developed countries most HIV infections have occurred in men, primarily as a result of sex with other men or intravenous drug use. However, the difference in numbers of men and women infected is narrowing as transmission by heterosexual intercourse becomes more common. In the Caribbean and some parts of Asia and Africa, transmission has largely been through heterosexual relations, and males and females are more or less equally affected. In Australia, sexual contact between men is the major means of transmission (see Table 19.4). Very low rates of transmission occur through injecting drug use or heterosexual contact. Occupationally acquired infection in healthcare workers, due mainly to needlestick injury, has been documented and is a definite risk. Unhygienic tattooing can also be a risk (see Spotlight box).

The transmission of HIV from mother to child may occur during pregnancy, at delivery, or through breast feeding. Without any intervention, the risk of transmission of HIV infection from infected mother to baby is at least 30 per cent.

Pathogenesis

HIV disease results from chronic infection of cells bearing the CD4 antigen. The gp120 envelope protein of the virus can bind tightly to this antigen (Figure 19.10). The CCR5 protein is present on the surface of some T cells and macrophages, and is an important co-receptor for HIV to enter the cells. T helper lymphocytes (T4 lymphocytes) are the main targets, but activated antigen-presenting cells (dendritic cells, monocytes and macrophages) are also infected.

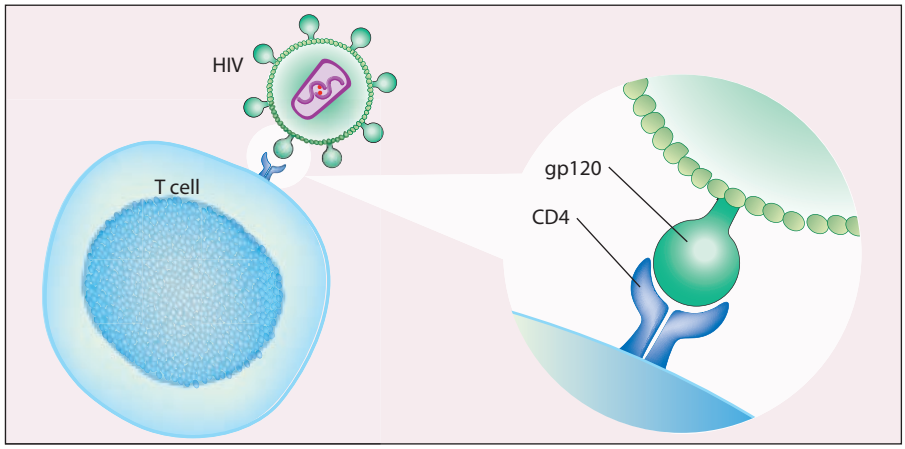


FIGURE 19.10

Binding of the HIV gp120 protein to the CD4 receptor of the human T lymphocyte

TABLE 19.4 **Diagnoses of HIV in Australia, cumulative to 30 June 2010**

EXPOSURE CATEGORY	MALE	FEMALE	% OF TOTAL
Men who had sex with men	19 423		75.1
Men who had sex with men and injecting drug use	1 092		4.2
Injecting drug use	773	240	3.9
Heterosexual contact	1 928	1 759	14.3
Receipt of blood/blood product/tissue	406	118	1.8
Healthcare setting	4	9	0.1
Child infection from mother	60	55	0.4

Source: Adapted from National Centre in HIV Epidemiology and Clinical Research 2010, *Australian HIV Surveillance* Report, 26(4).

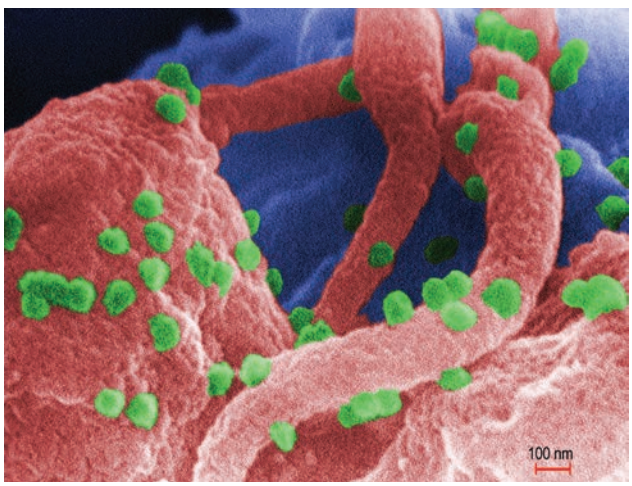


FIGURE 19.11

Human immunodeficiency virus on the surface of a human lymphocyte

This colourised scanning electron micrograph (SEM) shows the human immunodeficiency virus (green) on a lymphocyte (red). The finger-like extensions are the pseudopodia of the lymphocyte.

Source: CDC/C. Goldsmith, P. Feorino, E.L. Palmer, W.R. McManus.

Monocytes and macrophages appear to be relatively resistant to being killed by HIV, but they appear to be at least partly responsible for transporting the virus to different parts of the body, including the brain.

When the virus enters a cell, it uncoats and then, like all retroviruses, transcribes its RNA into DNA using its reverse transcriptase enzyme. The DNA is then converted into double-stranded DNA and integrated into the host cell genome using the integrase enzyme. Once the viral DNA is integrated, the cell is transformed into a factory for production of more viruses. HIV can replicate at a remarkable rate in an untreated person—around 10 billion virions a day! Complete viruses are released from the surface of infected cells by budding, and they can then infect other target cells (see Figure 19.11). In some cells the HIV DNA is dormant for a time, possibly for decades. Later it may become activated and direct the synthesis of new viral RNA and viral proteins.

The immunosuppression caused by HIV is a complex process that has still not been fully elucidated. The virus is thought to cause damage to the immune system in a number of ways, including:

- the direct killing of infected T4 cells by the virus
- the destruction of virus-infected immune cells, including T4 cells, by killer T cells
- the prevention of T4 cells responding to foreign antigens through the blocking of cell receptors (CD4) by detached viral proteins gp120 and gp41
- the release of a soluble suppressor factor by infected T4 cells
- the infection and killing or disruption of the function of other cells with the CD4 receptor, especially antigen-presenting cells
- virus-initiated autoimmune response to CD4 receptors and subsequent destruction of cells bearing those antigens
- viral damage to lymph nodes and other immune organs.

HIV link to Bali tattoo

SPOTLIGHT ON

On 23 December 2011 the Department of Health warned Western Australians of the potential health risks of getting a tattoo in Bali, following confirmation of a WA case of HIV where all the evidence pointed to a tattoo received recently in Bali as being the source of the infection.

Department of Health Communicable Disease Control Director Dr Paul Armstrong stated that getting body art and piercings done overseas, particularly in developing countries, was not recommended.

> *While tattooists in Western Australian must comply with strict regulations and a code of practice, tattoo parlours overseas may not meet the same standards … Western Australians who have had a tattoo done in Bali recently should consult their GP and consider the need for testing for HIV and other blood-borne viruses.*

Source: Adapted from Government of Western Australia, Department of Health media release: <www.health.wa.gov.au/press/view_press.cfm?id=1104>.

The killing or suppression of T4 lymphocytes greatly diminishes the ability of the host to produce adequate amounts of antibodies or lymphokines, since functional T4 cells are needed for the initiation of immune responses (see Chapter 9). The infected person thus becomes progressively more susceptible to infections and cancers that a healthy immune system would normally prevent.

In addition to immune system damage, the virus attacks the nervous system directly. It has been suggested that infected monocytes that carry the virus into the brain can damage cells by altering cytokine levels and causing release of toxic substances. The pathogenesis of AIDS is summarised in Figure 19.12.

The immune response to HIV typically (but not always) begins 2–4 weeks after infection. Neutralising antibodies are usually found, as well as a cell-mediated immune response. As the immune response gathers pace, the virus and its components become undetectable and early clinical manifestations disappear. However, the immune mechanisms do not completely clear the virus. The virus undergoes a high rate of mutation while it is causing infection, changing its antigens and thus allowing it to evade host responses.

Clinical features

Current treatment regimes (see later section) significantly alter the course of HIV infection. The 'natural' course of the disease (i.e. without treatment) is described here.

A wide spectrum of clinical manifestations is possible in an HIV-infected person. The first signs usually appear 1–3 weeks after infection and are typically an acute 'mononucleosis-like' illness with fever, headache, pharyngitis, nausea, mild CNS symptoms, mucocutaneous ulcers and a maculopapular rash. A lymphadenopathy may develop in the second week. These symptoms of acute infection occur in only 50–70 per cent of infected people. Shortly after acute infection, most patients undergo seroconversion—that is, produce antibodies. The symptoms subside within a few weeks and there follows a variable period of three years or more of reasonably good health. During this stage the rate of viral replication is at a lower and relatively stable level. The T4 cell count generally remains above 500 per μL of blood.

Apparent good health continues because T4 levels remain high enough to prevent infections by other organisms. But over time T4 levels gradually fall. An intermediate phase of T4 cell depletion (200–350 per μL) can be manifested by a variety of minor infections such as pharyngitis, sinusitis, gingivitis, tinea, seborrhoeic dermatitis, warts, reactivation of herpes zoster and oral candidiasis, and symptoms such as chronic diarrhoea, fever and weight loss.

Advanced immune depletion (T4 cell count falling below 200 per μL) heralds the likelihood of developing an AIDS-defining infection or malignancy. At this stage the person is said to have AIDS. The common AIDS-defining diseases are listed in Table 19.5. The most common secondary diseases in AIDS patients are *Pneumocystis jiroveci* pneumonia and Kaposi's sarcoma (see Figure 19.13). The microorganisms that cause secondary infections in people with AIDS are typically opportunistic pathogens; that is, they have low pathogenicity and only rarely cause infection in healthy people. Once such diseases appear, death usually occurs in a year or two. Progression from HIV infection to AIDS occurs in approximately 50 per cent of untreated people within ten years.

Infection with HIV can result in a large number of neurological manifestations throughout the course of the disease. These result from direct infection by HIV of the nervous system, secondary opportunistic infections and neoplasms involving the CNS, or a combination of these. Some of the more common manifestations are aseptic meningitis, peripheral neuropathy, recurrent seizures and the AIDS dementia complex, which is characterised by progressive cognitive dysfunction and motor and behavioural disorders.

Laboratory diagnosis

The laboratory diagnosis of HIV infection most often involves the detection of anti-HIV antibodies in serum. Antibodies are usually detected within 2–4 weeks of infection. ELISA (enzyme linked immunosorbent assay, see Chapter 15) is the most frequently used screening test for anti-HIV antibodies, but it can take up to two weeks to

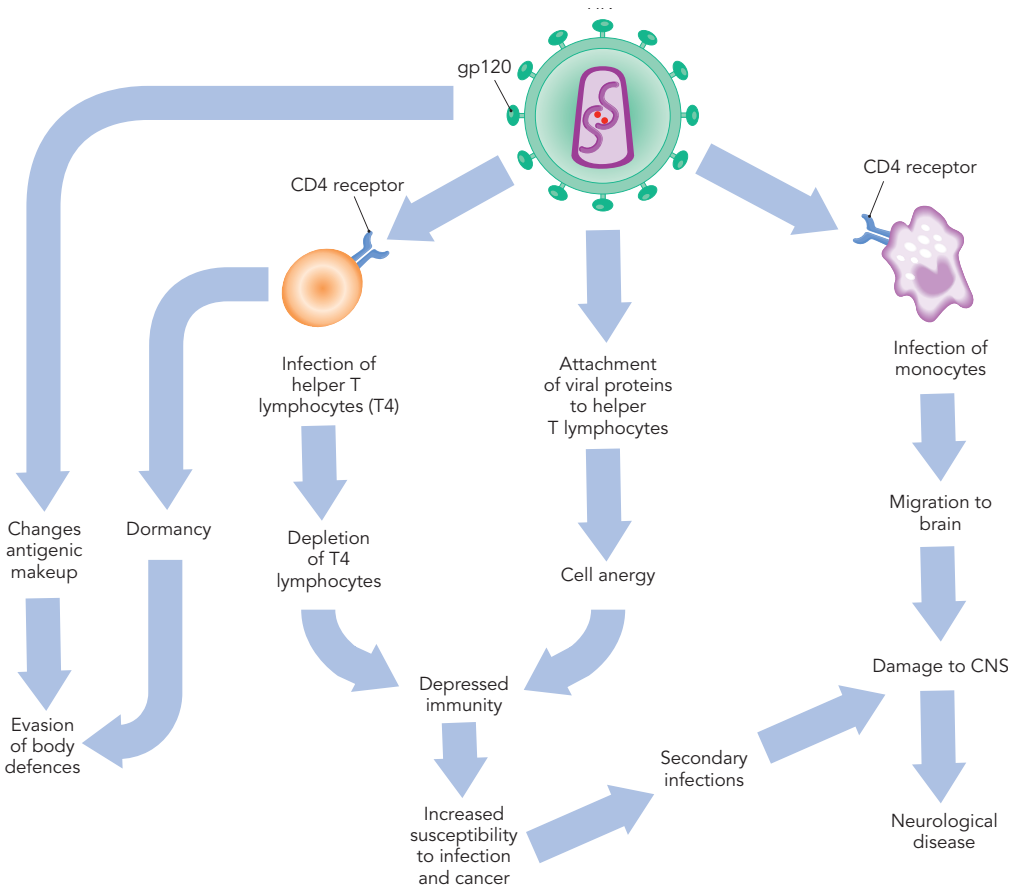


FIGURE 19.12

The pathogenesis of AIDS

TABLE 19.5 **Percentage of people with AIDS who have specific AIDS-defining illnesses**

AIDS-DEFINING ILLNESS	PERCENTAGE
Pneumocystis jiroveci pneumonia (PCP)	35
Kaposi's sarcoma (KS)	15
Oesophageal candidiasis	10
HIV wasting disease	6
Atypical *Mycobacterium* infection	5
Cytomegalovirus	< 5
HIV encephalopathy	< 5
Cryptococcosis	< 5
Non-Hodgkin's lymphoma	< 5
Cerebral toxoplasmosis	< 5
Herpes simplex virus	< 5
Cryptosporidiosis	< 5

obtain a result. Several simple, rapid assays have been developed that allow a screening result on blood or other body fluids (e.g. oral fluid) to be obtained within 20 minutes, and without the need for elaborate laboratory equipment.

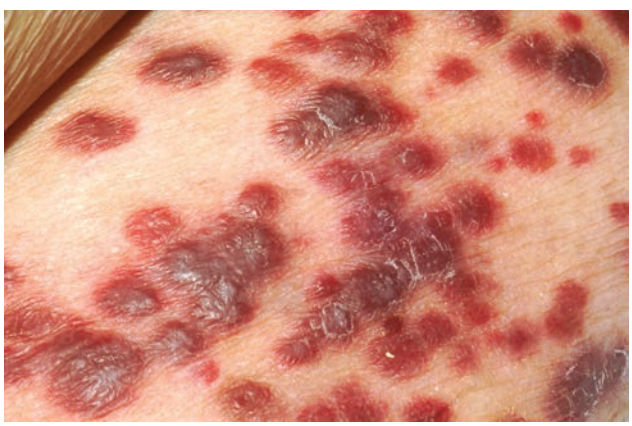


FIGURE 19.13

The purplish skin lesions of Kaposi's sarcoma

Source: National Cancer Institute.

Confirmation of positive screening test results is performed with the Western blot test.

One of the problems with these antibody tests is that the antibody-negative (window) period may be as long as several months in some infected people; so the diagnosis in these cases would be delayed. Also, the significance of

antibodies in neonates born to HIV-infected mothers is not always clear, given that antibodies are passed from mother to foetus.

A number of other diagnostic tests for HIV infection have been developed to supplement the antibody-detection methods. Assays for the detection of viral antigens in serum (e.g. HIV-1 p24 antigen test) are useful, since HIV antigens can, in some cases, be detected during the window period, in advanced disease when antibodies may not be detectable, and in infected neonates. Tests for detecting and assaying viral load in blood are also available. A polymerase chain reaction (PCR) test for HIV RNA is particularly useful because it can detect levels as low as 300 copies of RNA per millilitre of plasma and can quantitate the amount of viral RNA present. This test provides an accurate assessment of HIV disease progression. The progress of the disease can also be assessed by assaying the number of T4 cells in blood. Monitoring progress of the disease is useful in helping to determine the most appropriate time to begin treatment and in monitoring the effectiveness of anti-HIV treatment.

Treatment

There has been substantial progress in the use of chemotherapeutic agents for treatment of HIV infection. The drugs that have been developed can be grouped into four main types:

1. The nucleoside reverse transcriptase inhibitors compete with nucleosides during reverse transcription of viral RNA into DNA, thereby terminating reverse transcription before it is complete.
2. Non-nucleoside reverse transcriptase inhibitors target the same viral enzyme and bind directly to it, changing its conformation and reducing its function.
3. The protease inhibitors target the enzyme that the virus uses for splitting of viral proteins in the final stages of assembly of new virus particles.
4. Integrase inhibitors inhibit the integration of HIV DNA into the host genome.

All of these drugs are designed to prevent viral multiplication inside target cells. Some of the drugs in current use are listed in Table 19.6.

A major advance in the treatment of HIV infection came in the mid-1990s with the practice of using several of these drugs in combination, called highly active antiretroviral therapy (HAART). The combination used varies according to the situation, but typically includes two nucleoside reverse transcriptase inhibitors (often tenofovir and emtricitabine) plus a third drug with a different mode of action (often atazanavir, lopinavir or darunavir). Treatment regimens may be modified if the individual experiences unacceptable side effects to one or more of the drugs. HAART has led to substantial reductions in morbidity and mortality related to HIV infection. The effectiveness of treatment is variable, but in many people there is a marked decrease in viral load and a progressive improvement in T4 count. Patients who are able to adhere to this combination therapy can potentially achieve a lifelong suppression of HIV replication. However, this therapy prevents viral replication but does not eliminate HIV from the body. Dormant HIV DNA in cells is not affected by the drugs and, therefore, once therapy is stopped it is possible that a reactivation of the virus can occur. For this reason, current combination therapies must be continued for the person's lifetime. The other advantage of combination therapy is that drug resistance is less likely to arise.

TABLE 19.6 Antiretroviral drugs

Nucleoside reverse transcriptase inhibitors
abacavir
emtricitabine
lamivudine
stavudine
tenofivir
zidovudine (AZT)
Non-nucleoside reverse transcriptase inhibitors
efavirenz
nevirapine
etravirine
Protease inhibitors
atazanavir
fosamprenavir
indinavir
lopinavir
ritonavir
saquinavir
darunavir
Integrase inhibitors
raltegravir

When combination therapy fails, it is usually due to incomplete patient compliance and/or development of viral drug resistance. Although the commonly used drugs are generally well tolerated, non-compliance is a problem because most have short-term toxic effects, and the daily pill burden is difficult for some people to maintain for the remainder of their life. Drug resistance most often develops as a result of non-compliance. Unfortunately, drug therapy is not widely available in some underdeveloped countries, where it is needed most, due to the cost of the medications as well as the diagnostic and monitoring costs. These costs are also a growing concern in some developed countries.

Prevention

Numerous measures have been introduced to reduce the spread of HIV infection in the community. In Australia, preventive measures that have been adopted include:

- screening of blood donors for HIV and discouragement of people in high-risk groups from donating blood
- heat treatment of blood products
- public education programs encouraging safe sex practices (e.g. use of condoms) and discouraging promiscuous behaviour

- public education programs about the dangers of sharing needles, and the free distribution of clean needles and syringes
- drug therapy at the time of delivery, caesarean birth and formula feeding of the baby to reduce risk of transmission from infected mother to baby.

To reduce the chance of infection in healthcare staff, Standard Precautions procedures (see Chapter 13) should be followed in healthcare institutions. This includes appropriate hand hygiene, appropriate use of personal protective equipment, and safe use and disposal of sharps. Post-exposure prophylaxis with antiretroviral drugs, if given early enough (within 72 hours of exposure), significantly reduces (by about 80 per cent) the risk of infection following occupational exposure to HIV.

Numerous studies have demonstrated that male circumcision significantly reduces the risk of heterosexually acquired HIV. In light of this, the WHO currently recommends that properly performed male circumcision be considered in high-risk countries as part of a comprehensive HIV prevention package. However, it should be recognised that male circumcision does not provide complete protection against HIV or reduce the risk of transmission from a circumcised, HIV-infected man to a non-infected partner. Male circumcision should therefore only be considered to be an adjunct to other prevention methods.

Despite intensive and ongoing efforts to develop a vaccine, none is yet available. The ability of the virus to mutate rapidly, and the inability of the immune system to halt HIV infection, pose serious obstacles to the development of useful vaccines.

Ross River virus infection

Cause, incidence and transmission

Ross River fever is an important arbovirus (**ar**thropod-**bo**rne **virus**) infection in Australia. It is caused by the Ross River virus, an alphavirus of the family Togaviridae. The disease was first described in 1928 as an 'unusual epidemic' in Narrandera, New South Wales. Isolation of the virus was first achieved in 1963 from a mosquito caught on the Ross River in northern Queensland.

A less common virus, the Barmah Forest virus, causes a disease similar to the Ross River virus and is also spread by mosquitoes.

Annual notifications of Ross River virus infection in Australia are variable, ranging from 2000 to 6000 per year. The infection occurs throughout the country (see Spotlight box: Ross River virus), but the highest rates of notification tend to be from Queensland and the Northern Territory. Being a mosquito-borne disease, it predominates in regions with a warmer climate and higher rainfall, although it does occur throughout the country, including in Tasmania. Infection occurs in rural areas as well as in the major metropolitan areas of Australia. There are clusters of cases of Ross River virus infection every summer, but when mosquitoes reach plague proportions significant outbreaks may occur. The virus has been isolated from more than ten different species of mosquito from different regions in the country, explaining its country-wide incidence.

Clinical features, diagnosis and treatment

The intensity of Ross River virus infection varies considerably, with only approximately 30 per cent of people seeking medical advice. Symptoms usually start to appear 3–21 days after being bitten by an infected mosquito. The main manifestation is arthritis, with morning joint stiffness, although a maculopapular rash, lethargy and flu-like symptoms are also common. The symptoms wax and wane, usually lasting a few weeks. The arthritic symptoms and lethargy sometimes persist for more than three months. Complete recovery may take 12 months or more, but there is no residual damage to joints.

Laboratory confirmation of the disease is usually by serology, although virus isolation and PCR are possible. At present, only symptomatic treatment (e.g. an anti-inflammatory drug for arthritis) is possible. Prevention is based on avoidance of mosquito bites, especially during epidemics.

Dengue fever

Cause, incidence and transmission

Dengue virus is a flavivirus with four different serotypes. **Dengue fever** occurs mainly in tropical areas, and to a lesser extent in the subtropics. Globally, there are an estimated 50–100 million cases per year, with around 22 000 deaths, mainly in children. There has been a dramatic increase in incidence in recent decades, and it has spread to new areas of the world. Countries in South-East Asia and the Western Pacific are the most severely affected. In Australia around 300 cases are usually reported each year except when epidemics occur; many hundreds of cases may be reported in epidemic years (such as 1992, 1993, 1998, 2003 and 2009). The majority of cases are reported in Queensland. Dengue

SPOTLIGHT ON

Ross River virus

In 2002, Tasmania reported 117 cases of Ross River virus (RRV) infections. This was the largest number of infections ever recorded for the state in a year. Thirty-seven of the cases had lived in, or visited, the Sorell Municipal Area, 25 kilometres east of Hobart. In early 2002 a combination of spring tides and high summer rainfall created extensive salt marshes in the Sorell region, resulting in high densities of the mosquito *Ochlerotatus camptorhynchus*. This mosquito is a recognised vector for RRV. This is the furthest south that RRV has been found in Australia.

is only locally transmitted in northern Australia; all other cases are acquired overseas. The mosquito *Aedes aegypti* is the principal human vector.

Clinical features and complications

After an incubation period of 8–10 days, symptomatic disease is characterised by high fever, headache and a rash. Severe muscle and joint pain is also common, hence its other name, 'breakbone fever'. Except for its painful symptoms, dengue fever is a relatively mild and self-limiting disease that usually lasts about ten days, and over 50 per cent of cases may be asymptomatic. A much more serious form of dengue, called **dengue haemorrhagic fever**, is characterised by high fever, bleeding from the gums, skin and gastrointestinal tract, and sometimes circulatory failure, shock and death (see Case History 19.6). The WHO estimates that there are about half a million people hospitalised with dengue haemorrhagic fever a year, with a fatality rate of around 2.5 per cent. Without treatment, fatality rates can exceed 20 per cent.

The pathogenesis of dengue haemorrhagic fever is shown in Figure 19.14. After dengue virus infection, antibodies specific for the particular serotype are formed. If subsequently infected with a different serotype, the preformed antibodies bind to the virus, but fail to neutralise it. The antibodies actually enhance viral infection of monocytes, by first binding with the virus and then attaching to Fc receptors on the monocyte membrane. Increased infection of monocytes then induces an increased release of cytokines, leading to increased vascular permeability and leakage, hypovolaemia

CASE HISTORY 19.6

Dengue fever

A 40-year-old woman from Thursday Island presented to a hospital in north Queensland in February 2004 feeling generally unwell, including generalised body aches and fever, and vomiting and diarrhoea over the previous three days. She had been to a primary healthcare clinic the day before and was diagnosed clinically with dengue fever based on the typical symptoms, and the fact that there was an epidemic of dengue on Thursday Island at the time.

On admission to hospital, she was delirious and restless, with a pulse of 120. Petechiae were noted around the right elbow. She was immediately given intravenous fluid. Six hours after admission, she became more agitated and restless, with blood pressure of 123/55 mmHg and pulse of 150. Thirty minutes later, her blood pressure was too low to record. Despite being given intravenous fluids over the next seven hours, together with adrenaline and metaraminol, her blood pressure remained very low. Evacuation to a referral hospital was arranged. During transfer her pulse rate fell, her blood pressure became unrecordable, and she could not be resuscitated.

Antibody tests indicated that the woman had been infected at least twice with the dengue virus.

Source: Adapted from W.J.H. McBride 2005, Deaths associated with dengue haemorrhagic fever: The first in Australia in over a century. *Medical Journal of Australia* 183(1): 35–37.

Questions

1. What is the significance of this woman's multiple dengue infections?
2. What infection control measures should healthcare workers observe when involved in a case of dengue fever?
3. Why did this woman not receive any antiviral therapy?

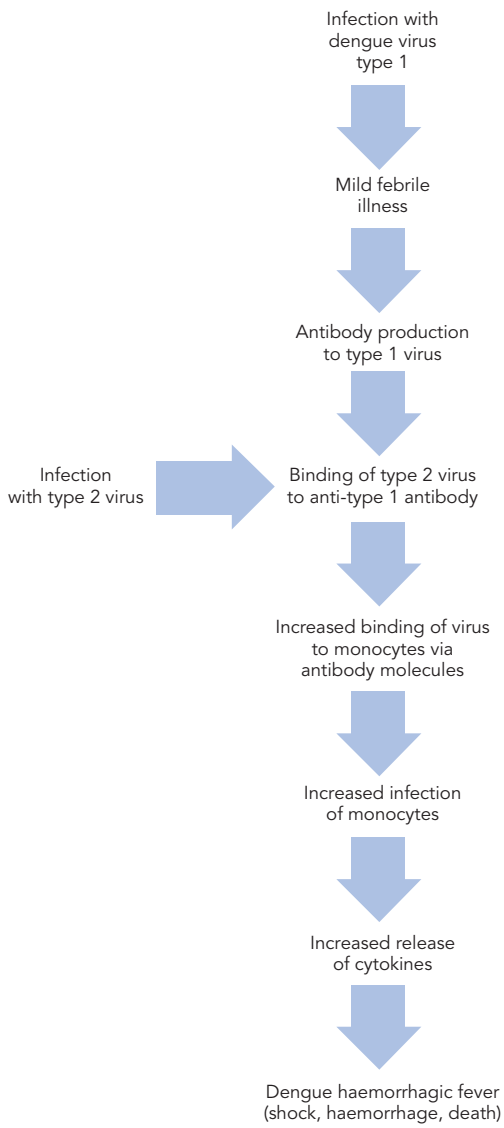


FIGURE 19.14

The pathogenesis of dengue haemorrhagic fever

There are four serotypes of dengue virus; types 1 and 2 have been used in this example.

and shock. Thus, dengue haemorrhagic fever becomes a possibility when dengue already exists in a region and subsequently a different serotype is introduced there.

Diagnosis, treatment and prevention

Diagnosis is often based on clinical and epidemiological grounds. Laboratory diagnosis is usually based on a four-fold increase in antibody titre in paired serum samples. Virus isolation is possible and a reverse-transcriptase PCR method has been developed, but is not yet readily available. No specific antiviral therapy is currently available, although with intensive supportive therapy mortality is significantly reduced. No effective vaccine for dengue exists. Prevention is therefore based on mosquito control programs and disease surveillance.

Chikungunya

Chikungunya virus is an RNA virus of the Alphavirus genus and Togaviridae family. The infection is transmitted by *Aedes* spp. mosquitoes, and was first identified in 1952 in Tanzania. It is endemic in Africa, India, the Middle East and South-East Asia, and a number of outbreaks have been described in the past 50 years. In an outbreak in India that began in 2006, 1.4 million cases were reported during that year.

Chikungunya infection is clinically similar to dengue fever. After an incubation period of 2–10 days, the illness is characterised by abrupt onset of high fever, rigors, severe joint pain and headache. A maculopapular rash appears in 50 per cent of people 2–5 days into the illness. Patients generally make a full recovery over several weeks, during which time they may be intensely tired and have difficulty in concentrating. However, 5–10 per cent of patients may experience persistent joint pain and swelling for a year or more. Serious complications are rare.

Laboratory diagnosis of chikungunya infection may be made by serological tests, by detecting viral RNA by RT-PCR, or by virus culture. No specific drug therapy is available, and treatment is supportive. No licensed vaccine is currently available.

Infectious mononucleosis

Infectious mononucleosis, or **glandular fever**, is caused by a herpes virus called Epstein-Barr virus (EBV) (see Figure 19.15). Infectious mononucleosis has been recognised clinically since the 1800s, but the identification of EBV as the causative agent was not made until the 1960s. In less developed parts of the world it is usually acquired in early childhood, whereas in developed countries a higher incidence occurs in teenagers and young adults. The usual route of infection is by transfer of saliva—for example, by sharing drinking vessels or eating implements, or by kissing—it is sometimes referred to as the 'kissing disease'. The virus may be shed intermittently in saliva for many years after infection.

Pathogenesis and clinical features

After entering the body via the oropharynx, the virus invades a number of organs such as the lung and parotid glands, where it specifically infects B lymphocytes. The B lymphocytes then spread the infection to other organs, including the liver, spleen and peripheral lymph nodes. Most cases are subclinical, but if symptomatic, mild symptoms of fever, headache, fatigue and malaise develop after an incubation period of 5–6 weeks. Most will develop a sore throat several days later, and after about a week of worsening symptoms

SPOTLIGHT ON

Dengue fever alert for Bali travellers

Western Australians are being urged to take precautions against mosquito bites when travelling to Bali, following a sharp increase in the number of cases of dengue fever in returning travellers. Acting Director of Communicable Disease Control Dr Gary Dowse said 54 Western Australian travellers contracted the disease in 2007, three to four times the usual number.

> *The trend has continued into 2008 with a further 16 cases reported in January … Almost 60% of cases reported between January 2007 and February 2008 were associated with travel to Indonesia, with 76% of these being infected in Bali. Smaller numbers of cases were reported in people who travelled to Thailand, Singapore, India, Vietnam, the Philippines and other South-East Asian countries.*

People travelling to affected areas were advised to take the following precautions to avoid being bitten by mosquitoes:

- Avoid outdoor exposure in areas of high mosquito activity, especially around dawn and dusk.
- Ensure accommodation is completely mosquito-proof. Use mosquito nets if available.
- Wear long, loose-fitting protective clothing when outdoors in mosquito-prone areas.
- Use personal repellents containing diethyltoluamide (DEET) or picaridin. The most effective and long-lasting formulations are lotions or gels. Some natural or organic repellents may provide a measure of protection.
- Ensure infants and children are adequately protected against mosquito bites, preferably with suitable clothing, bed nets or other forms of insect screening. Only infant-strength repellents should be used on children.

Source: Adapted from Department of Health 2008, Government of Western Australia, Press Release, 18 March.

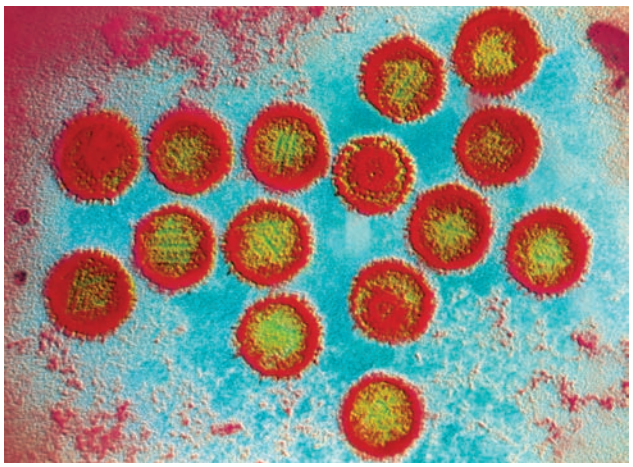


FIGURE 19.15

Electron micrograph of the Epstein-Barr virus

Source: CNRI/Science Photo Library.

the illness reaches its peak. The tonsils may be coated with a whitish-grey exudate and there may be inflammation of the rest of the pharynx. Palatal petechiae, swollen cervical and axillary lymph nodes, splenomegaly and hepatitis are also common. Although symptoms usually resolve within two months, the virus is not cleared, establishing a lifelong latency in B cells.

Diagnosis and treatment

The clinical diagnosis of infectious mononucleosis is complicated by the fact that it resembles other systemic diseases such as toxoplasmosis and cytomegalovirus infections. The name 'mononucleosis' refers to lymphocytes with abnormal morphology, and these can usually be found in a differential white cell count. Serologic tests for antibodies provide the usual diagnosis. Bed rest and symptomatic treatment are the basis of management of the disease. No vaccine is available.

Complications

The Epstein-Barr virus has been implicated in a number of different malignancies. Its association with Burkitt's lymphoma, B cell lymphomas in immunodeficient patients, Hodgkin's disease and nasopharyngeal carcinoma is well recognised. However, its part in the development of these tumours is not fully understood. The virus has also been implicated in **chronic fatigue syndrome**. People with this condition often have a history of an initial viral-like illness. Excessive fatigue, to the extent of having difficulty in performing routine tasks, and general malaise are the main complaints. Other possible symptoms include impairment of memory or concentration, sore throat, tender lymph nodes, fever, muscle pain and weakness, headaches, post-exertion malaise and non-refreshing sleep. Symptoms may last for six months or more. It is likely that other microorganisms, such as other herpes viruses, fungi and *Mycoplasma* spp., are also responsible for this condition.

Laboratory investigations in chronic fatigue syndrome are often remarkably normal, so diagnosis is usually based on clinical grounds and the exclusion of other causes. Bed rest is essential, together with symptomatic treatment. Attempts to exercise back to health are not recommended.

Cytomegalovirus infection

Cytomegalovirus (CMV) is a double-stranded DNA virus of the Herpesviridae family, and it therefore shares many attributes with the other herpes viruses. The majority of CMV infections occur in childhood and are often asymptomatic. In older children and adults, a mild malaise, fever, myalgia and lymph node inflammation may occur. The virus is not fully cleared from the body and establishes a lifelong, latent infection.

Transplacental transmission of CMV to the foetus may occur from a mother experiencing a primary or a reactivated infection. This leads to spread of the virus to multiple organs and a severe illness characterised by one or more of the following in the neonate: low birth weight, microcephaly, hepatosplenomegaly, growth retardation, impaired vision, hearing loss and haemolytic anaemia. Mortality among symptomatic neonates may be as high as 30 per cent within the first few months of life. Some babies infected *in utero* may not show signs of infection at birth, but later develop mental or sensory disorders.

CMV is responsible for severe disseminated infections in the immunocompromised—such as people with AIDS, cancer patients and organ transplant recipients. Infections in these patients may be due to primary infection by the virus from an exogenous source, or to the reactivation of latent virus. The virus may infect any organ but most often causes pneumonia, encephalitis, hepatitis, colitis or a sight-threatening retinitis.

CMV has been found in most body fluids, including saliva, urine, blood, breast milk, semen and cervical secretions, and thus can be transmitted in a variety of ways. It is usually spread by close contact but also by blood transfusions, organ transplants and during sexual intercourse. Shedding of the virus may occur intermittently for many months after infection, adding to its transmissibility.

PCR assays on blood or urine are the common methods for diagnosis of CMV infections. Other commonly used methods include serology and antigen detection assays. Culture of the virus from a body fluid may take up to six weeks and is thus too slow for clinical use.

Ganciclovir is the drug of choice for CMV-infected patients. However, it is not always effective and side effects can occur. Pregnant healthcare workers should be informed of the risk of infection and should not have contact with known cases. There is no vaccine for CMV registered in Australia.

Fifth disease (slapped cheek disease, or *Erythema infectiosum*)

Fifth disease is a common cause of rash in childhood throughout the world, caused by parvovirus B19. Epidemics are thought to occur in Australia every 2–3 years, although the majority of infected children are asymptomatic

in outbreaks. If symptomatic, it is a mild illness characterised initially by flu-like symptoms with pyrexia, headache, myalgia and chills, and about a week later the typical rash on the cheeks, giving the slapped cheek appearance. The rash may then extend to the trunk and limbs and may fade and reappear over several weeks. Foetal infection is usually benign, but occasionally severe, especially if infection occurs in the first 20 weeks of gestation. Foetal death occurs in a small percentage (<1 per cent) of cases. In adults, especially women, infection is more often characterised by an arthralgia that can affect any joint but usually the wrist, hand, knee or ankle. Transmission is by the respiratory route and maximal infectivity is in the week before the rash appears.

Diagnosis of fifth disease is generally by detection of specific IgM antibodies in serum. There is no specific treatment for fifth disease, and symptomatic treatment is only occasionally warranted.

Hand, foot and mouth disease

Hand, foot and mouth disease has a worldwide distribution, occurring in sporadic and epidemic forms. Children under 10 years are most commonly affected. Outbreaks have been reported in many countries, including Malaysia, Singapore, China, Taiwan, Thailand and Australia, most often occurring in schools and childcare centres. A number of viruses cause this disease, including coxsackie A virus (A16), some other coxsackie A and B virus subtypes, and enterovirus 71 (EV71).

Hand, foot and mouth disease usually begins with a fever, sore throat and poor appetite. One to two days later, vesicles begin to appear on the gums, palate and tongue. These painful lesions eventually blister and may ulcerate. A papular-vesicular skin rash on the palms, fingers, toes, soles of the feet and buttocks is also characteristic. Meningoencephalitis is a rare feature of the illness, although in some outbreaks it is common. This more serious form of the disease may be associated with significant morbidity and mortality. Generally, however, hand, foot and mouth disease is a self-limiting illness that lasts one to two weeks.

The disease is highly infectious. The virus is shed in faeces and saliva, and from skin blisters of infected people; thus, transmission is usually by direct contact or droplet spread. A person may still be infectious weeks after the symptoms have resolved. The virus can be transmitted to the foetus, particularly if the mother is infected late in pregnancy. Foetal infection may be severe, including meningoencephalitis, cardiomyopathy and hepatitis.

Diagnosis is usually based on clinical findings. Virus isolation, serology or PCR testing of appropriate specimens may be undertaken, but are rarely warranted. Symptomatic treatment (e.g. cold liquids for relief of painful oral lesions) is usually all that is required. Antiviral drugs may be used in severe infections, but there is little evidence of their effectiveness.

Yellow fever

Yellow fever virus is a flavivirus restricted to parts of Central and South America, Africa, and some Caribbean islands, where it is endemic. It is transmitted to humans by the mosquito *Aedes aegypti*. It causes an acute disease characterised by sudden onset of fever, vomiting and prostration 3–6 days after infection. Progression to extensive haemorrhagic manifestations and jaundice, leading to coma and death, occurs in about 15 per cent of infected people. Mortality in non-Indigenous people may be as high as 50 per cent. There is no specific treatment.

No case of yellow fever has been recorded in Australia, but the *A. aegypti* mosquito is present in north Queensland. Quarantine precautions to prevent introduction of the virus into Australia are therefore very important.

Preventive measures include protection from mosquito bites, and vaccination. The vaccine contains a live, attenuated strain of the virus and is highly effective. It is recommended for travellers to high-risk areas, and in some countries it is mandatory for visitors. Any person over the age of 12 months who is not immunocompromised, but who has been in or passed through an infected area six days before arrival in Australia, must have a current International Certificate of Vaccination against yellow fever.

Haemorrhagic fevers

Haemorrhagic fevers have received substantial attention in recent years. They are caused by a number of different viruses, including the dengue fever virus and yellow fever virus described above. Other viruses that have been associated with the haemorrhagic fever syndrome include Lassa fever virus, Rift Valley fever virus, Marburg virus and Ebola virus. These viruses tend to have a specific geographical distribution (see Table 19.7). Humans are not the natural reservoir of haemorrhagic fever viruses. The normal hosts are animals (especially rodents) or insects (e.g. mosquitoes). Humans are infected when they come into contact with infected hosts, although some types can spread from human to human by secretions, blood or tissues (e.g. Ebola

TABLE 19.7 Viral haemorrhagic fevers

VIRUS (DISEASE)	DISTRIBUTION	SOURCE
Ebola	Africa	Unknown
Marburg	Africa	Unknown
Rift Valley fever	Africa, Saudi Arabia, Yemen	Mosquito
Crimean-Congo haemorrhagic fever	Africa, Central Asia, Europe, Middle East	Tick
Hantavirus	Asia, Europe, worldwide	Rodent
Lassa	West Africa	Rodent
Machupo	South America	Rodent
Junin	South America	Rodent
Sabia	South America	Rodent
Guanarito	South America	Rodent
Yellow fever	Africa, tropical Americas	Mosquito
Dengue	Asia, Americas, Africa	Mosquito

and Marburg viruses). The natural hosts of the Ebola and Marburg viruses are not presently known.

Although these viruses differ in terms of their sources, modes of transmission and mechanisms for producing disease, they are grouped together on the basis of some common clinical manifestations. Haemorrhagic fever viruses typically infect cells of the monocyte-macrophage lineage, causing them to release excessive amounts of pro-inflammatory cytokines. Unchecked viral growth in other cell types may also contribute to the pathogenesis of the disease. Typically, patients suffer from fever, malaise, myalgia, prostration, multisystem involvement, and widespread haemorrhage due to endothelial cell damage. Severely affected patients may show signs of bleeding under the skin or from body orifices such as the mouth, eyes or ears. Severe infection can lead to shock, nervous system malfunction and death.

Ebola virus is one of two members of the filovirus family. It was discovered in 1976 in outbreaks in Africa, near the Ebola River. Its morphology of long filamentous particles (see Figure 19.16a) is unusual in viruses. The natural reservoir of the virus is not known, but in outbreaks the main form of transmission is person-to-person contact via body fluids (e.g. blood, semen, saliva, mucus). It has a fatality rate of 50–90 per cent. In recorded outbreaks, healthcare workers have often been infected (see Spotlight box: Ebola virus).

Marburg virus is is the other member of the filovirus family, discovered in 1967 in Marburg, Germany, when laboratory technicians were infected handling African green monkeys. This virus also has a long filamentous morphology (see Figure 19.16b), and has a 20–50 per cent fatality rate. Like Ebola, the reservoir of Marburg virus is unknown and transmission is person-to-person during outbreaks.

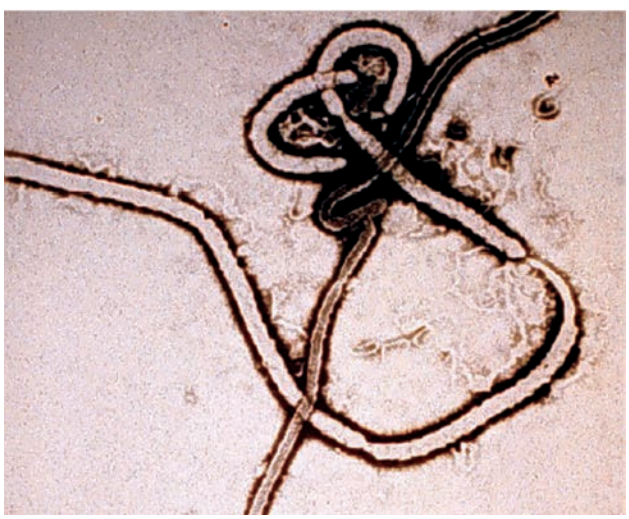
Ebola virus

SPOTLIGHT ON

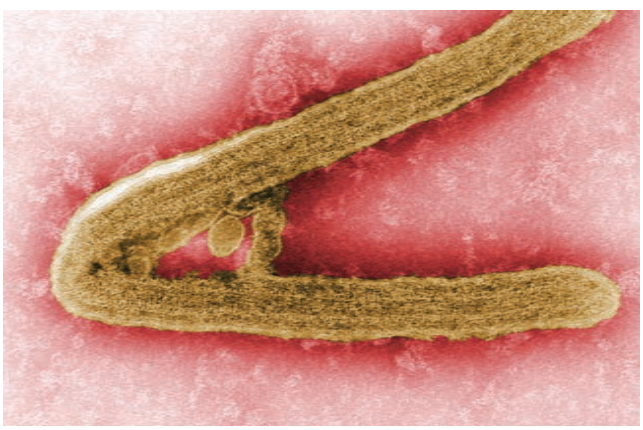
An outbreak of Ebola haemorrhagic fever occurred in Uganda between October 2000 and January 2001. The last reported case was a woman who fell ill on 11 January 2001 and was cleared of infection on 16 January. In all there were 426 cases, with 173 deaths, making it the largest recorded outbreak of Ebola virus. At least 14 medical/nursing staff were among the fatalities. Person-to-person transmission is known to occur via blood, semen, saliva and other body fluids. The spread of the virus in the community may have been aided by the nature of traditional funerals in many parts of Uganda. At these funerals, the body of the deceased is washed and mourners later dip their hands in the water.

These two viruses are among the most virulent of human pathogens, capable of causing death within days. However, they have been confirmed as the causes of relatively few clinical cases (less than 2500) since their discovery. Nevertheless, they have attracted much attention because of the severe clinical manifestations and their potential use as biological weapons.

Lassa fever is caused by a virus that is transmitted from infected rodents to humans. It is restricted to West Africa, where there are an estimated 100 000–300 000 cases per year, causing around 5000 deaths. Most human cases are asymptomatic, but about 20 per cent have severe multisystem disease. **Rift Valley fever** virus primarily affects livestock, but may also infect humans. It is transmitted by mosquitoes,



(a)



(b)

FIGURE 19.16

Haemorrhagic fever viruses of the filovirus family

(a) Transmission electron micrograph of Ebola virus; (b) transmission electron micrograph of Marburg virus

Source: (a) CDC/Cynthia Goldsmith; (b) CDC/Frederick Murphy.

and also by aerosols of blood or fluids from infected animals. It has a case-fatality rate of around 1 per cent. Hantavirus infection occurs worldwide, causing two major syndromes: hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome. It affects up to 200 000 people annually, particularly in Asia.

There is no specific treatment for most types of viral haemorrhagic fever. The antiviral drug ribavirin has some effectiveness against Lassa fever and hantavirus if commenced early in the course of the illness. Supportive therapy is the mainstay of patient management.

SYSTEMIC FUNGAL INFECTIONS

The frequency of disseminated (systemic) fungal infections has increased substantially in recent years, largely associated with the advances in the management of conditions such as cancer, autoimmune diseases and organ transplantation. Thus, the majority of these infections occur in immunocompromised hosts, since the organisms are typically opportunistic pathogens. The most commonly occurring infections are candidiasis, aspergillosis and cryptococcosis.

Candidiasis is the most frequent fungal infection among immunocompromised patients. Unlike the other opportunistic fungi, *Candida* spp. are part of the endogenous flora of humans. *Candida albicans* is the most common species causing infection, but non-albicans species, which are relatively resistant to antifungal agents, have emerged as significant pathogens in the last decade. The latter include *C. tropicalis*, *C. glabrata*, *C. parapsilosis* and *C. krusei*. Disseminated candidiasis occurs primarily in organ transplant recipients and neutropenic patients.

Aspergillus has emerged as an important systemic pathogen. Most infections occur in patients with neutropenia or in those receiving adrenal corticosteroids, which interfere with macrophage function. Several species are pathogenic, but *Aspergillus fumigatus* is the predominant cause. Most infections are acquired by inhalation of spores and therefore involve the lung. Haematogenous dissemination may then occur, and the central nervous system is a common secondary site of infection, resulting in cerebral infarction. The gastrointestinal tract may also be involved, especially the oesophagus and large bowel, which may lead to perforation or extensive haemorrhage.

Cryptococcus is a yeast-like fungus (see Figure 19.17). Two species are believed to cause human disease (called **cryptococcosis**). *C. neoformans* is commonly found in the environment, especially associated with the excreta of pigeons and some other birds, and mainly causes infections in immunocompromised hosts. It is acquired by inhalation of organisms into the lung. In the absence of adequate host defences the organism disseminates and meningoencephalitis is the most common infection. Other possible sites of infection include the skin, bone, prostate, liver and eye. *C. gattii* has a more restricted distribution in tropical and subtropical parts of the world, associated with the bark and flowers of certain tree species. *C. gattii* infections are rare and mostly occur in Australia in immunocompetent people.

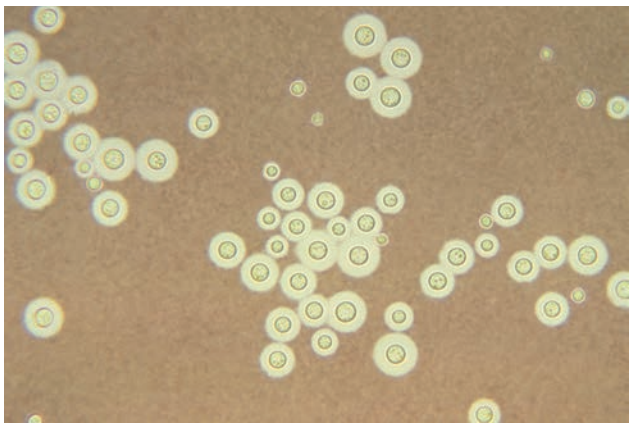


FIGURE 19.17

Cryptococcus neoformans

India ink stain showing yeast cells with characteristic capsules.

Source: CDC/Dr Leanor Haley.

After a primary infection in the lung it can invade the bloodstream of a small proportion of infected people, and spread to the brain and meninges.

Diagnosis of disseminated fungal disease is difficult. Signs and symptoms are non-specific, colonisation is difficult to distinguish from invasion, and blood cultures are slow and often negative. Non-culture-based methods, such as PCR, are being developed and offer some hope for more rapid and accurate diagnoses in the future.

Effective treatments for invasive fungal infections are often lacking. Amphotericin B has been the mainstay of therapy, but it is not always effective. Treatment failure is usually related to the severity of the patients' underlying diseases, poor organ function, impaired host defences or inadequate dosing (due to the drug's toxicity). Azoles (e.g. fluconazole, voriconazole) are a group of newer antifungal drugs that are less toxic and at least as effective as amphotericin B, and are now more frequently used. Echinocandins (e.g. capsofungin) are fungicidal drugs that may be used for resistant organisms or in patients who cannot tolerate the other drugs.

SYSTEMIC PROTOZOAL INFECTIONS

Although only a few protozoa cause systemic infections in humans, some are very important in terms of the number of people affected and the severity of the disease they cause. Malaria, in particular, continues to be a major problem in many areas of the world.

Malaria

Malaria is an ancient disease that is described in Chinese medical writings dating back to 2700 BC.

Incidence

The WHO estimated that there were 216 million cases of malaria in the world in 2010, resulting in 655 000 deaths, predominantly in children under 5 years of age. The vast majority of infections and deaths occur in Africa and parts of South-East Asia. For more than 30 years the WHO has

been trying to control malaria. In 1998 the WHO, the United Nations Children's Fund (UNICEF), the United Nations Development Program (UNDP) and the World Bank formed the Roll Back Malaria Partnership. While there has been a reduction in global malaria cases of around 17 per cent since 2000, the WHO warns that these gains are fragile and that inadequate funding threatens this and any future improvements. Additional threats are the increasing drug resistance in malaria parasites and the increasing resistance of vector mosquitoes to insecticides.

Environmental conditions in areas of Australia north of latitude 18°S (Townsville to north of Port Hedland) favour the transmission of the disease. This malaria-receptive zone coincides with the distribution of the most important malaria vector in Australia—the mosquito species *Anopheles farauti*. Therefore, malaria can be locally acquired in Australia (see Case History 19.7, page 503), but is currently rare. The great majority of cases are imported from overseas, especially in travellers who have been to the Solomon Islands, Papua New Guinea, Africa or India. Currently, about 500–800 notifications of malaria are made annually in Australia.

Causative organisms and transmission

The protozoa that cause malaria are sporozoans belonging to the genus *Plasmodium* (see Figure 19.18). There are five species that have been associated with human malaria: *Plasmodium falciparum, P. vivax, P. malariae, P.* ovale and *P. knowlesi*. Each species has defined areas of endemicity. Falciparum and vivax malaria are the most common. Both cause significant morbidity, but only *P. falciparum* is associated with a high mortality rate. The protozoa are transmitted to humans by species of *Anopheles* mosquitoes. Malaria can also be transmitted by blood transfusion or needlestick injury, although these modes of transmission are rare in non-endemic regions.

Pathogenesis and clinical features

Sporozoites in the saliva of an infected *Anopheles* mosquito are injected into the bloodstream when the person is bitten (see Figure 19.19). They enter the liver and infect parenchymal cells where enormous replication and development into the merozoite form occur over the next several days to weeks. The liver cell ruptures, releasing tens of thousands of merozoites into the bloodstream, where, after a few minutes, they invade red blood cells. Some species, especially *P. vivax*, may remain in the liver as dormant hypnozoites, which may reactivate some weeks or months later to cause relapses. Up to and including the liver stage of the infection the patient remains well, but symptoms begin once the merozoites invade red blood cells.

Over a 48- or 72-hour period, depending on the species of the parasite, the merozoite matures into a trophozoite; this reproduces asexually, forming many more merozoites which are again released into the bloodstream after rupture of the red cell. The number of parasites continues to increase as 48- or 72-hour cycles of red cell invasion, multiplication and release are repeated. This may continue for some months or even years.

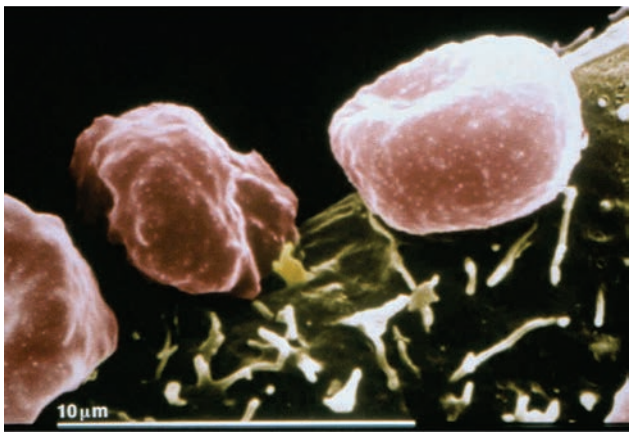


FIGURE 19.18

Electron micrograph of *P. falciparum*-infected red cells adhering to host cells

Source: Courtesy of Professor Stephen Rogerson, University of Melbourne.

Some merozoites enter the sexual reproductive phase within red blood cells to form male and female gametocytes, which are taken up by a mosquito when feeding. Gametocytes unite in the stomach of the mosquito to form zygotes, which become sporozoites as they move to the salivary glands. Sporozoites are then injected into a new host when the mosquito feeds. This sexual phase in the mosquito is essential for completion of the life cycle of the protozoan.

The gametocytaemic phase in humans is important because it is the means by which malaria may be introduced or reintroduced into a non-endemic area. Immigrants or travellers with established infection and gametocytes in their blood can infect local mosquitoes, which may then cause indigenous spread of malaria.

The clinical features of malaria are often vague and non-specific, and depend on the age and immune status of the patient. After an incubation period of 1–3 weeks, depending on the species of *Plasmodium*, the infected person usually develops a fever and a feeling of malaise, often accompanied by headache and muscular discomfort. If the cycle of red cell infection and rupture remains regular and synchronous, a characteristic pattern of recurrent chills followed by fever, drenching sweats, headache, muscular pain and vomiting occurs every 48 or 72 hours. Each symptomatic period tends to last for several hours.

The pattern of intermittent illness and well-being is usual in **benign malaria**—that is, infection caused by *P. vivax, P. ovale* and *P. malariae*—but less common in **falciparum malaria**. Without treatment, and if reinfection does not occur, benign malaria is normally self-limiting, resolving over a period of weeks or months, although relapses may occur months or years later. These relapses occur in vivax and ovale malaria if reactivation of dormant parasites in the liver occurs.

Falciparum malaria is a far more severe form. *P. falciparum* causes aggregations of red cells that can obstruct small

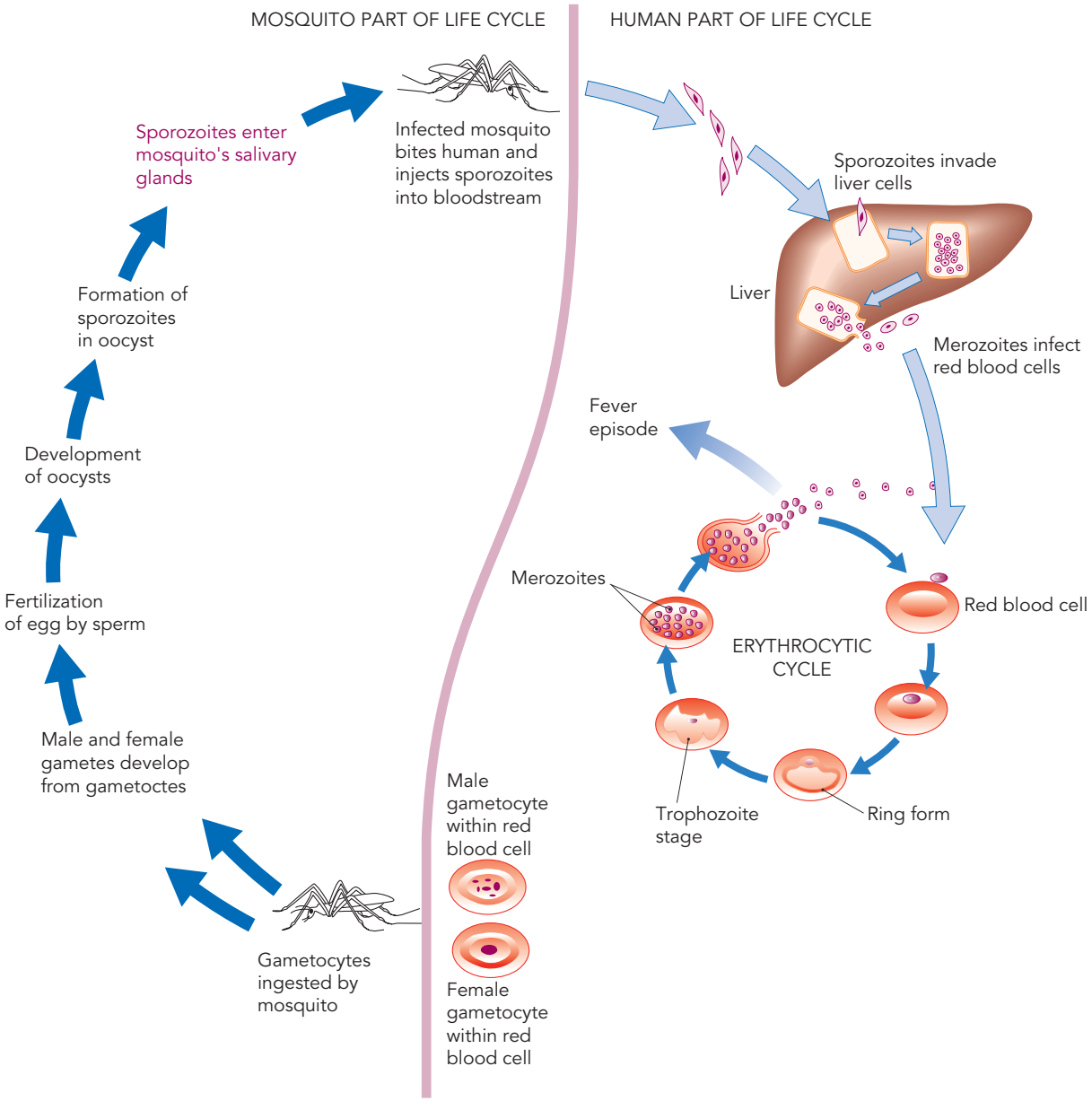


FIGURE 19.19

Life cycle of the malaria parasite, *Plasmodium*

Female Anopheles *mosquito bites human, transmitting sporozoites from its salivary glands. In the liver the sporozoites multiply and become merozoites, which are shed into the bloodstream. The merozoites enter red blood cells and become trophozoites, which eventually form many more merozoites. Merozoites are released by rupture of the red blood cells, accompanied by chills, high fever and sweating. They can then infect other red blood cells. After several such asexual cycles, gametocytes (sexual stages) are produced. A mosquito bites the infected human and ingests blood cells containing gametocytes. In the mosquito the gametocytes form a zygote, which gives rise to more infective sporozoites in the salivary glands of the mosquito. These can infect another person when the mosquito bites again.*

vessels throughout the body. The progress to severe disease can be rapid, particularly in children (see Figure 19.20). A patient with falciparum malaria may present with irregular fever and symptoms of cardiovascular, gastrointestinal, respiratory or urinary tract disease. The symptoms are persistent and there is often involvement of the brain. In cerebral malaria there may be gradual progression from periods of confusion to coma. Even with treatment, mortality can be as high as 20 per cent. Acute renal failure is also a major cause of death in falciparum malaria. An ominous sign is 'blackwater fever' (black urine) which is due to the excretion of large amounts of haemoglobin breakdown products in urine.

Diagnosis

Early diagnosis is a key component of malaria control. The main means of diagnosing the disease is by microscopic examination of a blood film. In a positive film, a ring form of the parasite is seen within the red blood cell. Repeated blood samples should be collected during or soon after a fever spike if the first sample is negative, particularly if the

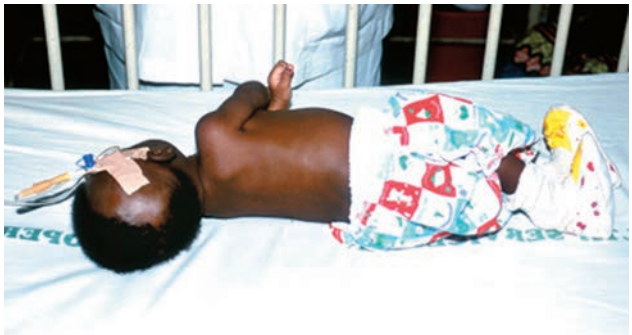


FIGURE 19.20

A child with severe malaria

Source: World Health Organization.

symptoms persist. Rapid diagnostic tests that detect malaria parasite antigens have been developed and are strongly recommended by the WHO, but their accuracy is currently a limiting factor. Molecular methods, such as PCR and nucleic acid sequence-based amplification (NASBA), have been developed and are highly sensitive, but they are expensive and require specialised equipment and personnel. In endemic areas, lack of resources is a major barrier to reliable and timely diagnosis.

Treatment and management

Increasing resistance of *Plasmodium* spp. to antimalarial drugs is a very serious problem and one of the major reasons for the persistence of malaria throughout the world. Chloroquine is one of the safest antimalarials and is the drug of choice for benign malaria, although some resistance has been reported. Artemisinin (or quinghaosu), a drug derived from the Chinese medicinal herb, *qing hao*, or sweet wormwood, is now commonly used for the treatment of falciparum malaria and drug-resistant benign malaria. The use of *qing hao* for the treatment of fevers was recommended in a Chinese handbook for medical emergencies published in AD 341. The WHO recommends the use of artemisinin or one of its derivatives, in combination with another antimalarial drug, to which there is not local resistance as first-line treatment for *P. falciparum* and chloroquine-resistant *P. vivax*. Artemisinin-based combination therapies are designed to prevent the development of drug resistance in the parasite.

In addition to chemotherapy, general measures such as physical cooling, antipyretics and fluid management may be necessary. In severe cases, other supportive treatments may be needed, such as blood transfusion (if red cell numbers are drastically reduced) and dialysis for renal failure.

Uncomplicated, benign malaria can be managed on an outpatient basis, but if falciparum is suspected then

CASE HISTORY 19.7

Malaria

A 57-year-old man contracted *P. vivax* malaria on a trip to Indonesia in May 2001. Although he had been prescribed mefloquine prophylaxis prior to this trip, he had not been fully compliant with the course. His infection was diagnosed in July 2001 and was treated with chloroquine and primaquine for an unspecified period.

In September 2002 he became unwell and presented to the hospital in Cooktown, north Queensland. Prior to that he had stayed for four nights at Noah Beach camping ground, about 70 kilometres south of Cooktown.

On 29 October, malaria in a 29-year-old man was reported to the Tropical Public Health Unit in Cairns. Over the next two weeks nine more cases were reported. It was found that all ten cases had stayed at Noah Beach camping ground between 3 and 18 October. Nearly all the infected people stated that they did not use mosquito repellents routinely or at all.

Comment

This outbreak shows how malaria can still be acquired in Australia. If a person with malaria acquired overseas (imported malaria) returns to Australia and is bitten by certain mosquito species at a time when he or she has malaria gametocytes in their blood, they can infect local mosquitos. These infected mosquitos can then infect other people that they bite. This only occurs in the 'receptive zone' in Australia—north of latitude 18°S—because this is where species of mosquitoes capable of transmitting malaria are present.

Source: Adapted from J.N. Hanna et al. 2004, An outbreak of *Plasmodium vivax* malaria in Far North Queensland, 2002. *Medical Journal of Australia* 180: 24.

Questions

1. How is malaria diagnosed in the laboratory?
2. What measures should be taken to prevent getting malaria?
3. Why are antimalarial drugs not always effective in preventing disease?
4. Can malaria be transmitted iatrogenically?

hospitalisation is usually considered. Severe falciparum is a medical emergency requiring intensive care attention and facilities, and immediate intravenous antimalarial therapy and supportive treatment.

Prevention

For people living in endemic countries the main objective is to reduce the number of malaria-bearing mosquitoes. The two core approaches recommended by the WHO are indoor spraying of a long-acting insecticide and use of insecticide-treated mosquito nets. Travellers risk acquiring malaria when they go to regions in the world where malaria is endemic. Protection of travellers against malaria is based on avoidance of mosquito bites and on chemoprophylaxis.

Malaria chemoprophylaxis is based on the use of chloroquine where the parasites are sensitive, and other drugs where resistant. Up-to-date information on malaria risk and drug susceptibility for travellers can be obtained from the World Health Organization <www.who.int> or Centers for Disease Control and Prevention <www.cdc.gov/travel> websites. The most important point, however, is that there is no single drug that is completely effective against all strains and species of malaria parasites. As a result, chemoprophylaxis should be regarded only as an adjunct to the avoidance of exposure to mosquitoes. Fake anti-malarial drugs are sold in pharmacies and shops in South-East Asia, so travellers are advised to obtain preventative medications before departing their home country.

Currently, no effective vaccine is available but intensive research is being undertaken. The limited effectiveness of current malaria control programs means that the development of a vaccine is extremely important. Development of an effective vaccine remains a challenge, but there is some hope that the first vaccine will become available within the next few years.

Toxoplasmosis

Toxoplasmosis is caused by the protozoan *Toxoplasma gondii*, which is a non-motile sporozoan. Cats are an essential part of the life cycle of *T. gondii* (Figure 19.21) because the organism undergoes its sexual phase in the intestine of the cat. Large numbers of oocysts are shed in the faeces of

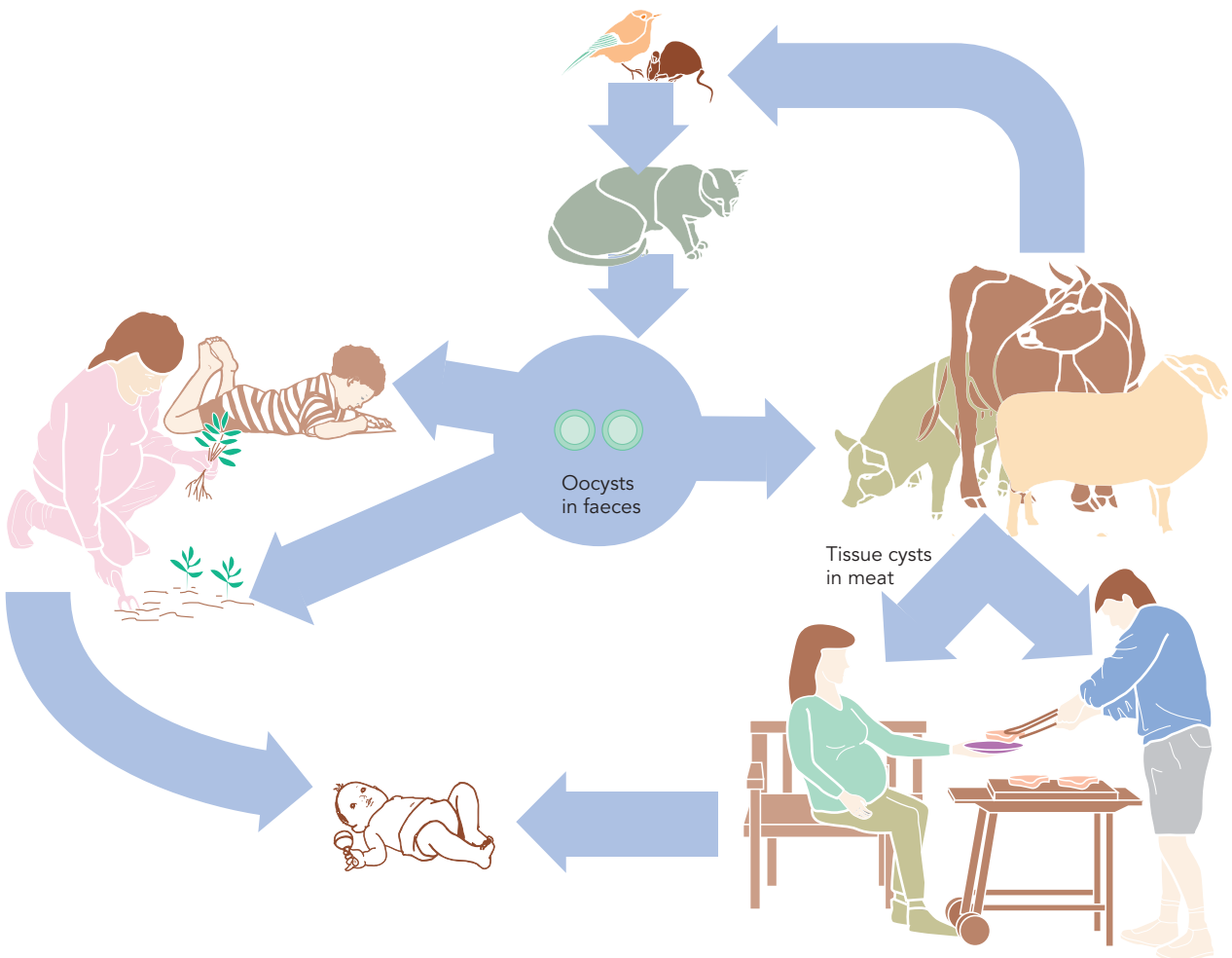


FIGURE 19.21

Life cycle of *Toxoplasma gondii*

Humans can be infected by cysts of this protozoan from cat faeces or animal meats. Infections in pregnant women can be passed on to the foetus, with severe outcomes.

an infected cat. They may then contaminate food or water, which can be ingested by other animals and humans. The oocysts contain sporozoites that invade cells to form trophozoites, called tachyzoites. The intracellular parasite reproduces rapidly and the increased numbers cause the rupture of the host cell, with release of more tachyzoites. As the immune system becomes increasingly effective, the disease enters a chronic phase in which the infected host cell develops a wall to form a tissue cyst. The numerous parasites within such a cyst reproduce very slowly, if at all, and persist for years. Loss of immune function (e.g. in AIDS) allows a reactivation of the infection from such cysts.

Humans generally acquire the infection by ingestion of undercooked meats (e.g. pork, lamb) containing tachyzoites or tissue cysts, although there is a possibility of contracting the disease more directly by contact with cat faeces. In adults, the disease is usually subclinical or an undefined, mild illness (e.g. low-grade fever, general malaise, myalgia), although occasionally there will be severe effects, especially when the organism attacks nervous tissue. Surveys indicate that 30–40 per cent of the Australian population carries antibodies for this organism. Severe infection may occur in immunocompromised persons (e.g. those who are HIV infected).

The primary danger is in the congenital infection of a foetus. The effects on the foetus can be drastic, including severe brain damage, blindness and death. The mother is usually unaware of the disease, as it is being transmitted across the placenta. In Western countries the rate of congenital toxoplasmosis seems to vary from approximately 1 in 500 to 1 in 10 000 births. A large study in Western Australia suggested a prevalence of approximately 1 in 4500 births.

Toxoplasmosis is usually diagnosed in the laboratory by serological tests demonstrating seroconversion or specific IgM antibodies, or by PCR testing on body fluids (e.g. CSF, blood, urine) or biopsy material. Pyrimethamine plus sulfadiazine and folinic acid are commonly used to treat symptomatic toxoplasmosis, but cannot reverse damage in congenital infection. Prevention of infection is important in pregnant women and the immunocompromised, and is based on avoidance of raw meat and eggs, washing fruit and vegetables, and avoiding contact with cat faeces.

SYSTEMIC HELMINTH INFECTIONS

Schistosomiasis

Schistosomiasis is a chronic disease caused by parasitic worms (blood flukes) of the genus *Schistosoma*. There are three major species—*S. mansoni*, *S. japonicum* and *S. haematobium*. The species vary with geographical location, but the diseases caused by each are similar. Over 200 million people in the world are thought to be infected, mostly in Africa, but the disease also occurs in Asia, South America and the Caribbean. Around 200 000 people die from the disease each year. The disease is not found in Australia, but has been diagnosed in travellers returning from countries where it is endemic.

When water becomes contaminated with *Schistosoma* ova excreted in human wastes, a motile, ciliated larval form of *Schistosoma* (called a miracidium) is released and enters certain species of freshwater snails. The lack of a suitable host snail is a primary reason why schistosomiasis is not transmitted in certain areas of the world (e.g. Australia). Eventually, the pathogen emerges from the snail in an infective form called the cercaria. When the cercaria contact the skin of a person working, washing or swimming in the water, they penetrate the skin (see Figure 19.22) with enzymes which break down its structure. They are then carried by the bloodstream to the liver or urinary bladder. There they mature into adults which live in the blood vessels. After mating, the females produce numerous eggs, some of which become trapped in tissues.

When egg production begins, acute symptoms develop, including fever, night sweats, abdominal pain, anorexia, diarrhoea, lethargy, headache, urticaria and a non-productive cough. Chronic disease is caused by the immune response to the eggs, which produces granulomas and tissue damage. The two major forms of the disease are intestinal schistosomiasis, caused by *S. haematobium*, and urogenital schistosomiasis, caused by the other species of *Schistosoma*. Intestinal disease is characterised by abdominal pain, diarrhoea, blood in the stool, and liver enlargement in advanced cases. Urogenital disease is characterised by haematuria, with fibrosis of the bladder and ureter and kidney damage in advanced cases. In women genital lesions and vaginal bleeding may occur; and in men, pathology related to damage of genital organs may be present. Eggs excreted in faeces or urine and into water continue the cycle of infection.

Laboratory diagnosis consists of microscopic identification of the flukes or their eggs in faecal or urine specimens or serological tests for antibody production.

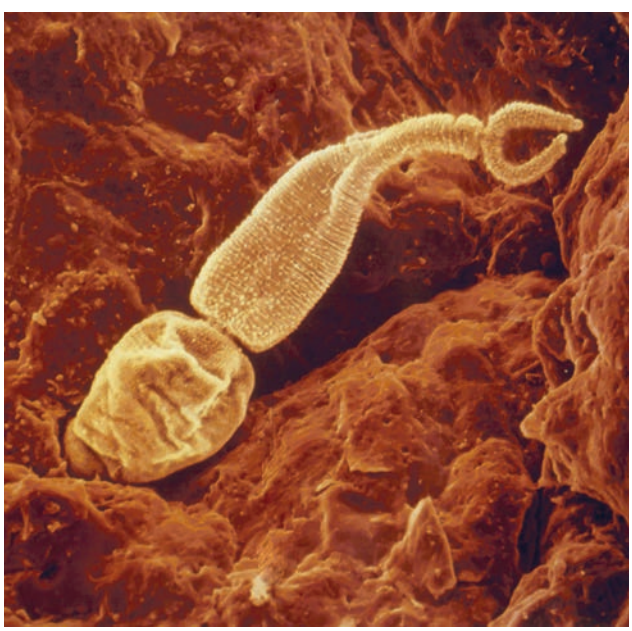


FIGURE 19.22

***Schistosoma mansoni*, a cause of schistosomiasis**

An electron micrograph showing a cercaria penetrating the skin.

Source: Dr Penny Bishop.

Praziquantel is the most readily available and effective treatment, although rapid re-infection can occur after completion of treatment. Other forms of control include improved sanitation and elimination of the host snail. Much work has been done towards the development of a vaccine, but it still appears to be some way off.

Filariasis

Filariasis affects more than 120 million people throughout Asia, Africa, the Western Pacific, the Caribbean and South America. **Filariasis** is caused by several different roundworms but most commonly by *Wuchereria bancrofti*, which causes lymphatic filariasis. This disease affects more than 90 million people. Other significant filarial parasites include *Onchocerca volvulus,* which causes a severe dermatitis or visual impairment, and *Loa loa*, which causes subcutaneous swellings, localised pain, pruritus and urticaria.

W. bancrofti is transmitted to humans by mosquitoes. After entering the tissues, the larvae develop into adult worms which can locate in the lymph glands and ducts of the infected person. Adult worms can be as long as 10 cm and can live in the lymph nodes of a person for years. Microfilariae are forms of the worm that are present in peripheral blood vessels during the night and which retreat to deep vessels, especially those of the lungs, during the day. When a mosquito bites an infected person, it ingests microfilariae that develop into larvae and migrate to the mosquito's mouth parts. When the mosquito bites again, the larvae can infect another person. They enter the blood, develop and reproduce in the lymph glands and ducts, thereby completing the life cycle. Adult worms cause inflammation in lymph ducts, fever and eventual blockage of lymph ducts. Repeated infections over a period of years can lead to **elephantiasis**, a gross enlargement of limbs (Figure 19.23), scrotum or sometimes other body parts, from an accumulation of fluid in the interstitial spaces and an increase in connective tissue.

Filariasis is diagnosed by finding microfilariae in thick blood smears made from blood samples taken at night. There are now rapid tests available for the detection of antigens of the worm in blood. PCR methods have also been developed for detection of worm DNA in blood. The drugs diethylcarbamazine or albendazole plus ivermectin are effective in treating the disease. Pressure bandages may be used to force lymph from swollen limbs and, if distortion is not too great, nearly normal size can be regained.

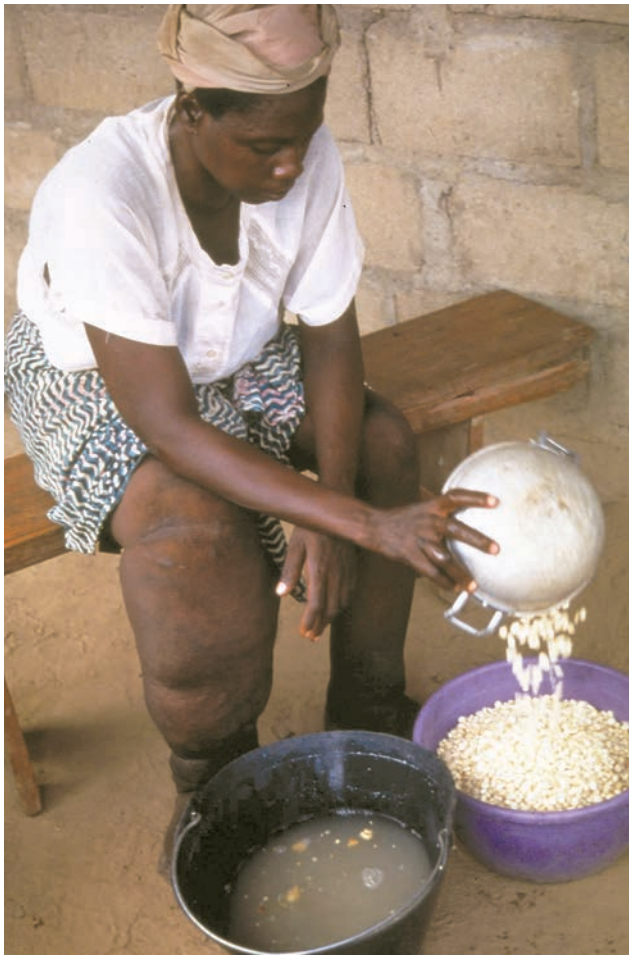


FIGURE 19.23

Elephantiasis of the leg caused by the roundworm *Wuchereiria bancrofti*

SUMMARY

- The circulating blood is normally sterile.
- Infections that spread through the body and reach more than one organ are called systemic infections.
- Bacteraemia refers to the presence of bacteria in the bloodstream.
- Septicaemia is a serious clinical syndrome resulting from bacteria in the bloodstream.

SYSTEMIC BACTERIAL INFECTIONS

- In most cases of septicaemia, the organisms enter the bloodstream from a focus of infection in the body.
- Endotoxic shock is a life-threatening form of septicaemia caused by Gram-negative bacteria.
- The diagnosis of septicaemia is made by blood culture.
- Rheumatic fever is a non-suppurative complication of *Streptococcus pyogenes* sore throat.
- Infective endocarditis is infection and inflammation of the endocardium. In most cases there is a predisposing heart defect.
- The major pathological feature of infective endocarditis is a growing clump of bacteria, fibrin and platelets on the endocardial surface of valves and surrounding structures.
- Osteomyelitis is infection and inflammation of the bone.
- The most common cause of osteomyelitis is *Staphylococcus aureus*.

- Lyme disease is caused by the spirochaete *Borrelia burgdorferi*.
- Lyme disease is transmitted to humans by ticks.
- The most common rickettsial infection in Australia is Q fever, caused by *Coxiella burnetii*.
- Scrub typhus and Queensland tick typhus are the most common arthropod-borne rickettsial infections in Australia.
- Leptospirosis is caused by the spirochaete *Leptospira interrogans*.
- Toxic shock syndrome is caused by certain strains of *Staphylococcus aureus* or Group A streptococcus.
- Toxic shock syndrome is a potentially life-threatening condition characterised by fever, shock and a sunburn-like rash.
- Anthrax is a zoonosis caused by *Bacillus anthracis* and is acquired mainly from sheep, goats and cattle.
- Brucellosis is caused by three species of the genus *Brucella*.
- Transmission of brucellosis from animals to humans occurs by direct contact with infected tissues or animal products, or via dust or aerosols.
- The causative agent of plague is *Yersinia pestis*.
- *Yersinia pestis* infects wild rodents, especially rats, and is transmitted from animal to animal and animal to human by flea bites.

SYSTEMIC VIRAL INFECTIONS

- Mumps is caused by a single-stranded RNA virus.
- Mumps now mainly occurs in young adults and older adolescents.
- The cause of AIDS is the human immunodeficiency virus HIV.
- HIV is transmitted by a variety of means, including sexual intercourse, through blood or blood products, from mother to child, and as a result of intravenous drug abuse.
- HIV produces disease by chronic infection of cells bearing the CD4 antigen. Helper T lymphocytes are the main targets.
- The most common secondary diseases in AIDS patients are *Pneumocystis jiroveci* pneumonia and Kaposi's sarcoma.
- Ross River virus fever is the most important arbovirus infection in Australia. It is spread by mosquitoes.
- The main manifestations of Ross River virus fever are arthritis, a maculopapular rash, lethargy and flu-like symptoms.
- Dengue fever has a worldwide distribution in tropical areas, and occasionally in the subtropics.
- The mosquito *Aedes aegypti* is the principal vector of dengue. The disease is characterised by high fever, headache and a rash, often with severe muscle and joint pain.
- Dengue haemorrhagic fever is characterised by high fever bleeding from the gums, skin and gastrointestinal tract.
- Chikungunya is a systemic viral disease that is clinically similar to dengue fever.
- Infectious mononucleosis, or glandular fever, is caused by the Epstein-Barr virus (EBV).
- EBV has been implicated in several malignancies and in chronic fatigue syndrome.
- The majority of cytomegalovirus (CMV) infections produce few symptoms.
- Transplacental transmission of CMV to the foetus leads to spread of the virus to multiple organs and a severe illness.
- CMV is responsible for severe disseminated infections in immunocompromised people.
- Fifth disease is a common cause of rash in childhood, caused by parvovirus B19.
- Hand, foot and mouth disease is caused by a number of viruses, particularly coxsackie A16 virus and enterovirus 71. It is characterised by painful oral lesions and a skin rash.
- Yellow fever virus is restricted to parts of South America and Africa. It is transmitted to humans by the mosquito *Aedes aegypti*.
- Yellow fever is characterised by sudden onset of fever and vomiting, and prostration 3–6 days after infection.
- Haemorrhagic fevers are caused by a number of viruses including the dengue virus, yellow fever virus, Ebola virus and Marburg virus.
- Symptoms of haemorrhagic fevers include fever, malaise, myalgia and widespread haemorrhage, leading to shock and death.

SYSTEMIC FUNGAL INFECTIONS

- The incidence of systemic fungal infections has increased because of greater numbers of immunocompromised people.
- Disseminated *Candida* spp. infections occur in transplant patients and patients with neutropenia.
- Disseminated *Aspergillus fumigatus* infections occur in neutropenic patients and people receiving adrenal corticosteroids.
- *Cryptococcus neoformans* is inhaled and causes a lung infection in the immunocompromised, which can spread to other organs including the brain.

SYSTEMIC PROTOZOAL INFECTIONS

- There are 300–500 million cases of malaria in the world each year. The estimated annual death toll from the disease is 2–3 million, mainly children.
- Five species of *Plasmodium* cause human malaria.
- The malaria protozoa are transmitted to humans by species of *Anopheles* mosquitoes.
- In malaria, a characteristic pattern of recurrent chills followed by fever, drenching sweats, headache, muscular pain and vomiting occurs every 48 or 72 hours.
- Severe falciparum malaria is a medical emergency.
- Toxoplasmosis is caused by the protozoan *Toxoplasma gondii*.
- *Toxoplasma* oocysts are shed in cat faeces and contaminate food or water.
- The primary danger of toxoplasmosis is the congenital infection of a foetus.

SYSTEMIC HELMINTH INFECTIONS

- Schistosomiasis is a debilitating disease caused by a fluke of the genus *Schistosoma*.
- More than 200 million people in the world have schistosomiasis, mostly in Asia, Africa, South America and the Caribbean.

- ❖ Filariasis can be caused by several different roundworms, most commonly *Wuchereria bancrofti*. The worms are transmitted to humans by mosquitoes.
- ❖ Repeated infections of filariasis over a period of years can lead to elephantiasis, a gross enlargement of limbs, scrotum or sometimes other body parts.

STUDY QUESTIONS

1. Explain the difference between 'bacteraemia', 'septicaemia' and 'sepsis'.
2. What is the usual source of organisms causing septicaemia?
3. What causes shock in patients with toxic shock syndrome?
4. What predisposing factor exists in the majority of people who develop endocarditis?
5. What is a vegetation in endocarditis?
6. Explain the difference between haematogenous osteomyelitis and exogenous osteomyelitis.
7. Explain why the existence of Lyme disease is uncertain in Australia.
8. How does Q fever differ from other rickettsial infections?
9. How is leptospirosis usually acquired?
10. Why can anthrax persist for long periods in the environment?
11. Why do outbreaks of mumps still occur in countries where vaccination programs exist?
12. What species causes most cases of brucellosis in Australia, and what is the usual source of the organism?
13. What are the major ways by which the human immunodeficiency virus is transmitted?
14. What cell types are targeted by the human immunodefiency virus?
15. How is HIV infection treated, and how does this treatment reduce viral multiplication?
16. Why is it that Ross River virus infection can be acquired almost anywhere in Australia?
17. Explain the difference between dengue fever and dengue haemorrhagic fever,
18. Explain the importance of B lymphocytes in glandular fever.
19. What is chronic fatigue syndrome?
20. What is the most serious form of cytomegalovirus infection?
21. Why is hand, foot and mouth disease so easily spread from person to person?
22. Explain how the haemorrhagic fever viruses produce the typical symptoms of the disease.
23. Which species causes the most serious form of malaria, and why?
24. Explain why malaria has been so hard to control worldwide.
25. What is the most serious form of toxoplasmosis, and how can it be prevented?
26. How would Australians contract schistosomiasis?
27. What is elephantiasis, and how are the symptoms produced?

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CHAPTER
20

Infections of the nervous system

CHAPTER FOCUS

- What microorganisms commonly cause infections of the nervous system?
- How do microorganisms gain access to the nervous system in order to cause disease?
- What are the important clinical aspects of nervous system infections?
- How are nervous system infections treated, and how may they be prevented?

INTRODUCTION

The central nervous system (CNS) consists of the brain and the spinal cord, which are surrounded by three layers of membranes or **meninges**, termed the dura mater, arachnoid and pia mater (see Figure 20.1). **Cerebrospinal fluid** (**CSF**), a clear fluid, circulates in the space between the inner two meninges, called the subarachnoid space, forming a liquid cushion around the CNS organs. This fluid also serves to maintain the chemical balance in the CNS and to nourish the brain. CSF is continuously produced from blood by masses of specialised capillaries (called choroid plexuses) in four ventricles (cavities) within the brain. The CSF circulates around the brain and spinal cord and returns to the blood via the arachnoid villi.

Normally, the CSF is produced and drained at a constant rate. However, if its circulation or drainage is blocked (e.g. by tumour or infection), it begins to accumulate and may exert pressure on the brain. This is termed 'hydrocephalus', or 'water on the brain'.

The **blood–brain barrier** comprises the tightly joined endothelial cells of the brain's capillaries (see Figure 20.1). These capillaries are the least permeable in the entire body. The blood–brain barrier protects the brain by ensuring that its internal environment remains stable. The capillaries form a highly selective barrier that allows some substances, such as nutrients (e.g. glucose and essential amino acids), to pass from the circulation to the brain. However, most substances are prevented from entering brain tissue, including wastes (e.g. urea and creatinine) and many drugs. Most antibiotics do not readily cross the blood–brain barrier, although chloramphenicol, a lipid-soluble drug, is able to cross it reasonably well. The blood–brain barrier is less effective in preventing the movement into brain tissue of substances such as alcohol and certain anaesthetics. Because of their lipid solubility, these substances diffuse easily through cell membranes and, therefore, across the blood–brain barrier.

INFECTIONS OF THE CENTRAL NERVOUS SYSTEM

In most CNS infections, microorganisms gain access to the meninges and/or brain from the bloodstream. However, infection may sometimes spread from the ears or sinuses, or via peripheral nerves.

When infection and inflammation of the meninges occurs, it is called a **meningitis**. It is most often caused by viruses or bacteria, and occasionally by fungi or protozoa. Infection and inflammation of the brain itself is called **encephalitis** and is almost always caused by viruses. In some circumstances, microorganisms may infect the brain tissue and cause an abscess to form. Brain abscesses are mostly caused by bacteria, but sometimes by fungi or protozoa.

Bacterial meningitis

Despite the availability of effective antibiotics and vaccines, bacterial meningitis remains a common disease worldwide, with high morbidity and mortality. Acute bacterial meningitis is a medical emergency requiring urgent and specific treatment. The mortality rate varies with the causative agent, ranging from 5 to 50 per cent. Up to half of the people who survive bacterial meningitis suffer permanent neurological damage, such as visual or hearing impairment or reduced mental development.

Causative agents, transmission and incidence

The most common causative agents have classically been *Neisseria meningitidis*, *Haemophilus influenzae* and *Streptococcus pneumoniae*; however, with the availability of effective vaccines for each of these organisms, their roles have markedly declined. *Escherichia coli* and Group B streptococci (*Streptococcus agalactiae*) are now the two most common causes of neonatal meningitis, and *Listeria monocytogenes* mainly affects neonates and immunocompromised patients. Patients with CSF shunts or severe head injury, or those who have had neurosurgery, may develop meningitis caused by bacteria such as staphylococci, *Propionibacterium* or *Corynebacterium*. *Mycobacterium tuberculosis* can cause meningitis, but is rarely seen in Australia.

Meningococcal meningitis

N. meningitidis, or the **meningococcus**, is an aerobic Gram-negative diplococcus. It is carried asymptomatically in the nasopharynx by up to 10 per cent of the population, although the carriage rate can be as high as 60 per cent in closed or crowded populations (e.g. military personnel in camps, dormitories). **Meningococcal meningitis** is transmitted from person to person via direct contact or respiratory droplets. In most cases this leads to a subclinical infection or mild symptoms. If it spreads from the nasopharynx to the blood, it can reach the meninges and cause disease. Worldwide, approximately 500 000 cases of meningococcal meningitis occur annually, resulting in about 50 000 deaths. A large proportion of these cases occur in the African 'meningitis belt', which extends east–west across the continent from Senegal to Ethiopia (see Figure 20.2). Currently, 300–400 cases per year of invasive meningococcal infection (meningitis and/or septicaemia) are reported in Australia. Most cases of meningococcal infection occur in the 0–4 years age group. The incidence in Western countries ranges from about 1 to 5 per 100 000 population. Why so many people are colonised and yet so few become infected is not clear, but the possession of antibodies to the organism's capsular antigens is protective. This would explain the higher incidence in young children, who have lost their maternal-derived antibodies and are yet to produce their own.

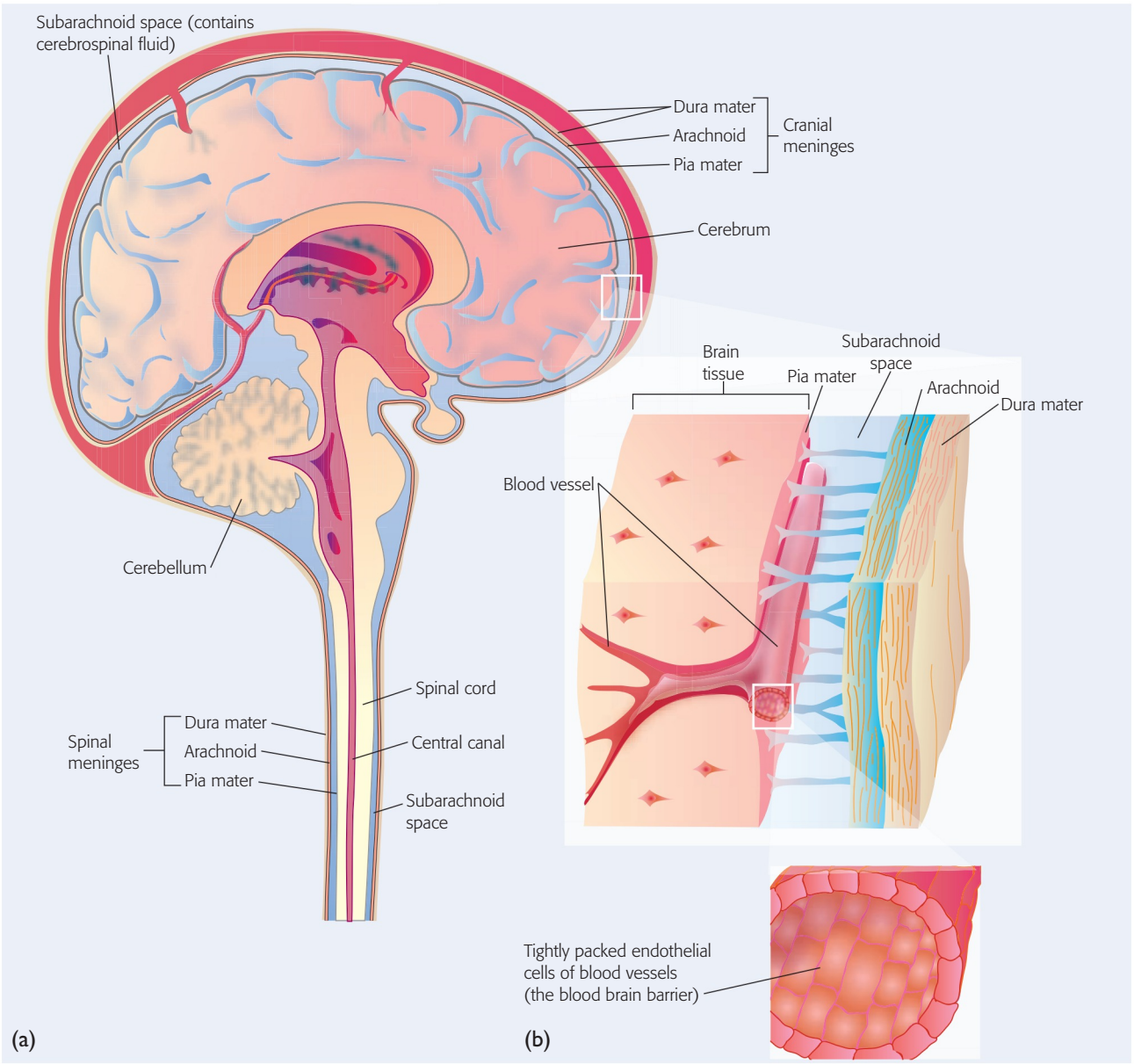

FIGURE 20.1

(a) The meningeal membranes covering the brain and spinal cord: dura mater, arachnoid and pia mater; (b) the blood–brain barrier: the endothelial cells of the blood vessels of the brain

There are 13 known serogroups of meningococcus; however, serogroups A, B, C, Y and W135 cause the majority of human infections. In Australia and other developed countries, serogroup B is the major cause of invasive infections. There has been a marked decline in serogroup C infections since the introduction in 2003 of serogroup C vaccination. Meningococcal meningitis is the only type of meningitis that can spread in an epidemic form. This can occur when carriage rates are high (see above). The persistence of serogroup B and C disease in many developed countries and recent outbreaks of serogroup A, Y, X and W135 in undeveloped countries confirm that meningococcal infection remains a worldwide threat.

Invasive meningococcal infection usually presents as a meningitis or a septicaemia, or a combination of the two. The meningitis is clinically similar to other forms of acute bacterial meningitis. Meningococcal septicaemia, however, is particularly severe, with a greater mortality than meningitis. The characteristic feature of meningococcal septicaemia is a haemorrhagic skin rash, although the rash may not be visible on presentation, or be only transiently present. The septicaemic form, sometimes called the Waterhouse-Friderichsen syndrome, can lead to organism invasion of multiple organs, the release of large amounts of endotoxin in the blood, and substantial cytokine release by host cells.

CASE HISTORY 20.1

Meningococcal infection

A 13-year-old girl from Western Sydney was taken to a local doctor on 18 October 2008 with fever and vomiting. She was initially diagnosed as having viral gastroenteritis. However, her condition deteriorated rapidly and she was admitted to the intensive care unit of a local hospital a few hours later. A lumbar puncture was performed and Gram-negative diplococci were identified in the CSF. The girl recovered after a week in hospital and antibiotic treatment. She had been fully vaccinated against meningococcal disease.

This case was considered to be part of a small outbreak involving two other children who lived in adjacent suburbs. A public health alert was issued to health professionals in the Sydney West Area Health Service on 20 October 2008.

Source: A. Jardine et al. 2009, A community outbreak of meningococcal serogroup B disease in Western Sydney: The challenges of identification and significance. *Communicable Diseases Intelligence* 33(2): 221–24.

Questions

1. What could be assumed from the Gram stain result of the CSF from the girl? Why is a Gram stain of CSF so useful in the diagnosis of meningitis?
2. How is meningococcal infection spread from person to person?
3. How can the spread of meningococcal infection be prevented?
4. If the girl had been fully vaccinated, how could she get meningococcal disease?

FIGURE 20.2

The African 'meningitis belt': areas with frequent epidemics of meningococcal meningitis

Source: Centers for Disease Control and Prevention 2012, *Travelers' Health*, Chapter 3. Edited by Gary W. Brunette. © Centers for Disease Control and Prevention. Reproduced with permission of Centers for Disease Control and Prevention. Available at: <wwwnc.cdc.gov/travel/yellowbook/2012>.

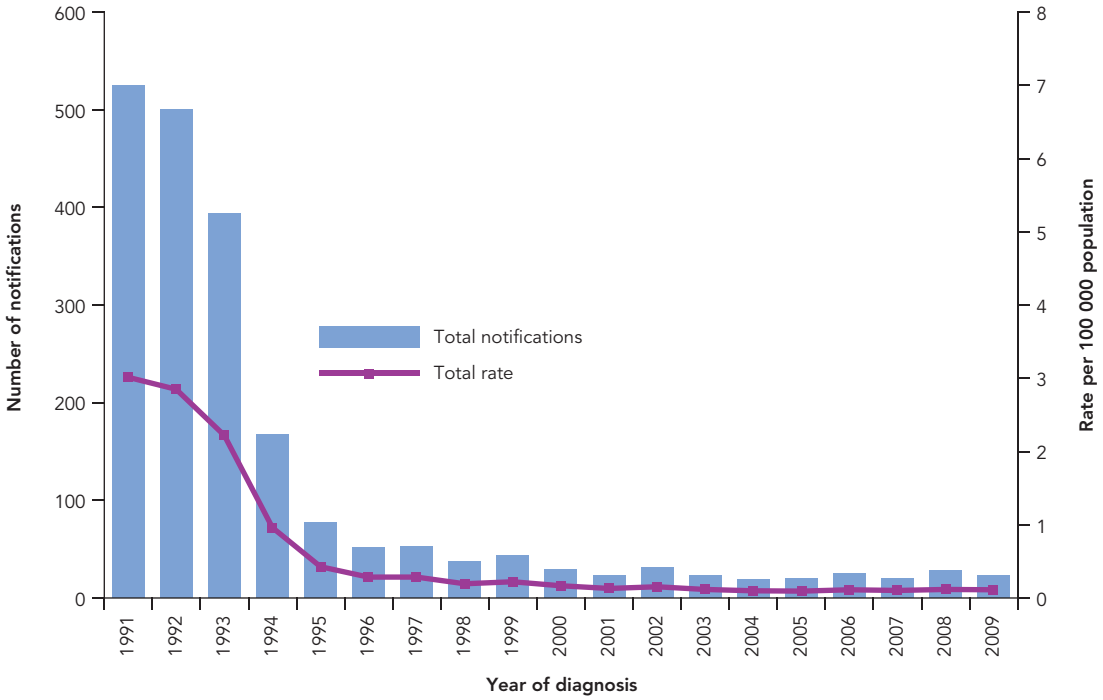

FIGURE 20.3

Notifications and rates for invasive *Haemophilus influenzae* type b infection, Australia, 1991–2009, by year of diagnosis

Source: NNDS Annual Report Writing Group 2011, Australia's Notifiable Disease Status, 2009: Annual Report of the National Notifiable Diseases Surveillance System. *Communicable Diseases Intelligence* 35(2): 101. Reproduced with permission of the Australian Government.

Endotoxin and cytokine release result in disseminated intravascular coagulation (DIC), skin and mucosal haemorrhages, renal failure, bleeding into the brain and adrenal glands, and shock; death is possible within hours. Meningococcal disease can range from mild or subclinical in some people to rapidly progressive and fatal in others. A rapid diagnosis and immediate commencement of antibiotic therapy are necessary.

Haemophilus meningitis

Haemophilus influenzae is an aerobic Gram-negative coccobacillus. There are a number of serological types, but invasive infections are almost always caused by encapsulated type b. The organism is a common inhabitant of the throat of infants and young children, but is found less frequently in adults. Invasion of the meninges is usually preceded by a viral infection of the respiratory tract, the resulting inflammation allowing the organism to invade the bloodstream and then the meninges. In the past, *H. influenzae* type b (Hib) caused about 70 per cent of all cases of childhood bacterial meningitis in Australia, especially in the 0–4-year age group. However, in the early 1990s an Hib vaccine became available and there has been a reduction of more than 90 per cent in the incidence of the disease in countries that have included the vaccine in their standard immunisation schedule (see Figure 20.3). The Hib vaccine was introduced in Australia in 1992. There is an average of 20 cases of Hib disease (respiratory or meningeal infection) reported annually in Australia.

Pneumococcal meningitis

Pathogenic *Streptococcus pneumoniae* are encapsulated, aerobic, Gram-positive cocci that are carried in the throat of many healthy people. Only rarely does the organism invade the meninges. It causes meningitis mainly in:

- children under 2 years of age
- debilitated or splenectomised patients
- head injury victims where there is a skull fracture communicating with the nasopharynx
- the elderly.

About 200 cases of pneumococcal meningitis occur each year in Australia. Children under 2 years and adults over the age of 80 have the highest risk, but the case fatality rate is highest in the elderly (up to 90 per cent).

Acute onset of meningitis may follow otitis media, pneumonia, septicaemia or head injury. Antibodies to capsular polysaccharides opsonise the organism and protect against invasion; however, there are 90 different capsule serotypes. Specific antibodies do provide immunity, but antibodies to one serotype do not protect against the other serotypes.

Neonatal meningitis

Neonatal meningitis can be caused by a wide range of bacteria, but *E. coli* (with capsular type K1) and Group B Streptococci (*Streptococcus agalactiae*) are the most frequent. The strains of *E. coli* and *S. agalactiae* that are pathogenic for the CNS owe their virulence, at least partially, to a polysaccharide

TABLE 20.1 Bacterial causes of neonatal meningitis

	% CASES
Streptococcus agalactiae	35
Escherichia coli	22
Neisseria meningitidis	6
Coagulase negative staphylococci	5
Listeria monocytogenes	<5
Other bacteria	<5

capsule. Other causes of neonatal meningitis are shown in Table 20.1.

The incidence of neonatal bacterial meningitis in developed countries, including Australia, is around 2–5 per 10 000 births. With appropriate antibiotic therapy and supportive care the mortality rate in developed countries has decreased from almost 50 per cent in the 1970s to around 10 per cent, currently. However, many of the infants that survive the infection have serious long-term sequelae, including cerebral palsy, learning disability, seizures and hearing problems.

Group B streptococci are part of the genitourinary flora of some women. They have been found to colonise about 20 per cent of women of child-bearing age. A baby born to a colonised mother may itself become colonised in the oral cavity, the rectum or the umbilical stump. A small percentage of colonised infants develop meningitis and/or pneumonia, usually within four weeks but sometimes up to three months later. Certain factors are known to place babies at increased risk of these infections, especially:

- prematurity (< 36 weeks)
- prolonged rupture of amniotic membranes
- maternal fever during delivery
- maternal urinary tract infection with Group B streptococci
- multiple births.

However, many babies have no apparent risk factors, apart from an immature immune system. Healthcare-associated transmission can occur, especially in crowded nurseries. *E. coli* meningitis in neonates is also often associated with one of the above risk factors, especially prematurity and prolonged rupture of membranes. Early intestinal colonisation by K1 strains of *E. coli* before the normal flora is established can lead to invasion of the blood and then the CNS.

CASE HISTORY 20.2

Neonatal meningitis

Baby Sarah was a 3950 gram full-term infant born to a 25-year-old woman. The baby appeared healthy at delivery, but 24 hours later started to show signs of respiratory distress. On examination she was found to be irritable and tachypnoeic. Her axillary temperature was 38.3°C and her pulse 200. The infant had a bulging anterior fontanelle. There was no apparent rash.

Initial CSF findings included: protein 1.15 g/L (normal 0.15–0.45 g/L), glucose 2.1 mmol/L (normal >2.5 mmol/L), many Gram-positive cocci and a few white blood cells.

Questions

1. What is the most likely cause and source of this infection?
2. What evidence is there to suggest that this is a bacterial infection?
3. What problems are associated with antimicrobial drug treatment of bacterial meningitis?

Listeriosis

Listeriosis is caused by *Listeria monocytogenes*, which causes meningitis mainly in the immunocompromised and the elderly. It is also an important cause of infection in pregnant women. Bacteraemic infection in a pregnant woman is not usually severe, but the organism can spread to the foetus *in utero* or be transmitted at birth, resulting in severe foetal or neonatal disease. Intrauterine infection can result in abortion, premature labour, intrauterine death or perinatal infection. Perinatal infection is most often manifested as a meningitis. The organism can also cause a febrile gastroenteritis in healthy adults. Listeriosis is a food-borne disease, so is covered in more detail in Chapter 18.

Tuberculous meningitis

Mycobacterium tuberculosis is a rare cause of meningitis in Australia. It can occur during active infection or in recrudescent infection (see Chapter 17). From a focus of infection elsewhere, the organism enters the bloodstream and disseminates to the subarachnoid spaces.

The causes, predisposing factors and incidence of bacterial meningitis are summarised in Table 20.2.

Pathogenesis

There are several critical events in the pathogenesis of many cases of acute haematogenous bacterial meningitis:

1. nasopharyngeal colonisation
2. invasion of the bloodstream and intravascular survival
3. penetration of the blood–brain barrier
4. damage and inflammation of the meninges.

Many of the bacteria that cause meningitis possess surface structures that enhance mucosal colonisation. For instance, both *N. meningitidis* and *H. influenzae* possess fimbriae which may facilitate their attachment to the nasopharyngeal mucosa.

Polysaccharide capsules are possessed by all the major pathogens and these may also assist them to attach. Immunoglobulin A (IgA), the antibody class found predominantly in mucosal secretions, may inhibit the attachment of bacteria, but many clinical isolates of *N. meningitidis*, *H. influenzae* and

TABLE 20.2 Bacterial meningitis: Causative agents, predisposing factors and incidence

CAUSATIVE ORGANISM	PREDISPOSING FACTORS	INCIDENCE (AUSTRALIA)
Neisseria meningitides	Young age Close contact Complement deficiencies	1.5 per 100 000 population Most frequent in 0–4 years age group
Haemophilus influenzae	Young age Non-immunised Respiratory tract infection	Fewer than 20 cases per year
Streptococcus pneumoniae	Young age Pneumococcal pneumonia Debility Skull fracture Old age	About 200 cases per year
Group B streptococci, *Escherichia coli*	Neonates: ■ prematurity ■ prolonged rupture of membranes ■ heavy maternal colonisation ■ maternal bacteraemia ■ multiple births	About 2 per 10 000 births
Listeria monocytogenes	Neonates—maternal infection Immunosuppression, e.g. transplant, cancer	0.25 per 100 000 population
Mycobacterium tuberculosis	Infection elsewhere which spreads to blood	Rare
Staphylococcus *Proprionibacterium* *Corynebacterium*	Entry of skin flora following: ■ head injury ■ CSF shunt ■ neurosurgery	Uncommon

S. pneumoniae secrete a protease which cleaves and inactivates IgA.

To invade the bloodstream, the bacteria must cross the mucosal epithelium of the nasopharynx. Different pathogens have different methods of invasion. For example, *N. meningitidis* is thought to enter non-ciliated epithelial cells by an endocytic process and pass through the cell in a membrane-bound vacuole. On the other hand, *H. influenzae* invades through separations in the tight junctions of columnar epithelial cells. Once in the bloodstream, the bacteria must then evade the host defence mechanisms there. Most of the pathogens (*H. influenzae*, *N. meningitidis*, *S. pneumoniae*, *E. coli* and *S. agalactiae*) are encapsulated, which protects them from phagocytes.

Pathogenic bacteria are able to pass from the circulation into the CSF by penetrating vulnerable sites in capillaries of the CNS. Once in the CSF, the bacteria have a good chance of survival because host defences, particularly immunoglobulin, neutrophils and complement activity, are limited in this body compartment. Even though blood leucocytes enter the CSF during infection, their effectiveness is limited by the lack of antibodies and complement, which normally enhance their activity.

In head-injured patients, organisms may gain direct access to the CNS. The cribriform plate at the anterior part of the base of the skull is in close proximity to the nasal sinuses. This bony plate may be damaged in head injury, and organisms found in the nares (particularly *Streptococcus pneumoniae*) may then gain direct access to the CSF.

The tissue dysfunction in bacterial meningitis may be due to focal lesions caused by bacterial growth or toxins. However, there is much evidence to suggest that the intense inflammatory response to the infection has a significant role in producing the neurological problems and death that are so often associated with bacterial meningitis. Lipopolysaccharides and other bacterial cell wall components are released into the subarachnoid space during natural or antibiotic-induced bacteriolysis and they elicit the local release of cytokines, especially interleukin-1 (IL-1) and tumour necrosis factor-alpha (TNF-α), from various cells (e.g. vascular endothelial cells, microglia and astrocytes). These cytokines lead to inflammation, vascular endothelial injury and increased blood–brain barrier permeability. The pressure in the subarachnoid space may rise markedly, as a result. This increased intracranial pressure can then cause a decrease in cerebral blood flow.

The pathophysiologic processes that contribute to the neurologic sequelae of bacterial meningitis are summarised in Figure 20.4.

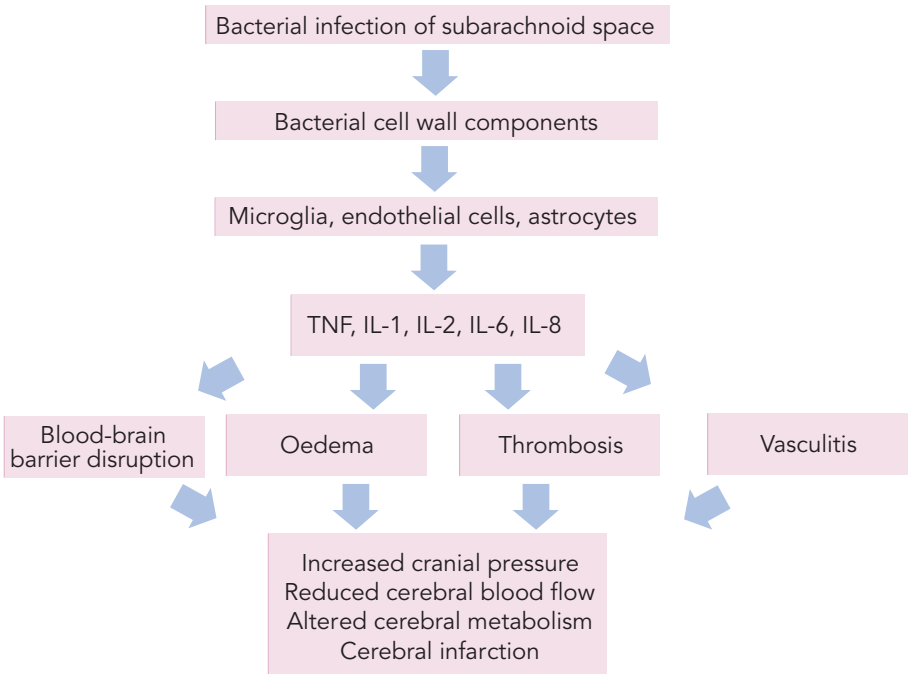

FIGURE 20.4

Pathophysiology of neurologic dysfunction in bacterial meningitis

Clinical features

The clinical presentation of bacterial meningitis is variable and depends on the patient's age, the duration of illness and the causative agent. Headache, fever, altered consciousness, vomiting and nuchal rigidity (neck stiffness) are the most common presenting symptoms. Lethargy, irritability, anorexia and photophobia are also common. These symptoms are often accompanied by upper respiratory symptoms such as a sore throat. In neonates, young infants and the elderly, the symptoms may be more subtle and usually include fever, lethargy, irritability, respiratory distress, lack of interest in food, vomiting and diarrhoea. A rapidly progressing, fulminant form may be caused by *N. meningitidis* and sometimes *S. pneumoniae*.

The presence of a haemorrhagic skin rash, associated with septicaemia, is suggestive of *N. meningitidis* infection, although *H. influenzae* can sometimes mimic this. The mortality rate varies from 5 to 50 per cent, depending on a number of factors—including the age of the patient, the causative agent, and the duration of illness before commencement of treatment. Up to 10 per cent of survivors experience long-term serious sequelae such as hearing loss, mental retardation and seizures.

Laboratory diagnosis

CSF is the most important specimen for the diagnosis of meningitis. It is usually collected by lumbar puncture, but sometimes other methods—such as a ventricular tap—are used. It should be remembered that CSF is a body fluid to which Standard Precautions apply. Laboratory-acquired cases of bacterial meningitis have been reported.

Rapid transport of the specimen to the laboratory is necessary for two important reasons. First, the disease is very serious and thus an accurate diagnosis is needed as soon as possible. Second, most of the common causes are fastidious organisms that may not survive variations in temperature or long transit times. Unlike many other specimens for the microbiology laboratory, CSF specimens should not be refrigerated but maintained at room temperature or at 37°C. Blood cultures should also be collected, since fastidious organisms not isolated from CSF may sometimes be cultured from blood.

Cerebrospinal fluid analysis in the microbiology laboratory usually includes:

- a macroscopic examination
- culture
- a Gram stain
- a cell count
- determination of glucose concentration
- determination of protein concentration.

Preliminary results of all of the above, except culture, should be available within an hour or two after receipt of the CSF specimen in the laboratory. These results may enable bacterial meningitis to be distinguished from other causes of meningitis, and differentiation of bacterial causes, as shown in Table 20.3. Figure 20.5 shows a Gram stain of CSF from a patient with meningococcal meningitis. However, not all infections produce the typical changes shown in this table. The differentiation between bacterial causes and viral meningitis may allow for early adjustment to treatment and patient management.

TABLE 20.3 Characteristic changes in cerebrospinal fluid in meningitis

	MACROSCOPIC APPEARANCE	CELLS (× 10^6/L)	PROTEIN (mg/dL)	GLUCOSE (mg/dL)	GRAM STAIN
Normal	clear and colourless	0–5	15–40	50–75	
Streptococcus pneumoniae	clear to turbid	elevated, PMN	elevated	reduced	Gram-positive diplococci
Haemophilus influenzae	clear to turbid	elevated, PMN	elevated	reduced	Gram-negative coccobacilli
Neisseria meningitidis	clear to turbid	elevated, PMN	elevated	reduced	Gram-negative diplococci
Escherichia coli	clear to turbid	elevated, PMN	elevated	reduced	Gram-negative rods
Group B streptococci	clear to turbid	elevated, PMN	elevated	reduced	Gram-positive cocci
Listeria monocytogenes	clear to faintly turbid	elevated (mainly lymphocytes)	elevated	reduced	Gram-positive coccobacillus
Mycobacterium tuberculosis	clear to faintly turbid	elevated, lymphocytes (PMN early)	markedly elevated	reduced	negative (may be seen in an acid fast stain)
Viruses	clear to faintly turbid	elevated, lymphocytes (PMN early)	slightly elevated	normal	negative
Cryptococcus neoformans	clear to faintly turbid	elevated, PMN or lymphocytes	elevated	reduced	yeast cells

PMN = polymorphonuclear leucocytes

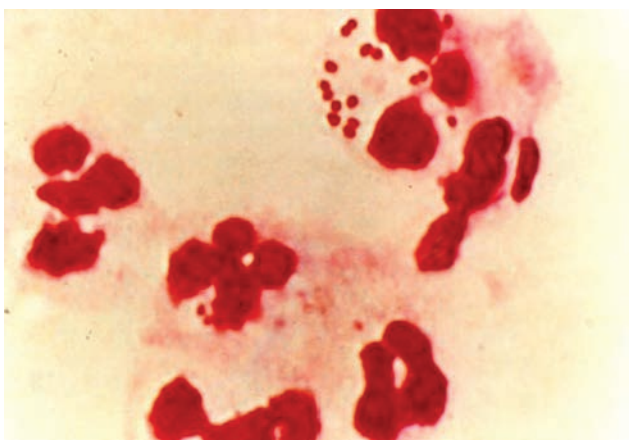

FIGURE 20.5

Gram stain of CSF from a patient with meningococcal meningitis

This Gram stain shows Gram-negative diplococci inside of polymorphonuclear leukocytes.

Source: CDC.

Obvious turbidity in a CSF specimen is suggestive of bacterial infection, and is usually due to the presence of large numbers of white cells and/or bacteria. The differential cell count typically shows polymorphonuclear leucocytes in acute bacterial meningitis. Bacteria may be detected in a Gram stain of CSF, but a negative Gram stain does not exclude bacterial meningitis. A reduction in the CSF glucose level is due to impaired transport of glucose from blood to CSF and its usage by the bacteria and the infiltrating polymorphs. There is usually a significant increase in the protein concentration because of alteration to the blood–brain barrier, resulting in an increased permeability to serum proteins which are normally excluded.

Antibiotic treatment of meningitis should not be delayed, so specimen collection often follows commencement of therapy. Once therapy is started, the ability to isolate bacteria from the CSF is rapidly reduced. The recent development of polymerase chain reaction (PCR) tests on CSF or blood has helped to overcome this problem. For example, meningococcal DNA can be detected in CSF for up to 72 hours after commencement of antibiotic therapy. Rapid latex agglutination tests for the detection of bacterial antigens in CSF, serum or urine have been developed and are increasingly being used for diagnosis of the major causes of bacterial meningitis. The advantage of these tests is also their theoretical capability of detecting bacterial antigens in CSF collected after antimicrobial therapy has commenced. The sensitivity of PCR and antigen detection tests is variable, so a negative result by one of these methods does not necessarily exclude bacterial meningitis.

If tuberculous meningitis is suspected, a Ziehl-Neelsen stain of centrifuged deposit of CSF is performed.

Treatment

Prompt treatment of any bacterial meningitis is required. Empirical therapy for acute meningitis is most often with ceftriaxone or cefotaxime plus vancomycin or ampicillin.

The third-generation cephalosporins are preferred because (1) they are effective against the three principal causes of bacterial meningitis—*N. meningitidis*, *H. influenzae* and *S. pneumoniae*—and (2) therapeutic levels can be reached in the CSF. It should be remembered that many substances, including antibiotics, do not cross the blood–brain barrier very well. The third-generation cephalosporins are highly potent and seem to enter the CSF in adequate concentration.

Therapy may be modified following Gram-stain findings and again once the identity of the organism and its susceptibility pattern is known. Benzylpenicillin is usually the drug of choice for *N. meningitidis* and *S. pneumoniae*, although some resistant strains have appeared in recent years, in which case cephalosporins would be used. Cephalosporins are usually maintained if *H. influenzae* is identified and found to be susceptible. Group B streptococcal infections are usually treated with penicillin, ampicillin or cephalosporins. The treatment of choice for *Listeria* meningitis is ampicillin or penicillin plus an aminoglycoside. Standard anti-tuberculosis drugs (see Chapter 17) are used for tuberculous meningitis.

Because the inflammatory response is at least partially the cause of the injury to the CNS in acute bacterial meningitis, the use of anti-inflammatory agents has been suggested as an adjunct to antibiotic treatment. Experimental and clinical trials have shown that anti-inflammatory agents, such as the corticosteroid, dexamethasone, can diminish the CSF inflammatory response and the subsequent oedema, increased CSF pressure and neurologic sequelae. However, dexamethasone is not recommended for neonates with meningitis. Overall, the routine use of anti-inflammatory drugs in acute bacterial meningitis remains controversial.

Prevention

There is an increased attack rate of *N. meningitidis* in close contacts of infected individuals, due mainly to higher rates of nasopharyngeal carriage. Antibiotic prophylaxis is therefore recommended in some settings. Household or intimate contacts may be given rifampicin, a quinolone or ceftriaxone to eliminate carriage and, therefore, the risk of infection. To prevent neonatal infection with Group B streptococci, administration of benzylpenicillin to high-risk women during labour has been shown to be effective. Screening of women for streptococcal Group B colonisation at 35–37 weeks' gestation, by culture of vaginal and rectal swabs, is strongly recommended by health authorities.

Vaccines are available for the three major causes of bacterial meningitis. Currently, there are two pneumococcal vaccines available in Australia. Prevenar 13® is recommended for children at 2, 4 and 6 months of age, with a fourth dose recommended for medically at risk children at 12 months of age. Aboriginal and Torres Strait Islander children living in the Northern Territory, Queensland, South Australia and Western Australia should receive a supplementary dose of Prevenar 13® at 12–35 months of age plus a dose of Pneumovax 23® between the ages of 18 and 24 months. Pneumovax 23® is also recommended for all people aged over 65 years and for Aboriginal and Torres Strait Islander people over 50 years of age and those of 15–49 years of age who are at high risk of invasive pneumococcal disease. See the section on pneumococcal pneumonia in Chapter 17 for a more detailed explanation of pneumococcal vaccines.

A meningococcal serogroup C vaccine has been part of the standard childhood immunisation program since 2003. A single dose of this vaccine is recommended at 12 months of age. A tetravalent vaccine for *N. meningitidis* serotypes A, C, Y and W135 is also available. It is recommended for the control of outbreaks, for travellers to high-risk areas (e.g. Nepal and Northern India), for close contacts of cases, for asplenic patients and people with complement deficiencies, and for at-risk laboratory personnel. It is not used for routine mass vaccination mainly because of inconsistent immunogenicity at younger ages. There is no effective vaccine for serogroup B.

Conjugate Hib vaccines to prevent *H. influenzae* infections have been available in Australia since 1992, and have been part of the standard childhood vaccination schedule since 1993. This provides a dramatic example of the potential success of good vaccines, with Hib infection rates decreasing by more than 90 per cent since introduction of this vaccine (see Figure 20.3, page 513). The National Health and Medical Research Council (NHMRC) recommends vaccination of all Australian children with PRP-OMP (purified Hib polysaccharide conjugated to meningococcal carrier protein) vaccine at 2 and 4 months, with a booster at 12 months.

The meningococcal and *H. influenzae* vaccines have the added advantage of reducing colonisation and carriage of these organisms. Thus, they also reduce the risk of infection for non-vaccinated individuals.

Viral meningitis

Overall, viral meningitis is the most common type of meningitis. Most of these infections are caused by enteroviruses (echoviruses, coxsackie viruses) (see Figure 20.6). The

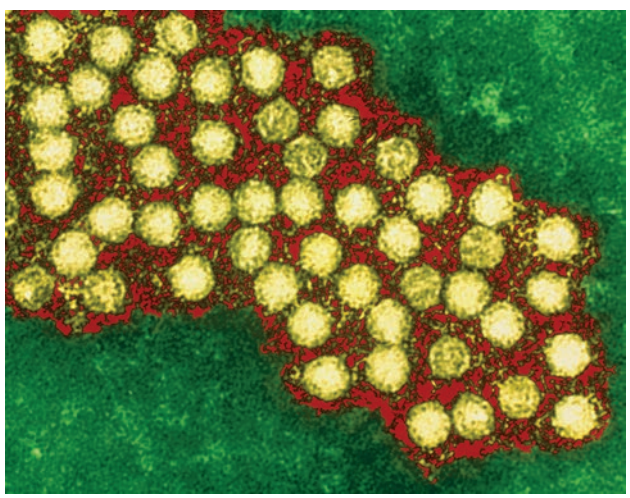

FIGURE 20.6

Electron micrograph of an enterovirus

Source: Dr Linda Stannard, UCT/Science Photo Library.

majority of people infected with enteroviruses are asymptomatic or have only minor symptoms of a cold, rash or mouth sores, with low-grade fever. Only a small proportion develop meningitis. Other possible causes include adenoviruses, herpes simplex viruses, the mumps virus and varicella zoster virus. The viruses usually enter the body via the gastrointestinal or respiratory route and, after local multiplication, may enter the bloodstream. Viraemia gives the viruses the opportunity to cross the blood–brain barrier and enter the subarachnoid space. As with bacterial meningitis, the incidence of viral meningitis is greatest in infants and young children. The immunocompromised are also at increased risk of viral meningitis.

Viral meningitis is sometimes referred to as **aseptic meningitis**, a general term for any meningitis where bacteria are not isolated by routine culture. Apart from viruses, which are the predominant cause, there are many other possible causes of aseptic meningitis, including bacteria (e.g. fastidious or unusual types), fungi, protozoa and non-infectious causes. Examples of causes of aseptic meningitis are listed in Table 20.4.

Viral meningitis is generally milder and progresses more slowly than bacterial infections. It is usually manifested by acute onset of headache, mild photophobia, low-grade fever, vomiting, general malaise and minor neck stiffness. This often follows upper respiratory symptoms, gastrointestinal symptoms, or both. The symptoms usually last 7–10 days, followed by complete recovery. Loss of alertness or normal mental status is not typical of viral meningitis but is suggestive of more serious disease, such as bacterial meningitis.

A distinction between viral and bacterial meningitis cannot always be made on clinical grounds. Laboratory examination of CSF in a case of viral meningitis generally shows a moderately increased white cell count with a predominance of mononuclear cells (lymphocytes), although early in infection polymorphs may predominate, mimicking acute bacterial infection. The protein concentration in CSF may be normal or elevated, but is usually lower than in bacterial meningitis. The CSF glucose is usually within normal limits. The characteristic laboratory findings for bacterial and viral infections are compared in Table 20.3 (page 517).

TABLE 20.4 Possible causes of aseptic meningitis

CATEGORY	EXAMPLES
Viruses	Echoviruses Coxsackievirus A Coxsackievirus B Poliovirus Adenoviruses Herpes simplex virus
Bacteria	Partially treated bacterial meningitis *Listeria monocytogenes* *Mycobacterium tuberculosis* *Treponema pallidum*
Parasites	*Ancanthamoeba* spp. *Angiostrongylus cantonensis* *Strongyloides stercoralis*
Fungi	*Cryptococcus neoformans* *Aspergillus* spp. *Candida* spp.
Non-infectious causes	Malignancy Autoimmune disease Chemical poisoning

Laboratory diagnosis of viral meningitis is difficult but can be sometimes achieved by virus isolation, antigen detection or microscopy of CSF. Although virus isolation is successful in some cases, it can take up to ten days. Antigen detection and microscopy methods generally have a low sensitivity. A definitive diagnosis can be made by the demonstration of a fourfold rise in antibody titre in acute and convalescent sera. However, this can take three weeks or more and is therefore of limited clinical value. Nucleic acid amplification techniques (e.g. PCR) have been developed for some meningitis viruses, including enteroviruses and herpes simplex viruses. These methods have much higher sensitivity than other diagnostic tests and thus are likely to be further developed and increasingly used. Often, however, no specific cause is identified.

Aseptic meningitis: warning on eating raw slugs

SPOTLIGHT ON

On 13 May 2010 the NSW Health Department issued a warning to people about the dangers of eating raw slugs. This followed the report of a 21-year-old man who ate a slug as a dare, and who was fighting for his life as a result. One of the variety of parasites carried by slugs and snails is *Angiostrongylus cantonensis* (also called rat lung worm). The parasite is found mainly in Asia and the Pacific Islands. It is rare for people in Australia to get infected, although a number of cases have been reported, including a fatal case involving a child. Infection with this worm can sometimes cause a life-threatening meningitis. It can take a person months to fully recover from the infection, even with medical care.

The adult form of the worm infects rodents, which pass worm larvae in their faeces which can be eaten by snails and slugs. New South Wales Health warned that not only should raw slugs or snails not be eaten, but that hands should be washed after touching them. The disease can also be acquired by eating raw, unwashed vegetables or fruit that have been contaminated by snails or slugs.

CASE HISTORY 20.3

Outbreak of viral meningitis

An outbreak of aseptic meningitis due to echovirus 30 occurred in the Wingecarribee Shire during October to November 1994. Thirty people had clinical presentations that were fairly typical of viral meningitis. Most patients had fever, headache and meningism. The onset was often short (a few hours) and patients were often debilitated, requiring admission to hospital. Other systemic features of myalgia and arthralgia were also typical.

There was no obvious geographical clustering of cases. They were distributed throughout Wingecarribee Shire, although the majority lived in the Mittagong postal area. The occurrence of some person-to-person transmission was suggested by the three clinical cases in one family and a healthcare worker who became ill after nursing a case. However, the other cases were not epidemiologically linked to a confirmed case, suggesting other means of transmission.

Source: Adapted from I. Gosbell et al. 2000, Outbreak of echovirus 30 meningitis in Wingecarribee Shire, New South Wales. *Communicable Diseases Intelligence* 24(5): 121–24.

Questions

1. What is the most likely way by which the healthcare worker became infected in this case?
2. What laboratory findings are suggestive of a viral meningitis?
3. What treatment would you expect to be provided for people involved in this outbreak?
4. How does the prognosis for viral meningitis compare with that for bacterial meningitis?

Therapy for uncomplicated viral meningitis usually involves symptomatic treatment only, although antibacterial drugs are normally given until a non-bacterial cause is confirmed. Herpes simplex meningitis may be treated with aciclovir. Complications such as chronic fatigue syndrome, chronic muscle weakness and ataxia may occur following viral meningitis, but are rare.

Fungal infections

Very few fungi infect the central nervous system of humans. They include *Coccidioides immitis*, *Histoplasma capsulatum* and *Cryptococcus neoformans*. Except for *Cryptococcus*, these organisms are rarely seen in Australia. Cryptococcal meningitis has become increasingly common in recent years and is most often found in people whose immune system has been compromised in some way.

Cryptococcal meningitis

Prior to the AIDS epidemic, cryptococcal meningitis was a relatively rare disease. It was usually associated with exposure to pigeon droppings and occurred in some immunosuppressed people. Today, it is far more familiar. It is now one of the diseases routinely anticipated in patients with AIDS and is also more common in other people because of the increasing use of corticosteroid therapy and immunosuppressive agents.

Cryptococcus is a yeast-like fungus with spherical cells. The cells reproduce by budding and have a thick polysaccharide capsule (see Figure 20.7), which is at least partly responsible for its virulence. The genus comprises over 50 species, but only *C. neoformans* and *C. gattii* are believed to be human pathogens. *C. neoformans* is distributed worldwide, and is found in soil and in the droppings of pigeons and other avian species, including chickens, parrots and canaries. It mostly causes disease in immunocompromised individuals. *C. gattii*, on the other hand, has a more restricted distribution in tropical and subtropical parts of the world, including parts of Australia, the US, South America and South-East Asia. Most cases of *C. gattii* are from Australia. It is associated with the bark, flowers and other parts of a number of species of eucalypt and other tree species. It has also been isolated from the dung and under the claws of some Australian native animals (e.g. koalas, possums and echidnas), which may act as secondary reservoirs. *C. gattii* also differs

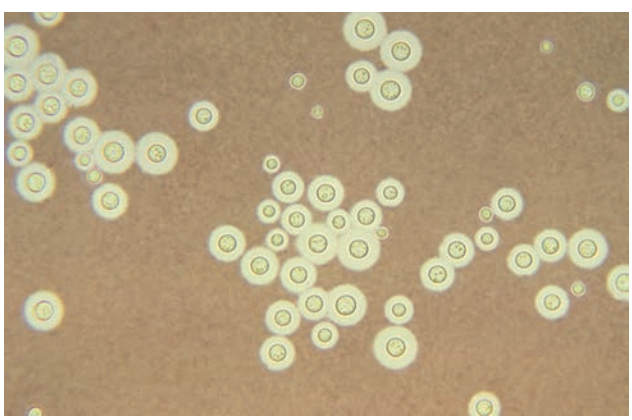

FIGURE 20.7

India ink stain of *Cryptococcus neoformans* showing the large capsule around the cell

Source: © The University of Adelaide. Reproduced with permission.

from *C. neoformans* in that it usually affects individuals with no immune impairment.

Infection caused by Cryptococcal species is called **cryptococcosis**. Humans generally become infected by inhalation of the organism, which then causes a primary infection in the alveoli. In people with normal defences, the disease is often contained within the lungs and is subclinical and regresses spontaneously. However, in *C. neoformans* infections in immunocompromised people and in a small proportion of infections involving *C. gattii*, the organism invades the bloodstream and spreads to other parts of the body, including the brain and meninges. This leads to a subacute or chronic type of meningitis with a slow onset (one or more weeks) that is often fatal if untreated, and with up to 20 per cent mortality, even with antimicrobial therapy. Complications, such as hydrocephalus, visual disturbances, hearing loss and seizures, may occur. Human-to-human transmission of cryptococcosis has not been demonstrated.

The typical preliminary CSF findings in cryptococcal meningitis are shown in Table 20.3. If cryptococcal meningitis is suspected, an India ink stain of centrifuged deposit may be performed. This stain allows the yeast cell and its characteristic large capsule to be seen. Confirmation is by culture of CSF and blood, or by a rapid latex agglutination test which can detect cryptococcal antigen in the CSF. A point-of-care test has recently been developed and may prove useful in resource-poor regions of the world. The standard treatment is with amphotericin B plus flucytosine for two weeks, followed by fluconazole for at least eight weeks.

Protozoal infections

Few protozoa infect the central nervous system. The notable one in Australia and other Western countries is *Toxoplasma gondii*. Amoebic meningitis caused by *Naegleria fowleri* is a rare disease that occasionally occurs in Australia. The flagellate *Trypanosoma brucei* is the cause of African trypanosomiasis, or sleeping sickness, which affects many thousands of people each year, mainly in Central and East Africa.

Toxoplasma infection of the CNS

Toxoplasma gondii has for many years been recognised as an important pathogen in congenital infection, but its importance has increased markedly since the AIDS epidemic struck. Cerebral toxoplasmosis is one of the more common neurological complications of HIV infection. The protozoan often produces multiple, well-demarcated lesions throughout the cerebral hemispheres. The clinical manifestations are highly variable and include headache, fever, clumsiness to hemiplegia, seizures, ataxia and cognitive changes. Toxoplasmosis is usually diagnosed in the laboratory by serological methods or by PCR testing on body fluids (e.g. CSF, blood, urine) or biopsy material.

Treatment is with pyrimethamine plus sulfadiazine (or clindamycin) and folinic acid.

Encephalitis

Encephalitis is almost always caused by viruses, with the herpes simplex virus and certain arboviruses (arthropod-borne viruses) being the most common. Other potential causes of encephalitis include rabies virus (discussed in a later section), varicella zoster virus (chickenpox and shingles virus) and cytomegalovirus, especially in immunocompromised individuals. Different encephalitis-causing arboviruses occur in different parts of the world (see Table 20.5). Each arbovirus has certain animal reservoirs and is transmitted by a particular arthropod vector. Arbovirus encephalitis in Australia (formerly called Australian encephalitis) is caused by two mosquito-borne flaviviruses—the Murray Valley encephalitis virus and, occasionally, the Kunjin virus. These two viruses mainly infect wild birds, and possibly other wild and domestic animals. Mosquitoes of the species *Culex annulirostris* become infected when they feed on these birds or animals, and then transmit the infection by biting a human.

Encephalitis may also occur as a rare complication of certain systemic viral infections (e.g. measles, mumps, cytomegalovirus) or, very rarely, after vaccination with some live attenuated vaccines (e.g. measles, mumps, rubella).

TABLE 20.5 Arthropod-borne encephalitis viruses

VIRUS	ARTHROPOD VECTOR	GEOGRAPHIC DISTRIBUTION
West Nile encephalitis	Mosquito	Africa, Asia, Europe, United States
Eastern equine encephalitis	Mosquito	United States
St Louis encephalitis	Mosquito	United States, Jamaica
Venezuelan equine encephalitis	Mosquito	United States, South America
Japanese encephalitis	Mosquito	Asia
Louping-ill	Tick	United Kingdom
Russian spring-summer encephalitis	Tick	Europe, Asia
Chandipura virus encephalitis	Sandfly	India
Murray Valley encephalitis	Mosquito	Australia, New Guinea
Kunjin	Mosquito	Australia, New Guinea

The characteristic manifestations of viral encephalitis represent cerebral dysfunction and include abnormal behaviour, seizures and altered consciousness. Fever, vomiting and nausea are also common. These symptoms reflect necrosis of neurones.

Murray Valley encephalitis

Murray Valley encephalitis (**MVE**) is a severe epidemic-type disease. The MVE virus is considered to be endemic in northern Australia (the Kimberley region of Western Australia and the Top End of the Northern Territory). The MVE virus is believed responsible for severe epidemics in 1917, 1918, 1922 and 1925. It was then known as Australian 'X' disease. The most recent major epidemic occurred in 1974, with a total of 58 cases occurring in all mainland states, resulting in 13 deaths. From 2004 to 2009, only ten cases were notified throughout Australia. The pattern of disease appears to be outbreaks a decade or so apart, with low numbers of cases between them. The primary hosts of MVE virus are water birds, which maintain infection in mosquitoes.

Following the 1974 epidemic, a number of sentinel chicken flocks were established by state health authorities. Currently, flocks are maintained in Western Australia, the Northern Territory, New South Wales, Victoria and northern Queensland. The birds in these flocks are regularly tested serologically for antibodies to the MVE and Kunjin viruses, to provide an early warning of any increase in virus activity.

Most infections with MVE virus are subclinical. However, occasionally the virus crosses the blood–brain barrier and causes damage to the brain tissue. The major presenting symptoms may include fever, headache, convulsions, coordination and speech difficulties, or loss of consciousness. The case fatality rate can be as high as 40 per cent. Residual neurological impairment, including spastic quadriparesis and facial nerve palsy, can occur in some survivors.

MVE is diagnosed by isolation of the virus from a clinical specimen, detection of MVE RNA by PCR in a clinical specimen or by serological evidence: the presence of IgM antibody in serum or CSF, or a fourfold rise in IgG antibody titre. There is no specific therapy for encephalitis caused by arboviruses, making prevention of infection very important. Australian health departments have contingency plans for disease prevention should virus activity, as indicated by sentinel flocks, be found to be increasing to dangerous levels. Although vaccines have been developed for some other encephalitis arboviruses (e.g. Japanese encephalitis), there is not yet one for MVE.

Kunjin virus disease

Kunjin virus is a flavivirus found in mainland Australia and Papua New Guinea. It is closely related to West Nile virus. The vast majority of Kunjin virus infections are asymptomatic, but a small number of people develop mild illness with fever, enlarged lymph nodes, rash, swollen and aching joints, headache, muscle weakness and fatigue. Some people with Kunjin virus disease may develop encephalitis, which may require hospitalisation. From 2004 to 2009, 14 cases of Kunjin virus infection were notified in Australia. Laboratory diagnosis is based on demonstration of a rising antibody titre on paired serum samples. There is no specific treatment available for Kunjin virus disease.

CASE HISTORY 20.4

Murray Valley encephalitis

On 15 February 2001, a 3-year-old boy was admitted to Mt Isa Base Hospital with a two-day history of an acute febrile illness and convulsions. He remained febrile over the following two days, had further brief seizures and then developed a left hemiparesis, agitation, confusion and subsequently became comatose. His CSF showed a white cell count of 200 x 10^6/L (39 per cent mononuclear cells). A provisional diagnosis of acute encephalitis was made, and he was commenced on broad-spectrum antibiotics and aciclovir.

Tests on sera showed a rise in antibody titre to Murray Valley encephalitis (MVE) virus between two specimens collected on days 4 and 6 of illness.

Two months after the onset he had persisting major neurological sequelae, and remained semi-comatose with a spastic quadriplegia.

During the presumed exposure period, the child had been resident in Mt Isa. On two occasions he and his family had visited Lake Moondarra, a popular recreational lake in the area. The boy's parents did not recall him being bitten by mosquitoes during the exposure period, although they had seen mosquitoes near their home and had seen other people applying insect repellent while at Lake Moondarra.

Source: Adapted from S. Hills 2001, Murray Valley encephalitis in Mt Isa, North Queensland. *Communicable Diseases Intelligence* 25(2), April.

Questions

1. What specific treatment would be effective in this case?
2. Why is this referred to as an arbovirus infection?
3. How is Murray Valley encephalitis controlled in Australia?
4. What are the risks for a healthcare worker attending to a patient with Murray Valley encephalitis?

Japanese encephalitis

Japanese encephalitis is caused by a flavivirus spread by mosquitoes. Its natural hosts are certain wading birds and pigs. Japanese encephalitis is a significant problem in some parts of Asia (e.g. India, Sri Lanka, China, Thailand) and since 1995 has occurred in low incidence in the Torres Strait Islands. In 1998 the first ever case was reported on mainland

Australia, on the west coast of Cape York. To date there have been fewer than ten documented cases of Japanese encephalitis in Australia.

Most infections are asymptomatic but, when symptomatic, are characterised by headache, fever, convulsions, lowered consciousness and coma, with a high case fatality rate (20–50 per cent). There is also a high incidence (up to 50 per cent) of neurological sequelae in those who survive the acute illness. The infection may be diagnosed in the laboratory by isolation of the virus or detection of viral RNA in clinical material, but antibody detection in CSF or serum is the usual method for diagnosis. There is no specific therapy for Japanese encephalitis, so patient management involves supportive measures. A vaccine is available for Japanese encephalitis and is currently recommended by Australian health authorities for travellers spending a month or more in rural areas of Asia or western Papua New Guinea, travellers spending a year or more in Asia (except Singapore), residents of the outer islands of the Torres Strait, and non-residents who will be on the outer islands of the Torres Strait for 30 days or more during the wet season. The Australian Quarantine and Inspection Service maintains herds of sentinel pigs in Torres Strait, Cape York and the Northern Territory to detect Japanese encephalitis virus activity.

Herpes encephalitis

Herpes simplex virus type 1 is most often associated with cold sores. Very rarely, the virus reactivates in the trigeminal ganglia, where it resides latently, and ascends into the brain. The spread of herpes simplex virus type 2 to the brain is even less common. **Herpes encephalitis** is a very severe disease that causes neurological damage with a high mortality (70 per cent if untreated, and 20 per cent in treated cases). Early treatment with aciclovir greatly reduces the mortality and permanent neurological damage. The early diagnosis of herpes encephalitis is therefore important because of the potential for effective treatment.

Diagnosis is usually made by computed tomographic (CT) or magnetic resonance imaging (MRI) scan and EEG. Polymerase chain reaction (PCR) on a CSF specimen provides conclusive evidence, but false negatives may occur. Enzyme immunoassays for IgM antibodies in CSF or serum may also aid in the diagnosis.

Other viral causes of encephalitis

A number of other viruses are capable of causing encephalitis in humans. West Nile virus is distributed across Africa, Asia, the United States and southern Europe. The natural hosts of the virus are birds, being transmitted between birds and to humans by mosquitoes. Although West Nile virus usually causes a relatively mild illness that only occasionally includes encephalitis, it has been responsible for some large outbreaks of encephalitis in southern Europe and Russia. A significant outbreak occurred in the United States in 2002, involving almost 4000 cases and causing over 200 deaths.

The Nipah virus can cause pneumonitis and encephalitis in humans. It has caused outbreaks of encephalitis in Malaysia and Singapore. The natural hosts are bats. Pigs are infected by contact with bat faeces or urine, and then humans are secondarily infected by droplet transmission, initially from infected pigs, and then from infected humans.

Dengue virus occasionally causes encephalitis, although it is more often associated with a systemic infection that does not involve the brain. Enterovirus 71, a cause of hand, foot and mouth disease, is also an occasional cause of encephalitis. Dengue fever and hand, foot and mouth disease are described in Chapter 19.

OTHER INFECTIONS INVOLVING THE NERVOUS SYSTEM

Tetanus

Tetanus was first described by the ancient Egyptians in around 3000 BC. It is now a rare, but serious, illness in developed countries, but much more common in underdeveloped areas of the world. **Tetanus** is caused by the bacterium *Clostridium tetani*, a strictly anaerobic, spore-forming, Gram-positive rod (see Figure 20.8). Its spores are widespread in soil and manure, because the organism is part of the bowel flora of various animals and some humans. Farmers have been found to carry tetanus spores on their skin. The spores are extremely resistant to drying, disinfectants and heat (they are resistant to boiling for 20 minutes) and can survive in soil for years.

The rarity of tetanus in developed countries is due to the development and widespread use of the tetanus vaccine. The highest incidence is often in the elderly because of inadequate immunisation. In Australia, fewer than ten cases are notified

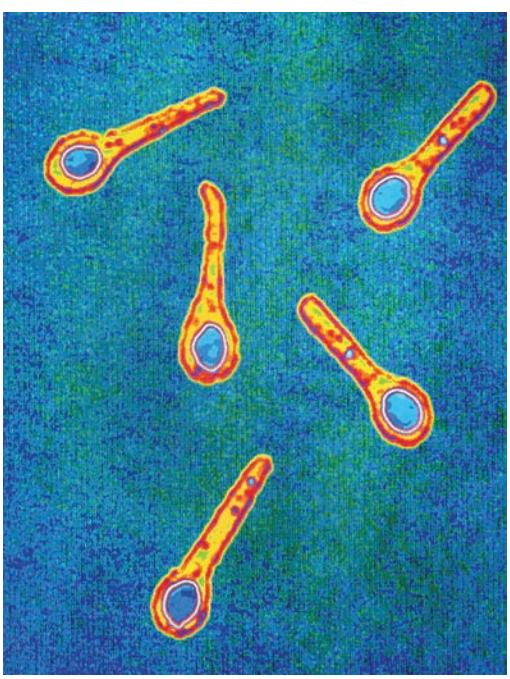

FIGURE 20.8

Electron micrograph of *Clostridium tetani* showing intracellular spores

Source: Pasieka/Science Photo Library.

annually. Worldwide, there are an estimated 1 million cases annually, resulting in around 50 000 fatalities.

Wounds contaminated with faeces or soil can become infected with *C. tetani*. Usually, the spores must be deposited into a deep, necrotic wound where oxygen is lacking, allowing growth of this strict anaerobe to occur. However, many cases of tetanus have occurred following only minor injuries, such as thorns or splinters acquired during gardening. In some developing countries, a high incidence in neonates is associated with the use of contaminated knives to cut the umbilical cord, or the use of mud to pack the umbilical stump to soothe the infant. Maternal tetanus, associated with aseptic obstetric practices, is also a problem in such countries. Tetanus is not spread from person to person.

Spores enter a wound and, when bacterial growth and multiplication occur, one of the most potent toxins ever identified is produced. The neurotoxin travels along peripheral nerves to the CNS where it blocks the release of transmitters, especially in inhibitory neurones. This causes an overactivity of motor neurones. Sympathetic nerves may also be affected, leading to a similar overactivity in the sympathetic nervous system.

After an incubation period of 3–21 days, muscle rigidity and uncontrolled muscle spasms occur. Uncontrolled contraction of the powerful masseter (jaw) muscles is common, leading to the classic 'lockjaw', or trismus. Contraction of the facial muscles may occur, causing the sneering appearance termed 'risus sardonicus'. Generalised contraction of the muscles of the back, neck, abdomen, arms and legs can give the whole body a stiff, ramrod appearance. In some cases, back muscle contraction may be sufficiently severe to cause the patient's back to arch and even to crush the spinal processes (see Figure 20.9). Patients experience severe pain. Death generally results from respiratory failure due to spasm of the respiratory muscles. Elevated body temperature and blood pressure, tachycardia and sweating may be present, due to the effects of the toxin on the sympathetic nervous system.

Diagnosis of tetanus is by clinical appraisal since the organism can rarely be isolated. Treatment of tetanus is difficult. Human tetanus immunoglobulin should be given immediately tetanus is suspected. The wound should be thoroughly cleansed and all devitalised tissue removed, and metronidazole given to prevent bacterial replication. Muscle relaxants may be used and, in severe cases, ventilatory support may be necessary. Despite treatment, the mortality rate can still be as high as 30 per cent.

Prevention of tetanus involves (1) immunisation and (2) prophylaxis after a tetanus-prone injury is sustained. Tetanus can be effectively prevented if full immunisation is followed. Immunisation is with tetanus toxoid vaccine. It is one of the safest vaccines in current use and is nearly 100 per cent effective. The NHMRC currently recommends that all people be immunised against tetanus at the ages of 2, 4 and

FIGURE 20.9

A painting of a man dying of the final stages of tetanus

Note the arched back and the sneering appearance of 'risus sardonicus', due to severe muscle spasm of the back and face, respectively.

Source: Royal College of Surgeons, Edinburgh.

6 months, with further booster doses at 4 years and 15–17 years. People aged 50 years who have not had a booster in the last ten years should receive one.

Certain types of wounds, termed 'tetanus-prone wounds', are likely to favour the growth of tetanus organisms. These include compound fractures, deep penetrating wounds, wounds with extensive tissue damage (e.g. burns), and any wound contaminated with soil, dust or horse manure. For tetanus-prone wounds there are guidelines for the use of tetanus toxoid and tetanus immunoglobulin as prophylactic measures (summarised in Table 20.6).

Botulism

Botulism is an acute neurologic disorder that has three different clinical courses: food-borne botulism, wound botulism and infant botulism. All three forms are caused by the anaerobic, spore-forming, Gram-positive rod *Clostridium botulinum*. Generally, the disease is rare in developed countries.

Spores of the organism are widespread in soil and are often found contaminating meat and vegetables. Spores can also be found in dust. The spores are very resistant to heat, being able to survive boiling for several hours, but are destroyed by standard sterilising processes.

Classically, food-borne botulism has been associated with the consumption of canned or preserved foods that have not been adequately sterilised. The foods usually implicated are those preserved at home, when appropriate sterilisation procedures have not been followed. The spores germinate inside the can or bottle during storage and the botulinum toxin is released. The ingestion of preformed toxin results in disease. It is absorbed in the intestine, causing gastrointestinal symptoms, and then enters the bloodstream. The botulinum toxin acts on peripheral nerve synapses, blocking the release of acetylcholine. The toxin paralyses the muscles in a relaxed state (flaccid paralysis). This neurotoxin is one of the most potent of all natural toxins—1 μg is sufficient to kill ten people. It is colourless, tasteless and odourless, and thus is not noticed when eating contaminated food.

Wound botulism results when a wound is contaminated with *C. botulinum* spores. This usually occurs when a traumatic wound is contaminated with soil. After an incubation period of 4–14 days, symptoms similar to those of food-borne botulism develop, except for the gastrointestinal effects.

Infant botulism affects children less than 1 year old. Unlike the food-borne form which involves the ingestion of preformed toxin in food, infant botulism involves the ingestion of *C. botulinum* cells or spores. Normally, the bacterium and its spores are unable to compete successfully with the intestinal flora, so their ingestion does not result in disease. But infants are more susceptible because their flora is not fully established. Such cases may be related to the consumption of honey (spores are picked up from flowers and plants by bees) or ingestion of soil. But in the majority of cases the source is unknown.

There are eight different serological types of toxin, which differ in potency. Types A, B and E are the most common causes of human disease, and A and B are the most potent.

Symptoms of food-borne botulism usually begin within 2–3 days of consumption of the contaminated food. Visual disturbances (e.g. double vision) and dysphagia (pain or difficulty in swallowing) are common early symptoms; as the disease progresses, muscles lower down the body become affected. Muscles in the trunk and limbs are weakened and then paralysed. Eventually, patients may have difficulty breathing and may die from respiratory failure due to dysfunction of respiratory muscles.

Diagnosis is mainly by clinical appraisal, and sometimes confirmed by culture or toxin assays of appropriate specimens. Possible specimens, depending on the type of infection, include serum, faeces, wound tissue and food material. Supportive care (e.g. ventilatory support, intubation of airways, cardiac support) is the most important aspect of patient management. Botulinum antitoxin should be administered urgently, if available. Antibiotics are not recommended, except in wound botulism.

A purified and diluted preparation of neurotoxin A of *C. botulinum*, called Botox, is now extensively used as a pharmaceutical agent. It has been used with moderate success in the treatment of disease conditions in which muscles are overactive or in spasm (e.g. in certain eye disorders, some stroke patients, carpel tunnel syndrome). It also has a very popular cosmetic use—as a beauty treatment. When injected into muscles of the face, it paralyses them and thereby smoothes away wrinkles. It nevertheless is a potent neurotoxin and thus should be used with care.

TABLE 20.6 National Health and Medical Research Council (NHMRC) guide to tetanus prophylaxis in wound management

HISTORY OF ACTIVE IMMUNISATION	TIME SINCE LAST DOSE	TYPE OF WOUND	TETANUS TOXOID	TETANUS IMMUNOGLOBULIN
Uncertain, or less than three doses		Clean, minor wound	Yes	No
		All other wounds	Yes	Yes
Three doses or more	less than 5 years	All wounds	No	No
	5–10 years	Clean, minor wound	No	No
		All other wounds	Yes	No
	more than 10 years	All wounds	Yes	No

Leprosy

Leprosy has been known since biblical times. It was a feared disease and, from the 6th century, spread through Europe, leading to the establishment of many isolated leper hospitals and colonies. It is no longer as feared as in the past but remains an important health problem in the world. The prevalence of leprosy has declined substantially over the last 25 years, largely as a result of the implementation of a multi-drug therapy regime recommended by the World Health Organization in 1981, and the provision by the WHO, since 1995, of free multi-drug therapy for all patients in the world. In 1985 there were an estimated 5.2 million people with leprosy worldwide. The global prevalence at the beginning of 2011 was estimated by the WHO to be under 200 000 cases. Leprosy has been eliminated from 119 countries where the disease was considered a major problem. However, pockets of high endemicity still remain in nine countries: Angola, Brazil, Central African Republic, Democratic Republic of Congo, India, Madagascar, Mozambique, Nepal, and the United Republic of Tanzania.

In Australia there are fewer than 20 notifications per year. Most of these infections are in migrants from endemic countries with occasional locally acquired cases in Indigenous communities (see Figure 20.10). In New Zealand, fewer than ten cases per year are generally notified.

Leprosy is a chronic infection caused by the acid-fast bacillus *Mycobacterium leprae*. The bacterium was first identified by Gerhard Armauer Hansen in 1873; hence the alternative name for leprosy is **Hansen's disease**. It has not been possible to culture the organism *in vitro*, so the study of it has been difficult. The disease appears to be confined to humans, so it is always acquired from another person. The organism is present in large numbers in the nasal secretions of people with severe disease and is possibly transmitted by nasal droplets followed by uptake through the respiratory mucosa. Skin-to-skin contact is not considered to be an important route. Transmission is enhanced by overcrowding and poor hygiene. However, the precise mechanism(s) of transmission are still not known. The disease has a long incubation period—usually 2–5 years but sometimes 20 years or more. Infected people may be shedding organisms for years before the onset of symptoms, making control in endemic regions very difficult. However, contrary to earlier belief, leprosy is not a highly contagious disease, requiring prolonged and close contact for transmission.

M. leprae is an obligate intracellular parasite that resides in histiocytes, endothelial cells, and Schwann cells in peripheral nerves. Its invasion of Schwann cells and the reduced myelin production by infected cells are the bases for the characteristic

CASE HISTORY 20.5

Leprosy

A 23-year-old male, born in North Queensland and of previous good health, presented to his general practitioner with a 12-month history of rash. He had been treated with topical antifungal and corticosteroid creams by other medical practitioners with no effect.

Examination showed numerous, asymmetrically distributed pink-to-brown plaques on the trunk and limbs, some with central healing. Ziehl-Neelson staining of skin biopsies showed numerous acid-fast bacilli. A diagnosis of borderline Hansen's disease was made.

A review of historical data on Hansen's disease in Queensland showed that the man's grandfather had been incarcerated on Peel Island (the Queensland leprosarium) from 1943 to 1949 and again from 1954 to 1958. It was also noted that in 1982 the grandfather had suffered a clinical relapse. Treatment, including rifampicin, was recommended, but smears were still strongly positive shortly before his death in 1984 at the age of 80.

The current patient was 10 years old when his grandfather died. He had had intermittent, usually short, contact with his grandfather. It was assumed that the grandfather was the source of disease presenting in the grandson 13 years later. The patient was treated with standard multi-drug therapy.

Source: H. Archibald, P.F. Fitzpatrick and G.H. Rée 1999, Locally acquired Hansen's disease in North Queensland. *Medical Journal of Australia* 170: 72.

Questions

1. What does this case demonstrate about the incubation period of leprosy?
2. Why does therapy for leprosy involve administration of multiple drugs?

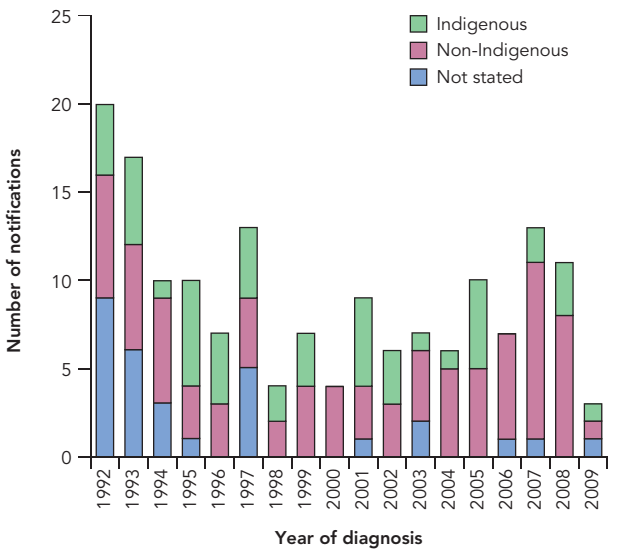

FIGURE 20.10

Notifications of leprosy in Australia, 1992–2009

Source: NNDSS Annual Report Writing Group, Australia's Notifiable Disease Status, 2009: Annual Report of the National Notifiable Diseases Surveillance System, June 2011, *Communicable Diseases Intelligence* 35(2): 121. Reproduced with permission of the Australian Government.

nerve damage in the disease. The organism grows extremely slowly, which helps to explain the incubation period of several years or more in human disease. The organism grows best at temperatures below 37°C, and thus has a predilection for cooler parts of the body such as the skin and peripheral structures such as the fingers, ears and nose.

Leprosy can develop with a spectrum of clinical manifestations. In **tuberculoid leprosy**, a strong cell-mediated immune response limits the multiplication of the organism and the disease is confined to patches of skin and certain nerve trunks. This form is characterised by blotchy skin lesions, which may be red or hypopigmented, and areas on the face, trunk and extremities which may have lost sensitivity (e.g. to a pinprick) because of damage to nerves and nerve endings.

Lepromatous leprosy is at the other end of the clinical spectrum and is due to the absence of cellular immunity to the organism. In this form, there is uncontrolled proliferation of the bacteria, which results in extensive skin lesions and nerve involvement. The skin lesions may eventually become very large and nodular in appearance. There is progressive destruction of bones in the hands, feet (see Figure 20.11) and nose. With the loss of local sensation, victims suffer frequent physical trauma to extremities which become infected with other organisms, causing further deformity. Blindness occurs in around 5 per cent of people with severe disease.

Between these two ends of the spectrum, there are intermediate forms referred to as borderline tuberculoid leprosy, borderline leprosy and borderline lepromatous leprosy. The less developed the cell-mediated immune response, the more severe the disease.

For practical and treatment purposes, the WHO recommends a simpler classification scheme of two types:

- paucibacillary (≤5 lesions)
- multibacillary (>5 lesions).

Diagnosis of leprosy is usually based on clinical grounds—that is, the finding of anaesthetic skin lesions (lesions with a loss of sensation) and thickened peripheral nerves. Laboratory confirmation is rarely required, but may be provided by the demonstration of acid-fast bacilli in skin smears or biopsies. The current treatment recommendations of the WHO are rifampicin plus dapsone for six months for paucibacillary leprosy, and rifampicin plus dapsone and clofazimine for 12 months for multibacillary leprosy. The multidrug approach is necessary to prevent the development of resistance in the bacterium. Prevention of leprosy is largely based on early diagnosis and treatment of cases.

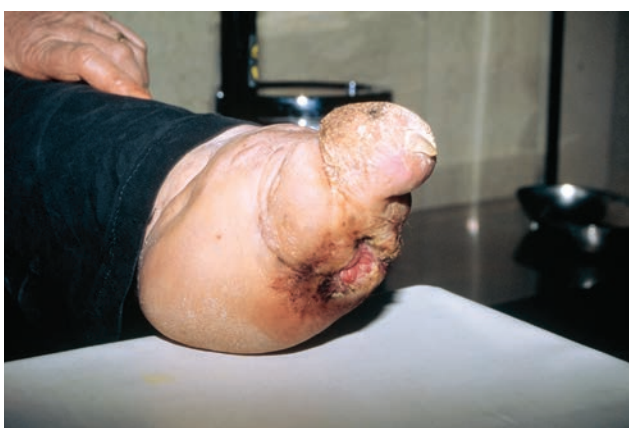

FIGURE 20.11

Leprosy

In this case a severe destruction of bones in the feet has occurred.

Source: A. Crump, TDR, WHO/Science Photo Library.

Rabies and Australian bat lyssavirus infection

Rabies ('rage' or 'madness' in Latin) has been the object of fear and fascination for thousands of years, with written accounts in ancient books of Mesopotamia and Babylon dating back to around 2300 BC. The disease often conjures up visions of a gentle, obedient pet suddenly becoming ferocious and drooling, or a human victim dying a horrible death in a state of delirium and rage.

Rabies is an encephalitis caused by a large, enveloped, bullet-shaped virus with single-stranded RNA, belonging to the genus Lyssavirus. It can infect almost all warm-blooded animals and is primarily a disease of animals. Most human cases are due to dog bites in countries where canine rabies is endemic. In some countries, other wild animals—including monkeys, foxes, bats and raccoons—are potential sources.

Rabies

SPOTLIGHT ON

A conservation catastrophe in India has become a human tragedy.

Since the 1990s, numbers of long-billed, slender-billed and oriental white-backed vultures have declined at an unprecedented rate. The cause is a veterinary drug called diclofenac, which was routinely given to cattle. When the cattle died, vultures that fed on their carcasses were poisoned by the drug. As vulture numbers crashed, the population of feral dogs across India surged, feasting upon cattle carcasses that would otherwise have been stripped bare by birds. Many of these dogs carry rabies. Scientists in the UK and India, led by Anil Markandya of the University of Bath, UK, calculated that the decline of vultures made way for at least 5.5 million extra feral dogs in India between 1992 and 2006. These extra dogs would have been responsible for at least 38.5 million bites. Based on national data of rabies cases related to dog bites, the researchers suggested that at least 47 000 people may have died as a result of the vulture die-off.

Source: Adapted from M. Walker 2008, Poisoned vultures clear the way for a plague of rabies. *New Scientist* 199(2668): 14.

Rabies occurs in many countries and is endemic in much of Africa, Asia, the Americas and Europe. The Global Alliance for Rabies Control (GARC) estimates that around 70 000 people throughout the world die each year from rabies, although this is likely to be a significant underestimate due to under-reporting and misdiagnosis. Recent increases in incidence in rabies deaths have raised concerns that it is re-emerging as a serious health issue. A number of countries are free of endemic rabies, including Australia and New Zealand, predominantly due to strict quarantine laws and, in some cases, animal vaccination programs. The occasional case of rabies identified in Australia results from infection overseas in endemic countries.

The rabies virus is usually acquired by the bite of an infected animal or, rarely, by exposure of mucous membranes or skin lesions to infected saliva or other secretions (e.g. urine). Once in the body, the virus infects local muscle cells where it may lie dormant for an extended period. The virus replicates in the muscle fibres and then travels via peripheral nerves and the spinal cord to the brain, causing an encephalitis. From there it can spread via nerves to a wide variety of sites, such as the salivary glands, respiratory tract, skin and cornea.

The incubation period varies considerably. The longest well-documented period is around two years, but in most cases it is usually 1–3 months. Clinical illness indicates the arrival of the virus in the CNS. The initial symptoms are headache, fever and partial paralysis at the bite site. One of two forms of the disease can follow. People with furious rabies exhibit signs of muscle spasms, hyperactivity, excited behaviour, hypersalivation, hydrophobia (fear of water) and hallucinations. After a few days, death occurs due to cardiorespiratory arrest. Paralytic rabies occurs in about a third of cases. This form has a less dramatic and usually longer course. The muscles gradually become paralysed, starting at the site of the bite or scratch, and the patient slowly becomes comatose. Both forms of the disease are almost always fatal once symptoms have developed.

Diagnosis of rabies can be made by detection of viral antigens in skin biopsy specimens using a microscopic immunofluorescence technique, by PCR testing for viral RNA in skin biopsy, saliva or CSF, or by examination of serum or CSF for specific antibodies. However, these tests generally do not detect infection until after the onset of symptoms, making them of limited clinical value.

Post-exposure prophylactic treatment is effective if given soon after (within a few days) suspected exposure, and must be before symptoms develop. These preventative measures are based on:

- prompt and thorough cleaning of the wound (even minor scratches and abrasions) with soap and water for a minimum of 15 minutes, followed by application of an antiviral antiseptic (e.g. 70 per cent ethanol or povidone-iodine)
- commencement of rabies vaccination regimen
- intramuscular injection of human rabies immunoglobulin if open wounds or contamination of a mucous membrane occurred
- thorough infiltration of open wounds with rabies immunoglobulin.

The most effective strategy for preventing rabies is vaccination of animals, especially dogs, in endemic countries. This has resulted in a reduction of human cases in several countries. Safe, effective human vaccines are available, but routine immunisation for Australians is not necessary. However, all workers and travellers who are at high risk of contracting rabies in an endemic area should be immunised, as should health professionals caring for infected patients.

The **Australian bat lyssavirus**, discovered in 1996, is related to the rabies virus and causes a similar clinical disease. It has been found to infect most types of Australian

CASE HISTORY 20.6

Rabies

On 21 April, a man aged 58 years began experiencing mild chest pain and went to the local hospital emergency department. ECG and blood tests did not support the initial provisional diagnosis of acute myocardial infarction. He was discharged, but on the next day he returned to the emergency department with panic attacks and anxiety when drinking water. When questioned, he reported that he had been bitten by a bat on the right hand about five weeks earlier. He was immediately given rabies immunoglobulin and a dose of rabies vaccine, and admitted for observation.

The next day (23 April) the man complained of continuing chest pain and numbness of his lower right arm. It was noticed that he became anxious when he tried to drink fluids offered to him. Serum, CSF, a skin biopsy and saliva were collected and sent to a reference laboratory, which confirmed the diagnosis of rabies by detection of viral RNA in the skin biopsy and saliva by reverse transcription–polymerase chain reaction (RT–PCR).

Despite supportive treatment for hypotension, bradycardia and increased intracranial pressure, his condition worsened over the next few days, and he died of heart failure on 30 April.

Questions

1. What early signs and symptoms did this man have that are typical of rabies infection?
2. Why was he given both rabies immunoglobulin and rabies vaccine, and what is the likely reason for their lack of effectiveness?
3. Would this man's infection have posed any risk for healthcare workers attending to him?
4. How is rabies prevented on a country-wide basis?

FIGURE 20.12

A red flying fox *Pteropus scapulatus*. Flying foxes are a potential source of the Australian bat lyssavirus

Rabies vaccine and immunoglobulin also protect against Australian bat lyssavirus infection, so the recommended treatment and preventive measures are similar for both diseases.

Source: Dr Raina Plowright.

FIGURE 20.13

A typical polio victim shown on an ancient Egyptian stone slab, circa 1580–1350 BC

Source: © Bettmann/Corbis.

fruit bats (flying foxes—see Figure 20.12) and insectivorous bats. However, transmission to humans occurs only rarely (one fatal case in 1996 and another in 1998).

Polio

Polio (poliomyelitis) has been known for thousands of years and was once a very common disease. Figure 20.13 shows a typical polio victim recorded on an ancient Egyptian stone slab from the 18th dynasty (1580–1350 BC). It is caused by the poliovirus, a single-stranded RNA virus, of which there are three distinct serotypes. Humans are the only known reservoir of the virus.

In 1988 the WHO launched the Global Polio Eradication Initiative with a view to eradicating the disease from the world. The program has been based on immunisation, surveillance and targeting of high-risk areas. As a result, there has been a progressive fall globally in the reported incidence of polio from an estimated 350 000 cases in 1988 to under 1500 cases in 2010. Wild poliovirus is now considered endemic in only three countries: Afghanistan, Pakistan and Nigeria. However, even in countries considered free of polio, it is still essential that population immunity be maintained at a high level as there is always the potential risk of an imported case, who could then infect unimmunised local children. An outbreak in China in 2011 resulting from importation from Pakistan demonstrates this risk. The last notified case of wild-type poliomyelitis in Australia was in 2007, and before that in 1978. The 2007 case was imported, occurring in a 22-year-old male student who had arrived from Pakistan in July 2007.

Vaccine-associated polio results from the use of the live, attenuated, oral polio vaccine (Sabin vaccine). Rarely, in about one case per million vaccinated children, the vaccine virus reverts to a virulent form and causes disease—called 'vaccine-associated paralytic poliomyelitis'. And more importantly, it is now recognised that vaccine-derived polioviruses that have become virulent can spread in poorly immunised populations and cause polio outbreaks. Vaccine-associated cases were reported in Australia in 1986 and 1995.

Poliovirus is excreted in the faeces of infected people and is usually transmitted via food, water or hands. It is a fairly stable virus which can remain infectious for long periods outside the body (e.g. in food or water). In the early stages of infection, it may also be present in the nose and throat and thus be transmitted by the airborne route.

After initial local multiplication in the intestine or pharynx, the virus reaches the bloodstream. The majority of cases are asymptomatic (90 per cent) or have only mild symptoms of headache, sore throat, fever and nausea. Around 1–2 per cent of people develop the paralytic form, in which the virus invades the CNS where it infects neurones in the spinal cord and brain. High fever, back or neck pain and muscle spasms are the early symptoms, and then hours to days later a flaccid paralysis occurs. One or more limbs may be affected, usually the legs, and some patients also experience difficulties in talking, swallowing or breathing.

Some patients with paralysed respiratory muscles used to be restricted to an 'iron lung' (a predecessor of modern ventilatory equipment) for the rest of their lives. Some paralysis may disappear, but 1 in 200 infections leads to permanent paralysis, usually in the legs (see Figure 20.14).

The diagnosis of polio is based on clinical appraisal. Laboratory confirmation involves the isolation and identification

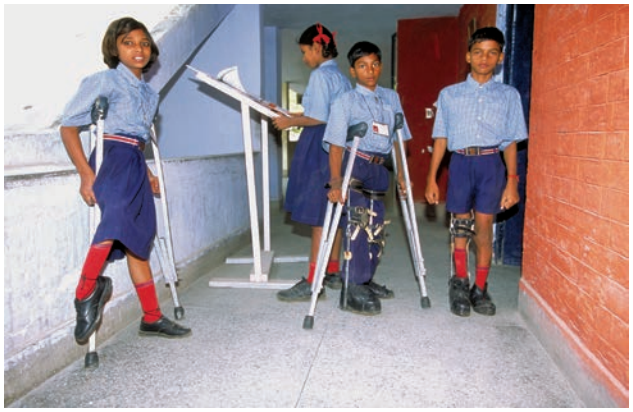

FIGURE 20.14

Polio victims in India

Source: World Health Organization.

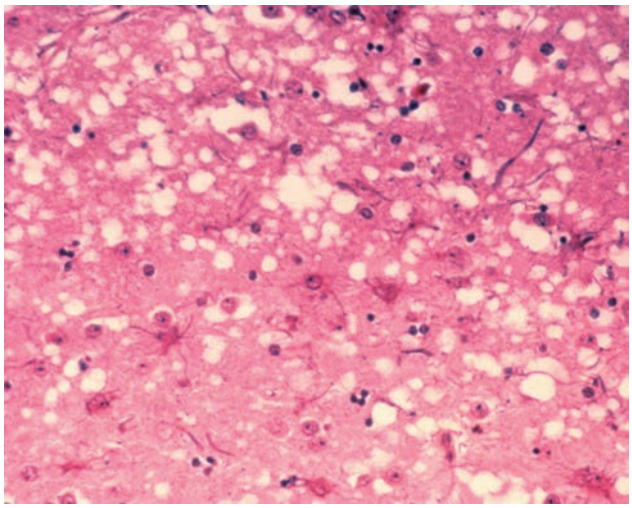

FIGURE 20.15

A section of brain tissue of a cow with BSE (a prion disease of cows)

Source: Dr Al Jenny, US Dept of Agriculture—Animal and Plant Health Inspection Service, APHIS.

of the virus in tissue culture from faeces or a pharyngeal swab. There is no cure for polio.

Post-polio syndrome is a term used to describe a condition that affects some people who have recovered from paralytic polio many years earlier. The symptoms, which typically appear 30–40 years later, are characterised by new and progressive muscle weakness, debilitating fatigue and loss of function, and pain in muscles and joints.

The first vaccine available for polio was the **Salk vaccine** (IPV—inactivated poliomyelitis vaccine) in 1955. It contains formalin-inactivated viruses and is 70–90 per cent effective. It is administered by intramuscular injection. In the early 1960s, the more effective (nearly 100 per cent) **Sabin vaccine** (OPV—oral poliomyelitis vaccine) was introduced. It contains attenuated, live viruses and is more effective because, unlike IPV, it induces a strong IgA antibody response at mucosal surfaces where infection first occurs. The OPV has the added advantage of oral administration, as well as providing a longer-lasting immunity if the correct number of doses is given. However, there is considerable debate about whether the IPV or the OPV should be used in mass immunisation campaigns, even given the greater efficacy and ease of administration of the latter. The debate is mainly due to the possibility of vaccine-associated paralytic poliomyelitis occurring in a recipient of OPV, and for this virulent virus to then circulate and possibly cause outbreaks of polio. The WHO currently supports the use of OPV, especially in countries with continued circulation of wild-type polio virus. However, many developed countries now use IPV.

IPV is part of the standard childhood vaccination schedule in Australia and is recommended for use at 2, 4 and 6 months, with a further booster dose at 4 years of age. OPV is no longer used in Australia.

Transmissible spongiform encephalopathies (prion diseases)

Transmissible spongiform encephalopathies (TSEs) are a group of infectious, neurodegenerative diseases in humans and animals. They are so called because transmission from human to human and animal to human is possible, and the diseases cause damage to the brain tissue that produces microscopic vacuoles, giving it a spongy appearance in histological sections (see Figure 20.15). These diseases typically have long incubation periods of months to years. Prion diseases are currently considered to be invariably fatal. There is no effective therapy presently known; however, some promising progress has been made and a number of potential therapeutic approaches are currently being investigated.

These diseases are believed to be due to an intracellular accumulation of an abnormal form of a normal protein found on the surface of many cells, especially neurons. The infectious protein, PrP, is referred to as a **prion**. The normal cellular isoform of the protein, PrP^{c}, is believed to be converted into an abnormal, misfolded, isoform, referred to as PrP^{sc} in affected brain cells. The normal function of PrP^{c} has not been elucidated. After entering a host cell, PrP^{sc} appears to act as a template causing conversion of PrP^{c} to aggregates of PrP^{sc}. The amino acid sequences of PrP^{c} and PrP^{sc} are identical, but their three-dimensional structure is different. Furthermore, different strains of prion proteins are known to exist, the strain differences related to the varied ways the prion protein can be folded. How the propagation of PrP^{sc} causes damage to brain cells is not known.

Human prion diseases can arise in three distinct ways: sporadic, inherited or acquired. Sporadic disease occurs in very low incidence throughout the world (annual incidence of one per million people). In sporadic diseases the abnormal PrP^{sc} protein is spontaneously generated. The inherited disease is the type found in people with a family history of TSE and is due to a mutation in the gene that codes for PrP^{c}. Acquired prion diseases are caused by the transmission of the infectious agent PrP^{sc} transcutaneously (e.g. during

medical or surgical procedures) or orally (by ingestion of contaminated material).

These infectious agents have a number of unusual properties, including an apparent lack of nucleic acid and a resistance to ultraviolet light, heat and certain chemical agents. Even years of storage in formaldehyde does not destroy their infectivity. Another remarkable feature of prions is that they do not appear to induce a classical inflammatory response or a specific immune response, normally the hallmarks of infection.

A number of different diseases of animals and humans have been attributed to prions. Scrapie is a prion disease in sheep and goats in which infected animals itch and scrape themselves against fences and posts, often until they bleed. Bovine spongiform encephalopathy (BSE), or 'mad cow disease' (discussed further below), has possibly affected more than 4 million cattle in the United Kingdom since 1986 and threatened the beef industry there.

Creutzfeldt-Jakob disease and variant Creutzfeld-Jakob disease are the most important of the human prion diseases and are discussed in the following sections. The other human prion disease that has been studied extensively is **kuru**. This disease has been found only in people of the Fore tribes in the highlands of Papua New Guinea. Kuru occurred in about one in ten people in these tribes and was found to be transmitted from person to person by their cannibalistic practices. Their ritual required the consumption of parts of the body of a dead member of the family who, given the incidence of disease, was likely to have died from kuru. No cases of kuru have been seen in those born after 1957, when the practice of cannibalism ceased. D. Carleton Gadjusek, an American scientist, won the Nobel Prize in 1976 for his work on this disease.

Gertsmann-Straussler-Scheinker syndrome is a rare human prion disease with an estimated incidence of less than two cases per 100 million people. This disease is mainly an inherited disorder. Fatal familial insomnia is also an exotic disease of humans attributed to prions.

Creutzfeldt-Jakob disease

Hans Gerhard Creutzfeldt first described a progressive dementing illness in a 22-year-old woman in 1920, and in the following year Alfons Maria Jakob described four similar cases. Worldwide, there is thought to be approximately one new case of **Creutzfeldt-Jakob disease (CJD)** per 1–2 million people each year.

CJD mainly affects people in the 50–75 years age group. The majority of cases (about 85 per cent) are considered to be of the sporadic type, in which there is a spontaneous change to the prion protein or to the gene that codes for it. About 10 per cent of cases appear to be inherited and the remainder are due to transmission of infectious material. Transmission from human to human has been shown to occur through the transfer of infected tissue or body fluids, or via contaminated surgical instruments. Iatrogenic transmission of CJD in treatments involving pituitary hormones and dura mater grafts, and via corneal transplants and neurosurgical equipment has been reported. The use of pituitary-derived growth hormone and gonadotrophin, collected and pooled from cadaveric pituitary glands in the 1980s, was responsible for more than 140 cases of CJD in the United States, the United Kingdom, France, New Zealand and Australia. There is no evidence that CJD is transmissible by blood or blood products, although it is theoretically possible.

The Department of Health and Ageing established the Australian National Creutzfeldt-Jakob Disease Registry (ANCJDR) in October 1993. From 1993 to 31 March 2010 there were 866 definite, probable or possible cases of CJD reported to the ANCJDR (see Table 20.7). This includes an average of 24.5 definite and probable CJD deaths per year.

There is no evidence to suggest that CJD is transmitted during normal social contact or sexual contact, from mother to foetus, or via food. A number of reports of CJD in health-care workers (including neurosurgeons, doctors, dentists, nurses and histopathology technicians) suggests the possibility of workplace infection, although precise evidence of this has not been demonstrated in most of these cases.

From iatrogenic infections it has been established that, when the agent is introduced into or near the brain, the incubation period is in the order of months, but in peripheral transmission the incubation period may be years or even decades. The dominant features of CJD are muscle weakness and dementia. Early symptoms may include loss of balance and muscle coordination, slurred speech, failing vision and myoclonic (quick, jerky) movements of the extremities or head. The symptoms become progressively more severe, leading to dementia and a variety of other neurological disturbances such as dysphasia, spasticity and seizures in

TABLE 20.7 Cases of CJD on the Australian National Creutzfeldt-Jakob Disease Registry to 31 March 2010

CLASSIFICATION	SPORADIC	FAMILIAL	IATROGENIC	UNCLASSIFIED	TOTAL
Definite	366	42	5	0	413
Probable	203	10	4	0	217
Possible	11	0	1	0	12
Incomplete	0	0	0	224	224
Total	580	52	10	224	866

Source: Adapted from G.M. Klug et al. 2010, Surveillance of Creutzfeldt-Jakob disease in Australia: 2010 update. *Communicable Diseases Intelligence* 32(2): 96–101. Reproduced with permission of the Australian Government.

a period of only months. Myoclonic jerking movements are the most frequent physical sign. The patient eventually becomes comatose. The disease is uniformly fatal, usually in less than a year from the onset of symptoms.

A definitive diagnosis of CJD is made at autopsy with the demonstration of spongy neuropathological changes and the demonstration of PrP^{sc} deposits in brain tissue. A pre-mortem presumptive diagnosis of CJD can be made with reasonable accuracy based on clinical evidence, together with EEG and MRI scans and examination of CSF for specific proteins.

Because prions are unusually resistant to heat and many chemical disinfectants, specific methods for sterilisation and disinfection are followed when contamination with these agents is suspected. Local or organisational guidelines should be followed. Typical guidelines specify:

- incineration of disposable instruments and clinical waste
- autoclaving of instruments for reuse at 134°C for 3–18 minutes
- immersion of heat-sensitive instruments in 5 per cent sodium hypochlorite solution or 1M NaOH for one hour
- disinfection of surfaces with 5 per cent sodium hypochlorite or 1M NaOH, allowed to stand for one hour.

Variant Creutzfeld-Jakob disease

Variant Creutzfeld-Jakob disease (**vCJD**) was first described in the United Kingdom in 1996, associated with the consumption of meat from cattle with **mad cow disease** (**bovine spongiform encephalopathy—BSE**). As of June 2012, 176 people in the UK had been diagnosed with definite or probable vCJD, all of whom have died.

The BSE epidemic began in the UK in 1986 and is thought to have originated from the similar disease in sheep known as scrapie. It is considered likely that the disease crossed the species barrier as a result of the practice of using sheep carcasses to produce protein-rich supplements for cattle feed. The height of the epidemic was in 1993, when 1000 new cases per month were being identified. In the next two to three years the epidemic was largely brought under control by the banning of feed containing cattle or sheep products and by the slaughter of 200 000 diseased cattle and 4.5 million asymptomatic cattle (due to the anticipated long incubation period). BSE had by then had a crippling effect on the British livestock industry and other industries reliant on bovine-derived products (e.g. gelatin and pharmaceutical industries). BSE had also spread to other European countries, presumably due to their importation of live animals or livestock food supplements from the UK. Australia and New Zealand are considered to be free of scrapie and BSE.

FIGURE 20.16

Mad cow disease

Source: Dr Art Davis, US Dept of Agriculture—Animal and Plant Health Inspection Service, APHIS.

An infected animal typically loses weight, has a depressed appearance with lowered head and arched back, and suffers increasing difficulty in walking, eventually being unable to stand (see Figure 20.16).

The first cases of vCJD in the UK were observed in 1995. Mortalities peaked in 2000 with 28 deaths.

Variant CJD is a clinical syndrome that is somewhat different from classical CJD:

- vCJD occurs in much younger people (mean 28 years) than is seen in the classical form (mean 62 years)
- vCJD tends to have a longer duration of illness (average 14 months) than classical CJD (average 8 months)
- the histological changes in the brain tissue are more common in vCJD.

There is evidence that vCJD may be transmitted by transfusion of blood or blood products. As a result, a number of countries, including Australia, have banned blood donation from anyone who visited the UK for a cumulative period of six months or more between 1980 and 1996.

The diagnosis of vCJD is similar to that of classic CJD, although the clinical criteria for a presumptive diagnosis are somewhat different. A prototype blood test for the diagnosis of vCJD has recently been reported, and holds promise as a means for assessing therapeutic approaches as well as for the screening of blood and tissue donors.

SUMMARY

- The brain and the spinal cord are surrounded by three membranes, or meninges.
- Cerebrospinal fluid (CSF) circulates in the space between the two inner meninges.
- The blood–brain barrier consists of the tightly joined endothelial cells of the brain's capillaries.
- Meningitis is an infection and inflammation of the meninges, most often caused by viruses or bacteria.
- Encephalitis is an infection and inflammation of the brain and is almost always caused by viruses.

INFECTIONS OF THE CENTRAL NERVOUS SYSTEM

Bacterial meningitis

- *Haemophilus influenza* was a major cause of meningitis until an effective vaccine became available in 1992.
- Most cases of meningococcal meningitis (*N. meningitidis*) occur in the 0–4 years age group.
- Pneumococcal meningitis (*Streptococcus pneumoniae*) usually follows septicaemia or head injury.
- Neonatal meningitis is caused mainly by *E. coli* (capsular type K1) or *Streptococcus agalactiae*.
- *Listeria monocytogenes* mainly causes meningitis in neonates or immunocompromised people.
- *Mycobacterium tuberculosis* can cause meningitis by entering the bloodstream and disseminating to the meninges.
- The tissue dysfunction in bacterial meningitis results from focal lesions due to bacterial growth or toxins, and the host inflammatory response.
- Headache, fever, altered consciousness, vomiting and neck stiffness are the common presenting symptoms of bacterial meningitis.
- The mortality rate in bacterial meningitis varies from 5–50 per cent.
- Up to 10 per cent of those who survive bacterial meningitis experience serious long-term sequelae.
- CSF is the most important specimen for the diagnosis of meningitis. Blood cultures should also be collected.
- Prompt treatment of any bacterial meningitis is required.
- Vaccines are available for some causes of bacterial meningitis.

Viral meningitis

- The major causes of viral meningitis are enteroviruses.
- The incidence of viral meningitis is greatest in infants, young children and the immunocompromised.
- Viral meningitis is generally milder and progresses more slowly than bacterial infections.
- A definitive diagnosis of viral meningitis is usually made by culture of CSF or serology.
- Therapy for viral meningitis usually involves symptomatic treatment only.

Fungal infections

- Fungal meningitis is rare in Australia, except for cryptococcal meningitis.
- Humans generally become infected with *Cryptococcus neoformans* by inhalation of the organism.
- Cryptococcal meningitis is confirmed by culture of the organism and detection of cryptococcal antigen in CSF.

Protozoal infections

- Cerebral toxoplasmosis is one of the more common neurological complications of HIV infection.
- Diagnosis of toxoplasmosis is based on serological methods or PCR testing on body fluids.
- Treatment of cerebral toxoplasmosis is with pyrimethamine, sulfadiazine and folinic acid in combination.

Encephalitis

- Encephalitis is almost always caused by viruses; herpes simplex virus and arboviruses are the most common.
- Viral encephalitis is characterised by cerebral dysfunction, abnormal behaviour, seizures and altered consciousness.
- Murray Valley encephalitis (MVE) is a severe, epidemic-type disease found mostly in the northern parts of Australia.
- Most infections with the MVE are subclinical, but occasionally the virus causes damage to the brain tissue.
- MVE is diagnosed by isolation of the virus, detection of viral RNA or serological evidence.
- Japanese encephalitis is caused by a flavivirus spread by mosquitoes. It is a significant problem in parts of Asia.
- There is no specific therapy for encephalitis caused by arboviruses.
- Herpes encephalitis causes neurological damage and has a high mortality rate.
- Other viral causes of encephalitis include the West Nile virus, Nipah virus, dengue virus and enterovirus 71.

OTHER INFECTIONS INVOLVING THE NERVOUS SYSTEM

Tetanus

- Tetanus is a rare, but serious, illness caused by the bacterium *Clostridium tetani*.
- Tetanus spores are widespread in soil and manure.
- Tetanus spores usually cause infection when deposited into a deep, necrotic wound where oxygen is lacking.
- The tetanus toxin blocks the release of transmitters, resulting in an overactivity of motor neurones.
- Diagnosis of tetanus is by clinical appraisal.
- Human tetanus immune globulin should be given immediately tetanus is suspected.
- Prevention of tetanus involves immunisation and prophylaxis after a tetanus-prone injury is sustained.

Botulism

- Three types of botulism, caused by *Clostridium botulinum*, occur: food-borne, wound and infant botulism
- Spores of *C. botulinum* are widespread in soil and are often found contaminating meat and vegetables.
- The botulinum toxin acts on peripheral nerve synapses, blocking the release of acetylcholine and causing a flaccid paralysis.

- Supportive ventilatory and cardiac care is the most important aspect of patient management.

Leprosy

- Leprosy is caused by the acid-fast bacillus *Mycobacterium leprae*.
- Invasion of Schwann cells by the organism is the basis for the characteristic nerve damage in leprosy.
- Multi-drug therapy is used in the treatment of leprosy.

Rabies

- Most cases of rabies in developing countries are due to dog bites.
- Rabies virus replicates in muscle fibres and then travels to the brain, where it causes an encephalitis.
- Diagnosis of rabies is based on detection of viral antigens or RNA in skin biopsy specimens, or by detection of specific antibodies.
- The Australian bat lyssavirus is related to the rabies virus and causes a similar clinical disease.

Polio

- Poliomyelitis is caused by the polio virus.
- The polio virus produces symptoms in only about 10 per cent of infected people.
- Laboratory diagnosis of polio involves the isolation of the virus from faeces or a pharyngeal swab.
- In Australia, the inactivated polio vaccine (IPV) is preferred for mass immunisation.

Transmissible spongiform encephalopathies (prion diseases)

- Prions are transmissible agents that cause a number of fatal, neurodegenerative diseases in humans and animals.
- The prion diseases are believed to be due to an accumulation of an abnormal form of a cell protein in brain cells, called PrP (prion protein).
- Human prion diseases can occur sporadically, can be inherited or can be transmitted.
- Prions appear to lack nucleic acid and have an unusual resistance to ultraviolet light, heat and certain chemical agents.
- Creutzfeldt-Jakob disease (CJD) and variant CJD are the most important of the human prion diseases.
- Variant Creutzfeldt-Jakob disease (vCJD) is associated with consumption of meat from cattle with mad cow disease (BSE).
- Definitive diagnosis of CJD and vCJD can only be made at autopsy.
- There is no treatment for CJD or vCJD and no vaccine.

STUDY QUESTIONS

1. What is the blood–brain barrier?
2. Define the terms 'meningitis' and 'encephalitis'.
3. What are the two most common causes of neonatal meningitis, and how do these organisms reach the meninges?
4. What are the critical events in the pathogenesis of acute bacterial meningitis?
5. What are the major clinical features of acute bacterial meningitis?
6. What specimens should be collected from a patient suspected of having meningitis?
7. What preliminary laboratory results would be suggestive of a bacterial meningitis?
8. What effect has the Hib vaccine had on the incidence of bacterial meningitis in Australia?
9. What is aseptic meningitis?
10. Clinically, how does viral meningitis compare with bacterial meningitis?
11. What is the most common cause of fungal meningitis in Australia, and what are the common predisposing factors?
12. What protozoan most commonly causes infection of the central nervous system, and what are the common predisposing factors?
13. What are the causes of Australian encephalitis and how is this disease transmitted?
14. What factors usually predispose to the development of a brain abscess?
15. What is the causative agent of tetanus, and where is this organism commonly found?
16. Describe the pathogenesis of tetanus.
17. How does the treatment of tetanus differ from its prevention?
18. Name the causative agent of botulism and describe the pathogenesis of the disease.
19. Name the causative agent of leprosy and describe how the disease is transmitted.
20. Differentiate between tuberculoid leprosy and lepromatous leprosy.
21. What is rabies?
22. Describe the pathogenesis of paralytic poliomyelitis.
23. Why is polio a rare disease in Australia?
24. What is a spongiform encephalopathy? Give two examples.
25. What is a prion protein and how is it formed?

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CHAPTER

21

Infections of the urinary and reproductive systems

CHAPTER FOCUS

- What microorganisms cause urinary tract infections?
- How do urinary tract infections develop, and what forms of infection occur?
- What are the major types of infections that involve the reproductive system?
- How can sexually transmissible diseases be prevented?

INTRODUCTION

In this chapter we examine the infectious diseases of the urinary tract and reproductive system. The urinary tract is one of the more common sites of infection, especially in females. It is the most common site of hospital-acquired infection. Many infections of the genital tract are acquired during sexual activity and are therefore called **sexually transmissible infections** (**STIs**). STIs are a major public health problem throughout the world.

DEFENCES OF THE URINARY AND REPRODUCTIVE SYSTEMS

In a healthy person, the upper urethra, the urinary bladder, and the ureters and kidneys are sterile. In contrast, the lower urethra has a normal flora that usually includes *Staphylococcus epidermidis*, *Enterococcus faecalis*, corynebacteria, species of *Neisseria* and *Bacteroides*, and members of the *Enterobacteriaceae*, such as *Escherichia coli* and *Proteus*.

The upper regions of the urinary tract are normally kept free of microorganisms by a variety of defence mechanisms. One of the more critical of these is the normal flow of urine that washes over the surface of the urethral epithelium, flushing away microorganisms. The lower urethra is also cleansed by this mechanism, but tends to be recolonised quickly. Urine has several antimicrobial features, including a low pH and a low osmolarity.

In the genital tract of males, normal flora organisms are found only in the anterior urethra—that is, near the external opening. The genital tract of females—in particular, the vagina—has a very complex normal flora. This microbial population is significantly influenced by sex hormones and is thus different in neonates, pre-pubertal girls, and pre- and post-menopausal women. Its composition also varies during the menstrual cycle.

In adult females, the major residents of the vagina are the lactobacilli. These bacteria assist in maintaining the acidic pH of the vagina (around pH 4.4 to 4.6) by breaking down the glycogen in vaginal secretions with the formation of lactic acid. Thus, only microorganisms capable of growth at this pH are found in the normal flora. Apart from the lactobacilli, these include enterococci, corynebacteria, a variety of anaerobic bacteria and the yeast *Candida albicans*. In healthy females, protection against infection of the genital and urinary tracts is afforded by the low pH and the normal flora of the vagina. In situations where the pH and/or the normal flora are modified (e.g. with broad spectrum antibiotic therapy or use of spermicides), infection often ensues.

URINARY TRACT INFECTIONS

Urinary tract infections occur much more frequently in females. Around 20–50 per cent of women experience a urinary tract infection (UTI) at some time in their life, and up to 5 per cent suffer recurrent infection. In contrast, less than 5 per cent of males under 60 years of age suffer an episode of urinary tract infection, although the incidence of UTI increases markedly above that age in association with an increased incidence of prostatic hypertrophy (causing urinary obstruction). UTIs represent 30–40 per cent of all hospital-acquired infections, and are often related to urinary catheterisation. Although the majority of infections are short-lived, recurrences are possible, and severe infections can result in renal damage.

Causative organisms

The vast majority of infections are caused by microorganisms that ascend the urethra and thereby reach the bladder and sometimes the kidneys (see Figure 21.1). In patients with septicaemia, organisms may spread from the bloodstream to the kidneys to cause infection.

Ascending infections are most often caused by the enteric bacterium, *E. coli*. Other common causes include *Staphylococcus aureus*, coagulase negative staphylococci (especially *S. saprophyticus*), *Enterococcus faecalis*, *Pseudomonas*, and Enterobacteriaceae such as *Proteus*, *Klebsiella* and *Enterobacter*. These organisms are derived mainly from

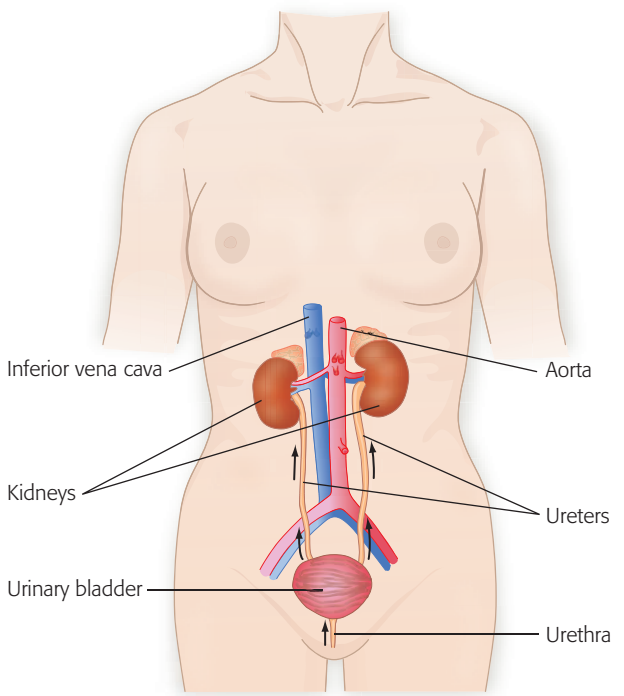


FIGURE 21.1

Organs of the urinary system (*coloured red*)

Arrows show the usual direction in which microorganisms spread.

the patient's own gastrointestinal tract. *E. coli* is the most common cause of both community-acquired and hospital-acquired UTI, but organisms such as *Proteus mirabilis*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Pseudomonas aeruginosa* have a more prominent role in hospital infections than in community-acquired infections (see Table 21.1). Hospital-acquired organisms are often multiply resistant to antibiotics and therefore difficult to treat.

Viruses are uncommon causes of UTI, but are responsible for some infections in immunocompromised patients. Strictly anaerobic bacteria, fungi (other than *Candida albicans*), protozoa and helminths are rare causes of UTI. A number of sexually transmitted organisms cause urethritis (inflammation of the urethra). These are discussed in a later section on reproductive system infections.

Pathogenesis of urinary tract infections

Host factors predisposing to infection

The much higher incidence of urinary tract infection in women is attributed to the proximity of the anus to the urethral opening and the shortness of the urethra. The proximity of the anal and urethral orifices favours the colonisation of the lower urethra with gastrointestinal flora. The short urethra means that bacteria have less distance to travel to reach the bladder and establish infection (see Figure 21.2). Good personal hygiene is important in preventing the transfer of organisms from the anus to the vagina and periurethral areas. Sexual intercourse can facilitate the movement of organisms into and up the urethra in females, explaining a higher incidence of UTI in women aged 18–30 years—associated with sexual activity and pregnancy.

Because the flushing of microorganisms from the urethra during the normal passage of urine is an important defence mechanism, any factor that prevents normal urine flow or complete bladder emptying makes the individual more susceptible to UTI. Pregnancy, renal calculi and tumours are common causes of urinary obstruction and are therefore predisposing factors of urinary tract infection. And, as stated earlier, there is a marked increase in the incidence of UTI in men above the age of 60 due to urinary obstruction caused by hypertrophy of the prostate. Loss of neurological control of the bladder leading to incomplete emptying, such as can occur in paraplegia, also makes the individual more prone to UTI.

TABLE 21.1 Common causes of urinary tract infection

CAUSE	PERCENTAGE OF CASES: COMMUNITY-ACQUIRED	PERCENTAGE OF CASES: HOSPITAL-ACQUIRED
■ *Escherichia coli*	80	40
■ Other organisms, including: *Proteus mirabilis*, *Klebsiella*, *Enterobacter*, *Pseudomonas aeruginosa*, Coagulase negative staphylococci, *Staphylococcus aureus*, *Candida albicans*	20	60

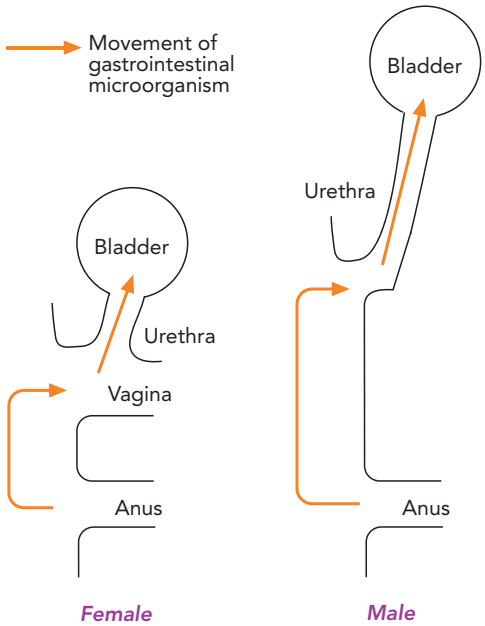


FIGURE 21.2 Susceptibility of females to urinary tract infections

Females are more susceptible than males because of the proximity of the anus to the urethra and the shorter urethra.

Urinary catheterisation

The high incidence of urinary tract infection in hospitalised people is partly due to the use of indwelling urinary catheters. Up to 25 per cent of catheterised patients develop a UTI. During insertion of the catheter, bacteria already in the urethra may be carried into the bladder or, if proper aseptic technique is not used, organisms from the hospital environment or on the hands of a staff member may be introduced. Generally, the longer a catheter is in place the greater the risk of infection, because the catheter provides several potential sites of entry for microorganisms.

The catheter also prevents the normal flushing of the urethra with urine, and bacteria are thought to be able to move upwards between the outside of the catheter and the surface of the urethra, travelling by capillary action in the thin film of mucous. An indwelling catheter increases the risk of urinary tract infection by irritating the urethral and bladder mucosa and providing a suitable surface for formation of a bacterial biofilm. As described more fully in Chapter 10, a biofilm is a collection of bacteria embedded in an extracellular matrix that they have secreted. The bacteria located deep within a biofilm on a catheter surface are protected from host defences and antibiotics.

CASE HISTORY 21.1

Urinary tract infection

An 18-year-old female presented with symptoms of left flank pain, fever, chills and increased urinary frequency. The patient said that she knew how to collect a urine specimen and did so herself. The specimen was sent to the laboratory and the results of urinalysis included:

- >50 white cells per high power field
- >10 squamous epithelial cells per high power field
- $>10^8$ *Escherichia coli* and $>10^8$ *Streptococcus faecalis* per litre.

Comment

The interpretation of these results that was included in the laboratory report stated that the patient could have a urinary tract infection, but that the specimen was probably contaminated.

Questions

1. What do the urinalysis results indicate?
2. Why are the numbers of organisms in urine quantitated?
3. Why are urinary tract infections more common in women than in men?
4. What would be the appropriate action following receipt of these laboratory findings?

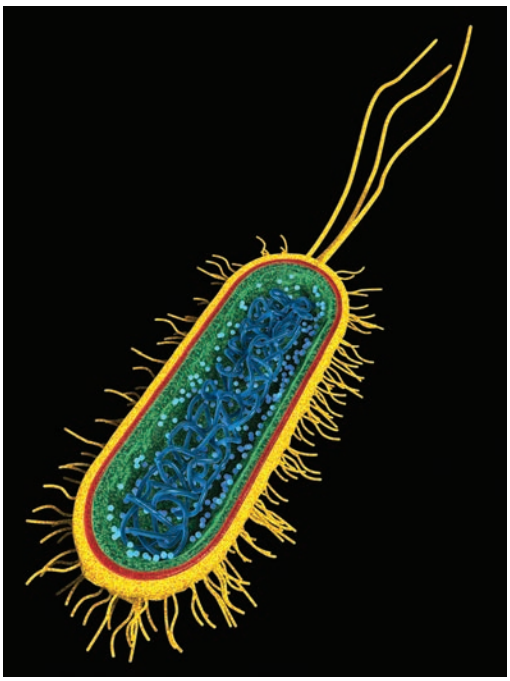


FIGURE 21.3

Computer-generated image of *E. coli*

The numerous pili (fimbriae) of E. coli *assist it to adhere to tissue surfaces in the urinary tract and hence cause infection.*

Source: Pasieka/Science Photo Library.

Bacterial virulence factors

Since *Escherichia coli* is the most common cause of UTI, the bulk of the research into virulence factors of urinary pathogens has centred on this organism. Only certain strains of *E. coli* are able to cause UTI, and this has largely been attributed to their possession of pili (see Figure 21.3). These filamentous protein structures allow bacteria to attach to surfaces (see Chapter 4)—in this case to the urethral and bladder epithelium. Non-piliated strains of *E. coli* that cause UTI are thought to have other adhesins that allow them to stick to epithelium. Adhesins have also been identified in other urinary tract pathogens.

Other virulence factors of urinary pathogens include the capsular polysaccharides of *E. coli*, which help it to evade being phagocytosed by tissue macrophages. *Proteus* and some strains of *E. coli* have an enzyme called *urease* and are thought to survive well on the outer surface of catheters by encrusting themselves with minerals released from the breakdown of urea, thereby protecting themselves from host defences.

Sites and clinical features of urinary tract infections

Infection of the urinary tract may initially be in the urethra, resulting in an inflammation of that site, called **urethritis**. As the organisms ascend the tract, subsequent infection of the bladder may occur. The bladder tissue becomes inflamed, and the patient is said to have **cystitis**. In some cases, the organisms may travel further up the urinary tract and infect the kidney(s), resulting in inflammation of one or both kidneys, which is called **pyelonephritis**.

Asymptomatic bacteriuria

It is possible for individuals to have large numbers of bacteria in their urine without displaying any symptoms. This is termed an **asymptomatic bacteriuria**. As there are no symptoms present, it is usually detected during routine screening of a person. It is thought to represent multiplication of bacteria in the bladder urine without significant involvement of tissues. In most cases, treatment is unnecessary because infection never occurs. However, in pregnant women, young children and people about to undergo instrumentation of the urinary tract there is a significant risk of infection. In such cases treatment is warranted. Treatment of asymptomatic bacteriuria in pregnant women not only reduces the risk of pyelonephritis, but also reduces the risk of premature labour and low birth weight.

Cystitis

Acute infections of the bladder are usually characterised by symptoms of:

- **dysuria**—difficult or painful (burning) urination
- **urgency**—an urgent need to urinate
- **frequency**—the need to urinate more frequently than usual.

In addition, the urine is cloudy due to the presence of large numbers of leucocytes (**pyuria**) in response to infection, and may also contain blood (**haematuria**) because of tissue damage.

Cystitis in the elderly can be difficult to diagnose because of the vague and atypical symptoms they can have. For example, urinary incontinence, lethargy or confusion may be the only obvious sign of a UTI in an older person. Recurrent cystitis can result in scarring and permanent damage to the urinary tract.

Pyelonephritis

The clinical presentation of pyelonephritis is similar to that of cystitis, although more serious. In pyelonephritis, fever and haematuria are more common, and flank pain and tenderness in the kidney region may also be present. The patient may also suffer vomiting, dehydration and hypotension. If the pyelonephritis becomes chronic, permanent damage and loss of kidney function may occur.

Laboratory diagnosis

The specimen most commonly collected for the diagnosis of UTI is a 'clean-catch' or midstream specimen of urine (MSU). Urine may be collected by other methods, depending on the circumstances—for example, suprapubic aspiration of urine from young children, a specimen from an indwelling urinary catheter, or a specimen collected during cystoscopy.

An amount of 2–50 mL should be collected into a sterile, plastic, wide-mouth container. Because urine is a good growth medium and quantitative bacterial counts are required (see below), the specimen should be transported to the laboratory without delay. The specimen may be refrigerated if a delay is unavoidable or if another specimen cannot be collected at a more appropriate time.

Patients are often started on antibiotic therapy before the results of the laboratory tests are available. Where possible, it is important that the urine specimen is collected before antibiotic therapy is initiated, so that an accurate laboratory diagnosis is obtained (see Chapter 15).

In the laboratory the urine specimen is examined microscopically and cultured. A microscopic examination of urine allows the presence of white cells, red cells, epithelial cells, casts and bacteria to be determined. The presence of any one of these does not necessarily indicate infection, but they, together with culture results, are used to build a clinical picture from which a diagnosis can be made. Most patients with symptomatic infection have pyuria, but pyuria may also be present in the absence of infection. The clinical significance of these microscopic elements is summarised in Table 21.2.

Since the lower urethra is usually colonised by bacteria, even properly collected MSU specimens may be contaminated with (non-infecting) periurethral organisms. Since some species of bacteria that colonise the lower urethra are also potential urinary pathogens, the *number* of bacteria in a urine sample is more important than their mere presence.

TABLE 21.2 The clinical significance of urinary elements found during microscopic examination of urine

ELEMENT	POSSIBLE CLINICAL SIGNIFICANCE
White cells (>4 per hpf* or $>10^8$/L)	■ bacterial infection
	■ acute glomerulonephritis
	■ bladder tumour
	■ drug therapy
Red cells (>2 per hpf)	■ infection of the kidney or bladder
	■ renal trauma
	■ renal calculi
	■ malaria
	■ strenuous exercise
	■ endocarditis
	■ carcinoma of the urinary tract
Epithelial cells (>10 per hpf)	■ poor specimen collection (i.e. not a midstream urine)
Casts (more than occasional)	■ infection
	■ inflammatory conditions of upper urinary tract
	■ many other types of renal disease

* High power field.

Therefore, infection is assessed and distinguished from contamination by quantitative culture methods.

Significant bacteriuria is usually defined as a microbial count of 10^8/L in a properly collected MSU. However, it should be recognised that, in certain situations, fewer than this number may represent infection. Less than 10^8/L can be significant in:

- some symptomatic women with cystitis
- infections caused by Gram-positive bacteria, fungi, or slow-growing or fastidious organisms
- catheter specimens
- specimens collected during cystoscopy
- specimens collected by suprapubic aspiration
- specimens from patients already receiving antibiotic therapy
- specimens from patients with high urine output (causing dilution of the bacteria).

A number of laboratory findings indicate improper specimen collection. One or more of the following in a specimen of urine casts doubt on its quality:

- greater than normal numbers of epithelial cells
- culture of more than one type of bacterium (since most UTIs are caused by a single organism)
- the presence of organisms without white cells (in some cases)
- the presence of Gram-positive rods (i.e. lactobacilli from vaginal flora).

There are also several rapid methods in use for detecting bacteriuria, such as urine dipstick. This method has low sensitivity, and therefore is useful only as a screening test.

See Chapter 15 for further information on the diagnosis of urinary tract infections.

Treatment and management

Although an uncomplicated cystitis may resolve spontaneously, appropriate antibiotic treatment reduces the duration of infection and the likelihood of progression to the upper urinary tract. Empirical therapy is usually with cotrimoxazole, nitrofurantoin or ciprofloxacin, but ultimately the choice of drug should be based on culture and sensitivity results. This is particularly important in healthcare-associated infections or recurrent infections, both of which may be caused by antibiotic-resistant organisms. Supportive measures such as the raising of the pH of the urine with urinary alkalinisers (to slow bacterial growth) and high fluid intake (to help to flush out organisms) may also be instituted. Management of catheterised patients with UTI usually includes the removal or replacement of the catheter, since it is likely to be acting as a source of infection.

Because pyelonephritis is often associated with substantial morbidity, more aggressive therapy (e.g. intravenous rather than oral antibiotics) for a longer period (e.g. ten days) is often warranted.

Prevention

The incidence of community-acquired UTI in women may be reduced by such measures as promotion of good personal hygiene, adequate fluid intake and regular emptying of the bladder. Dietary organic acids, such as derived from fruits and fruit juices (e.g. cranberry juice), may help to reduce the risk of UTI, possibly by aiding in the maintenance of a urinary pH at 5.5 or less. In women with recurrent UTIs, voiding urine after intercourse may reduce the frequency of infection. In hospitals, prevention is based on similar principles, plus avoidance/minimisation of catheterisation wherever possible, using a closed drainage system and proper catheter care.

Sample guidelines for catheter care are listed in Table 21.3.

TABLE 21.3 Procedures for the prevention of catheter-associated urinary tract infection

- Avoid catheterisation of a patient where possible.
- Catheter and associated equipment must be sterile before use.
- Wash hands thoroughly before and after inserting a catheter or other manipulation of the catheter.
- The external meatal opening should be cleansed prior to insertion of the catheter.
- The catheter must be inserted using good aseptic technique.
- The catheter should be inserted in such a way as to cause minimal damage to mucosal surfaces.
- A closed drainage system should be used wherever possible.
- The drainage bag should be kept below the level of the bladder.
- The catheter tubing should not be allowed to come loose from the collecting bag.
- The collecting bag must not be allowed to fill to the level of the inlet tube.
- Keep duration of catheterisation to a minimum.
- Use intermittent rather than continuous catheterisation where possible.

INFECTIONS OF THE REPRODUCTIVE SYSTEM

Most diseases of the reproductive (genital) system are transmitted during sexual activity and are thus referred to as **sexually transmissible infections**. They were formerly called venereal diseases, after Venus, the Roman goddess of love. It is important to recognise that a number of STIs can also be transmitted by other body fluids, especially blood and blood products (e.g. the human immunodeficiency virus and the hepatitis B virus).

Table 21.4 lists the common STIs and syndromes and their causative agents. Notice that some syndromes (e.g. urethritis, genital ulcer disease and pelvic inflammatory disease) can be caused by a number of different organisms.

TABLE 21.4 Sexually transmissible infections and their causative agents

SYNDROME	CAUSATIVE AGENTS
Gonorrhoea	*Neisseria gonorrhoeae*
Syphilis	*Treponema pallidum*
Urethritis	*Neisseria gonorrhoeae* *Chlamydia trachomatis* *Mycoplasma genitalium* *Ureaplasma urealyticum* Herpes simplex virus
Vaginitis	*Trichomonas vaginalis* *Candida albicans*
Cervicitis	*Neisseria gonorrhoeae* *Chlamydia trachomatis* Herpes simplex virus
Genital warts and carcinoma	Human papillomaviruses
Genital ulcer disease	Herpes simplex virus *Treponema pallidum* *Haemophilus ducreyi* *Chlamydia trachomatis* L1, L2, L3 *Calymmatobacterium granulomatis*
Pelvic inflammatory disease	*Neisseria gonorrhoeae* *Chlamydia trachomatis* *Mycoplasma hominis* Vaginal normal flora
AIDS	Human immunodeficiency viruses 1 and 2
Hepatitis	Hepatitis B virus

This can make the diagnosis and treatment of infection much more difficult. All of these syndromes are discussed in this chapter, except for HIV (see Chapter 19) and hepatitis B (see Chapter 18), because these two infections predominantly involve body systems other than the genital tract.

Although many of the genital infections are preventable if appropriate precautions (e.g. use of condoms) are taken, and many are readily cured with antimicrobial treatment, they still represent a major health problem throughout the world in both developed and developing countries. The World Health Organization estimates that worldwide there are almost 450 million new cases of STI each year (excluding HIV). Reasons commonly given for the continuing high incidence of STIs include:

- increased sexual activity at a younger age
- frequent partner change
- the production of few or no symptoms by some pathogens
- the social stigma of these diseases and the non-seeking of medical advice and/or notification of sexual partners
- the sexual freedom resulting from contraception
- ignorance
- the failure to use barrier preventive measures (e.g. condoms)
- the lack of effective vaccines for most STIs.

STIs are usually unevenly distributed in a population, with higher rates of infection occurring in commercial sex workers, homosexual men, adolescents and young adults, and sexual contacts of these groups. In some Indigenous communities in the northern parts of Australia there is a high rate of STI.

The numbers of cases of STIs reported by health authorities generally represent only a fraction of the actual cases, because many are not diagnosed. As you will see, in some STIs a large percentage of infected people may be asymptomatic. Furthermore, many symptomatic people do not seek medical advice, because of the social stigma associated with sexually transmissible infections.

STIs are also of major health importance because the presence of a genital infection, whether ulcer-forming or not, increases the risk of both acquisition and transmission of HIV, by as much as ten times.

Gonorrhoea

Causative organism and incidence

Gonorrhoea is an infection caused by the bacterium *Neisseria gonorrhoeae*, sometimes called the 'gonococcus'. It is named after Albert Neisser, who first described it in 1879. The disease was somewhat misnamed as gonorrhoea (after the ancient Greek *gonos* meaning 'seed', and *rhoia* meaning 'flow') because the male urethral discharge was mistaken for semen. The bacterium is a Gram-negative round or oval-shaped diplococcus with flattened sides (see Figure 21.4), so that it often has the appearance of a pair of coffee beans.

Gonorrhoea is a major global disease with estimates of around 200 million new cases annually, although infection rates in developing countries are largely unknown. In many developed countries the highest rates occur in men who have sex with men. In Australia, around 8000 notifications of gonococcal infection are received per year by the National Notifiable Diseases Surveillance System, with the highest rates of infection in the Northern Territory. The vast majority of infections occur in Indigenous Australians—

SPOTLIGHT ON

Sexually transmitted infections

'Nearly a million people acquire a sexually transmitted infection (STI), including the human immunodeficiency virus (HIV), every day. The results of infection include acute symptoms, chronic infection, and serious delayed consequences such as infertility, ectopic pregnancy, cervical cancer, and the untimely deaths of infants and adults. The presence in a person of other STIs such as syphilis, chancroid ulcers or genital herpes simplex virus infection greatly increases the risk of acquiring or transmitting HIV. New research suggests an especially potent interaction between very early HIV infection and other STIs. This interaction could account for 40 per cent or more of HIV transmissions. Despite this evidence, efforts to control the spread of STIs have lost momentum in the past five years as the focus has shifted to HIV therapies.

Prevention and control of STIs should be an integral part of comprehensive sexual and reproductive health services in order to contribute towards the attainment of the Millennium Development Goals and respond to the call for improved sexual and reproductive health as defined in the programme of action of the United Nations International Conference on Population and Development (Cairo, 1994).

The *Global strategy for the prevention and control of sexually transmitted infections: 2006–2015* has two components: technical and advocacy. The technical content of the strategy deals with methods to promote healthy sexual behaviour, protective barrier methods, effective and accessible care for STIs, and the upgrading of monitoring and evaluation of STI control programmes. The steps needed to develop health systems capacity to deliver the programme are explained. Emphasis is placed on a public health approach based on sound scientific evidence and cost-effectiveness.'

Source: Extract from the World Health Organization's *Global strategy for the prevention and control of sexually transmitted infections: 2006–2015*, p. 9.

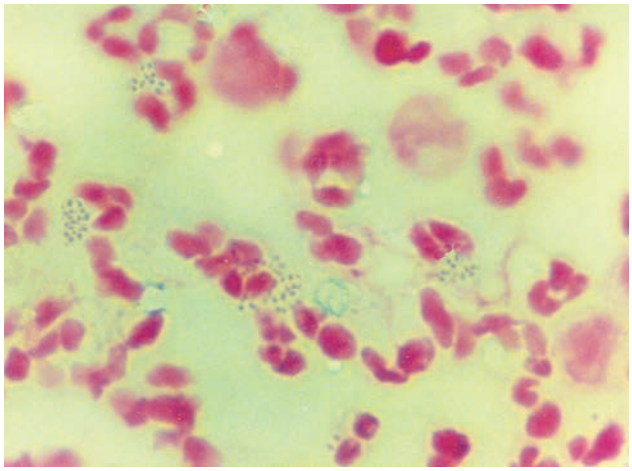


FIGURE 21.4

Neisseria gonorrhoea

Gram stain of the gonococcus in pus cells (polymorphonuclear leucocytes).

Source: Dr Gary Lee.

a ratio of Indigenous to non-Indigenous of 30:1. Highest rates of infection occur in the 15–40-year age group. These data include gonorrhoea and other forms of gonococcal infection (e.g. eye infections), but the vast majority are genital infections. There has been a gradual, increasing trend of gonococcal infection over the past decade.

Transmission

N. gonorrhoeae is sensitive to drying and survives for only a short time on dry surfaces. It can, however, survive for several hours in pus. Given its inability to survive well outside the body, it is usually transmitted by direct person-to-person contact.

The efficiency of transmission appears to be gender-dependent. It is thought that an infected male will transmit the infection 60 per cent of the time to non-infected contacts, whereas a female will transmit it only 30 per cent of the time. This difference seems to be related to the high organism load in semen. There are several high-risk transmission factors, the most important being multiple sexual partners and disregard of safe sex practices. Pharyngeal and rectal infections have become more common in both homosexuals and heterosexuals because of more varied sexual practices.

N. gonorrhoeae can also be transmitted vertically from an infected mother to baby at birth. This usually results in an eye infection referred to as **ophthalmia neonatorum** (see Chapter 16). While this infection has been controlled in many Western countries through the use of antibiotics or silver nitrate eye-drops, it remains a major problem in developing countries.

Pathogenesis

Once the gonococci are introduced into the vagina, or the urethra of males, they attach by way of pili to the mucosal cells. This attachment prevents them from being washed away by the flow of urine or secretions. The bacteria multiply rapidly and spread up to the cervix in females or up the urethra in males. Survival of the bacteria is aided by their production of an IgA protease, which inactivates IgA antibodies found in mucous secretions.

The bacteria invade the epithelial cells lining the cervix and urethra and multiply within them. Inside these cells they are protected from phagocytes and antibodies. Eventually, they are discharged from the epithelial cells into the sub-epithelial connective tissue. There they cause local damage and elicit an inflammatory response. In a small proportion of people the organisms invade blood vessels and then spread to other parts of the body, such as joints—termed disseminated gonococcal infection. Persistent infections cause chronic inflammation and fibrosis of the tissues.

Clinical features

Symptoms usually develop within a week (often in 1–2 days) of infection. In men, gonococcal infection is usually characterised by a urethral discharge (see Figure 21.5) and **dysuria** (pain on passing urine). The infection may be very mild or asymptomatic in up to 50 per cent of cases. A small percentage of untreated cases can develop an **epididymitis** (infection and inflammation of the epididymis) or **orchitis** (infection and inflammation of the testicles).

Up to 90 per cent of infected women also have very mild symptoms or are completely asymptomatic. Symptomatic infection is usually characterised by a cervico-vaginal

CASE HISTORY 21.2

Gonorrhoea

An 18-year-old female university student attended the sexual health clinic for the second time in six months. On both occasions it was confirmed that she had gonorrhoea. While counselling the young woman the nurse learns that she has only one sexual partner. Both she and her boyfriend were treated with ceftriaxone on the first occasion, but she suspects that her boyfriend might also be having sex with someone else. He refuses to wear a condom. The girl does not want to confront her boyfriend because she is afraid of losing him. She seems unconcerned, because she believes the infection is easily treated.

Questions

1. Would you expect the girl and her boyfriend to have been successfully treated on the first occasion with ceftriaxone?
2. What course of action would you recommend on this second occasion, in terms of treatment and prevention of cross-infection?
3. What advice would you give the girl regarding recurrent infections and the possible long-term effects of these on her body?

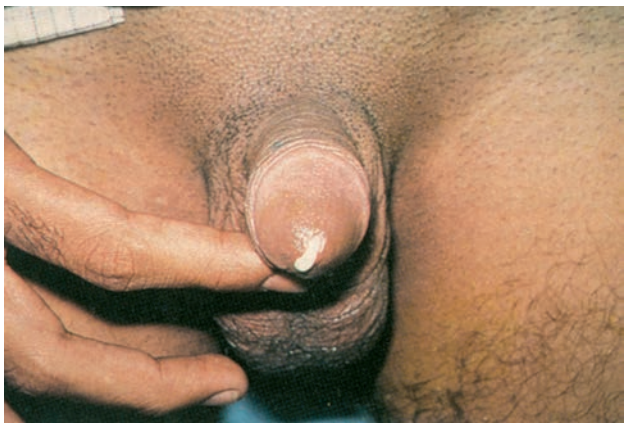


FIGURE 21.5

A purulent urethral discharge in a male with gonorrhoea

discharge and cervical oedema. Abnormal menses, dyspareunia (painful intercourse) and dysuria (due to urethral infection) are also common.

The asymptomatic nature of infection in many women is important for two main reasons. First, these women are an important, unrecognised source of infection. Second, in up to 30 per cent of untreated women the infection spreads further up the genital tract to cause inflammation in the fallopian tubes (**salpingitis**) and sometimes the ovaries (**oophoritis**), or in the whole of the peritoneal cavity (**peritonitis**). Inflammation in one or more of these sites is called **pelvic inflammatory disease** (**PID**).

Persistent infection, especially in the fallopian tubes, can cause scarring and blockage of the tubes, leading to infertility or ectopic (tubal) pregnancy, as well as increased susceptibility to recurrent attacks of PID. Other organisms, especially *Chlamydia trachomatis*, can also cause it, alone, or as a co-infection with the gonococcus. PID is dealt with more fully on page 554.

Anorectal infection is often asymptomatic, but otherwise can be characterised by rectal pain, discharge, constipation and tenesmus (painful defecation). Gonococcal pharyngitis, acquired during oral sex, is clinically indistinguishable from any other bacterial pharyngitis, but is also usually asymptomatic. Disseminated gonococcal infection can occur but is rare. Usually, joint symptoms (tenosynovitis or arthritis), a skin rash and fever are the major manifestations. A meningitis or endocarditis is also possible.

Diagnosis

Diagnosis has classically been based on microscopy and culture of an appropriate specimen. In men, this is a urethral swab. In women, a cervical swab is usually collected and, additionally (not alternatively), a sample of vaginal discharge if purulent. A rapid, presumptive diagnosis can be made if intracellular Gram-negative diplococci are seen in a Gram stain of exudate. It should be remembered that the organism is sensitive to drying and extremes of temperature, so the specimen should be inoculated directly on to culture media at the bedside or transported immediately to the laboratory in an appropriate transport medium, and must not be refrigerated. A pharyngeal or rectal swab may also be collected for culture if appropriate. Given the variability in antibiotic sensitivity of different strains of gonococci, culture and sensitivity results are important.

Other more rapid tests are available, as an adjunct to culture. An antigen detection test that uses a probe to detect *N. gonorrhoeae* DNA in specimens has recently been developed and is becoming widely used. Polymerase chain reaction (PCR) tests for gonorrhoea (and *Chlamydia trachomatis*—see later section) are commercially available and have the major advantage of being suitable for use on urine. Gonococci and *Chlamydia* are found in urine because these organisms often colonise the urethra. Urine is a less invasive specimen than the swabs above, and urine PCR tests for gonorrhoea and *Chlamydia* infections have made possible mass screening programs in high-risk populations. The disadvantages of the PCR test for diagnosis include the cost, false positives, and the inability of the methodology to provide antibiotic sensitivity information. Dual testing, by culture and PCR, increases the likelihood of detecting the microbe in an infected person.

All patients found to have an STI should be evaluated for other STIs. As noted earlier, *Chlamydia* co-infection is common in a person with gonorrhoea. In addition, all patients should be evaluated for syphilis, HIV infection and hepatitis B.

Treatment and prevention

Since the early 1980s there has been a marked and disturbing increase in the incidence of antibiotic resistance in *N. gonorrhoeae*. Pencillins were one of the standard treatments for gonorrhoea, but a high proportion of infections are now caused by penicillin-resistant strains. Increasing resistance to the quinolone antibiotics (e.g. ciprofloxacin) and to tetracyclines has also been observed. Resistance to later-generation cephalosporins (e.g. cefixime, ceftriaxone) is increasingly being reported, and there is concern that the gonococcus may develop widespread resistance to these drugs as well, with their increasing use. The WHO established the Gonococcal Antimicrobial Surveillance Programme to monitor susceptibility and resistance patterns of the gonococcus in different regions of the world.

Currently, treatment is usually with cefixime, although penicillins are still appropriate for use in regions where resistance is low. The advantage of these groups of antibiotics is that they can be administered in a single oral dose. Ceftriaxone is preferred for disseminated infections. Follow-up cultures 4–7 days after treatment are strongly recommended to confirm the effectiveness of treatment. Since many patients have a coexistent infection with *Chlamydia trachomatis*, treatment should also include an effective anti-chlamydial drug, usually azithromycin.

Prevention of gonorrhoea is based on the use of condoms and the tracing and presumptive treatment of patient

contacts. Despite extensive effort, no vaccine is currently available.

Syphilis

Causative organism and incidence

Syphilis is an STI that has been recognised for centuries and still remains a global health problem, especially in parts of Africa and Asia. Recent outbreaks in a number of developed countries (e.g. Canada) have occurred. It is caused by the spirochaete *Treponema pallidum*, a thin, coiled and highly motile bacterium with fastidious growth requirements. It is a fragile organism that can survive only briefly outside the body. Between 2000 and 3000 cases of syphilis are notified in Australia per year. The highest notification rate occurs in the Northern Territory. As in other developed countries, infection rates continue to rise in Australia, predominantly in men who have sex with men.

Transmission

Transmission of syphilis requires close, personal contact. Transmission via fomites (e.g. toilet seats) is virtually impossible, because the organism survives poorly outside the body—it is very sensitive to drying, heat (as low as 42°C) and disinfectants. The organism is present in the skin or mucous membrane lesions of an infected person and enters the body of a sexual contact through the mucous membranes or through abrasions (even minute) in the skin. An infected woman may also transmit the organism to her foetus *in utero*, especially after the first trimester, often resulting in congenital malformations.

Pathogenesis and clinical features

The treponemes multiply slowly and so the average incubation period is three weeks before symptoms develop. The course of infection may be divided into four stages, but only some patients experience all stages.

1. **Primary syphilis** is characterised by the development of a papular lesion at the site of infection, usually on the genitals, but sometimes on the rectum, lip or hand. The lesion appears after an average incubation period of three weeks. In males, there is usually an obvious lesion on the penis, but in females the primary lesion usually occurs on the cervix and therefore often goes unnoticed. The papular lesion breaks down to form a painless, hard-based ulcer called a **chancre** (see Figure 21.6). The papule and ulcer contain large numbers of organisms and are highly infectious. Also, inguinal lymph nodes may be enlarged. In 2–6 weeks the lesion heals spontaneously. Unfortunately, for many this is only a temporary respite.
2. In approximately 50 per cent of untreated cases, **secondary syphilis** develops 2–10 weeks later. From the time of initial infection, the treponemes begin to spread from the site of entry to local lymph nodes and then to the bloodstream. The secondary stage involves the multiplication of bacteria and the production of lesions, especially in skin and mucous membranes. It is characterised by the appearance of a red-brown maculopapular rash anywhere on the skin, anogenital region, mouth, throat or cervix (see Figure 21.7). The rash may even occur on the palms or soles of the feet. This may be accompanied by malaise and mild fever. These lesions are highly infectious. The symptoms again subside spontaneously after a few weeks, followed by a prolonged asymptomatic period of 3–30 years.
3. **Latent syphilis** follows resolved secondary syphilis. It is a prolonged asymptomatic period that can last anywhere between 3 and 30 years. Some patients may experience recurrence of the symptoms of secondary syphilis during this period.
4. After this latent period, signs of disease reappear in about 30 per cent of untreated cases. This is called **tertiary** (or late) **syphilis**. Renewed multiplication, dissemination and a cell-mediated hypersensitivity

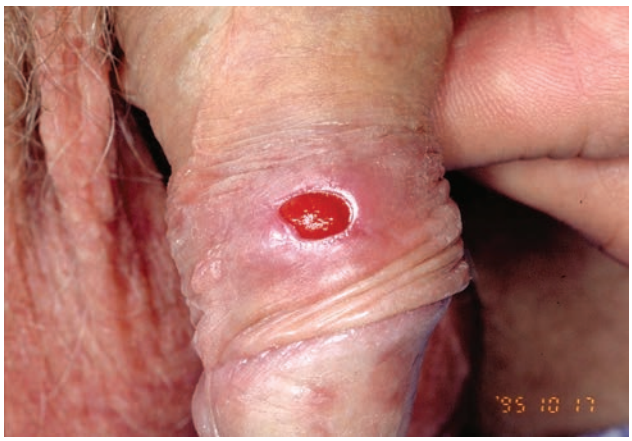


FIGURE 21.6

The chancre of primary syphilis on penis

Source: © www.ihotauti.net.

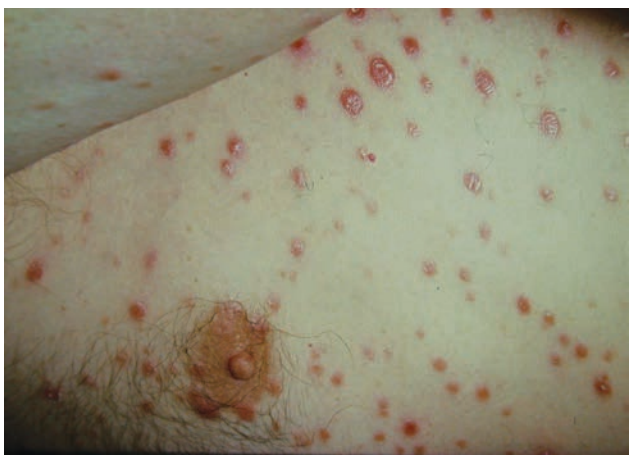


FIGURE 21.7

Secondary syphilis showing a papular rash

Source: Reproduced with permission of New Zealand Dermatological Society Incorporated at DermNetNZ.org.

response of the immune system combine to form lesions in various organs. Soft, granulomatous lesions (**gummas**) may form in the skin, bones, liver or other organs. If lesions form in the central nervous system, **neurosyphilis** is the result. Degenerative changes may lead to mental illness, paresis (weakness or partial paralysis) or *tabes dorsalis* (poor muscle coordination and unstable gait). The paresis plus gradual loss of higher integrative functions in tertiary syphilis is sometimes referred to as 'general paralysis of the insane'.

In **cardiovascular syphilis**, aortic aneurysm and blood vessel and valve damage may occur, leading to heart failure. The treponemes are rarely found in the lesions of tertiary syphilis because the symptoms appear to be due mainly to a hypersensitivity reaction to the microbes.

The pathogenesis and clinical features of syphilis are summarised in Table 21.5. Congenital infection occurs when the organism crosses the placenta in a woman with secondary syphilis. Globally, about 1 million pregnancies each year are affected by syphilis, of which almost half end in foetal death, about a quarter give rise to babies with congenital syphilis, and the remaining babies are premature and/or have low birthweight. Congenital infection may not become apparent for many months after birth, but in some it may cause severe symptoms at birth, including hepatosplenomegaly, prolonged jaundice, thrombocytopaenia and failure to thrive. Other possible congenital abnormalities include mental retardation, blindness, deafness, bone disease and, later, facial and tooth deformities.

As noted earlier, diseases such as syphilis, in which genital ulcers are produced, have the added problem of increasing the risk of HIV transmission and acquisition. Not only do the ulcers provide a site of entry for the HIV, but there is evidence that activated CD4 lymphocytes are attracted to the site of infection. These lymphocytes are the key target of the HIV. In addition, HIV infection can increase the likelihood of clinical progress of syphilis.

Diagnosis

Treponema pallidum cannot be readily grown in the laboratory. Therefore, the laboratory diagnosis of syphilis relies on microscopic and serologic methods. The quickest and most direct method is microscopy. The primary and secondary lesions of syphilis usually contain large numbers of organisms. The bacteria may be seen in freshly collected exudate from the lesions when examined by dark-ground microscopy (see Figure 21.8) or in a UV microscope after treating the specimen with fluorescein-labelled anti-treponemal antibodies. The exudate is highly infectious and must be handled with care.

FIGURE 21.8

Dark-field micrograph of *Treponema pallidum*

Source: Dr Gary Lee.

TABLE 21.5 Pathogenesis and clinical features of syphilis

STAGE OF DISEASE	PATHOGENESIS	SIGNS AND SYMPTOMS
Incubation period (2–10 weeks)	Multiplication at site of entry	
Primary syphilis (lasting 2–6 weeks)	Spread to regional lymph nodes and bloodstream	■ primary chancre ■ enlarged inguinal nodes
Asymptomatic period (2–10 weeks)	No or very slow multiplication of organisms	
Secondary syphilis (lasting 2–6 weeks)	Multiplication in extra-genital sites	■ maculopapular rash anywhere on skin or mucous membranes ■ malaise and mild fever
Latent syphilis (lasting 3–30 years)	Organisms dormant in liver, spleen and central nervous system	
Tertiary syphilis	Renewed multiplication and invasion plus a cell-mediated hypersensitivity response	■ gummas in skin, bone, liver ■ cardiovascular syphilis: – aortic lesions – heart failure ■ neurosyphilis: – general paresis of insane – tabes dorsalis

Serology is the mainstay of diagnosis. The serological tests used are of two types: the non-specific (non-treponemal) tests and the specific (anti-treponemal antibody) tests. The non-specific tests do not detect antibodies to treponemal antigens, but rather detect an antibody-like substance called *reagin*. The two non-specific tests in common use are the VDRL (Venereal Diseases Research Laboratory) and the RPR (Rapid Plasma Reagin). False positive results are possible with these non-specific methods, so a positive result is confirmed using a specific test, such as the fluorescent treponemal antibody absorption test (FTA-ABS), the *Treponema pallidum* particle agglutination test (TPPA) or enzyme-linked immunosorbent assay (ELISA). Unless seroconversion is demonstrated, these serological methods do not distinguish between past and current infection.

Treatment

Penicillin is very active against *Treponema pallidum* and is the drug of choice. A single intramuscular injection of benzathine penicillin remains active in the body for two weeks and is usually effective for syphilis of less than two years' duration. For older infections, three intramuscular injections at weekly intervals, or daily injections of procaine penicillin for three weeks are the usual regimes. If the patient is allergic to penicillin, oral doxycycline or ceftriaxone may be used. In pregnant women, azithromycin is generally used.

Prevention

Prevention is based on the practice of safe sex, and contact tracing and screening. Prevention of secondary and tertiary disease depends on early diagnosis and treatment. Congenital infection is preventable if women are screened (by serology) early in pregnancy and treated. Re-screening in the third trimester is warranted in high-incidence regions.

CASE HISTORY 21.3

Syphilis

A 27-year-old male presented to the medical clinic with penile pain. He explained that he had the pain for several days, following catching the skin of his penis in the zipper of his pants. He stated that he had not had a sexual partner for six months, and before that was in a monogamous relationship with a woman for two years. On examination he was found to have a circular lesion of 1 cm diameter on the shaft of the penis and bilateral inguinal adenopathy. He appeared otherwise healthy. The doctor prescribed an antibiotic ointment. Four weeks later, the patient returned to the clinic because he then had a macular rash on his chest and on the palms of his hands. The lesion on his penis had resolved. The doctor took a blood sample and requested a VDRL test. The VDRL test was positive, and a diagnosis of syphilis was confirmed by the fluorescent treponemal antibody absorption test (FTA-ABS).

Questions

1. What stage of syphilis did the man have on his second visit, and what characteristic clinical features did he have of that stage?
2. Why were two serological tests necessary to confirm the diagnosis?
3. What treatment should the man have received, and what other actions should the doctor have undertaken?
4. Is there a cross-infection risk for a health professional attending to this patient?

Chlamydial infections

Causative organisms and incidence

Chlamydia are very small bacteria that are obligate intracellular parasites. Four species are currently recognised, but only *Chlamydia trachomatis* infects the genital tract. This species can be subdivided into different serotypes (serovars), which are associated with different infections:

- Serotypes A, B and C cause an eye infection called trachoma (see Chapter 16).
- Serotypes D to K cause genital and associated infections.
- Serotypes L1, L2 and L3 cause a specific genital infection called lymphogranuloma venereum.

Genital chlamydial infections caused by serotypes D to K have a high incidence in many countries and are currently one of the most prevalent of the bacterial STIs. The WHO estimates that worldwide there are 140 million cases of *C. trachomatis*. The Centers for Disease Control and Prevention in the United States estimates that around 2.5 million Americans are infected by *Chlamydia* each year. In Australia, *Chlamydia* infection has the highest incidence of any notifiable disease, with over 62 000 cases notified in 2009. The number of notifications has increased every year since 1991 when it became a nationally notifiable disease. Between 2004 and 2009, notification rates increased by 61 per cent. Notifications include all types of chlamydial infection although the majority are genital infections. The real incidence is probably many times the reported number because many cases are asymptomatic.

Most infections occur in the 15–29 years age group, with a predominance in females (see Figure 21.9). Geographically, highest rates of notification come from Indigenous populations in the Northern Territory and parts of Queensland and Western Australia. The large number of people who are infected asymptomatically facilitates transmission.

Lymphogranuloma venereum (LGV) is a serious disease found in restricted regions of the world, particularly tropical and subtropical Africa, Asia, and South and Central America. It is less common in developed countries, although outbreaks have recently been reported among men who have sex with men in Europe and North America.

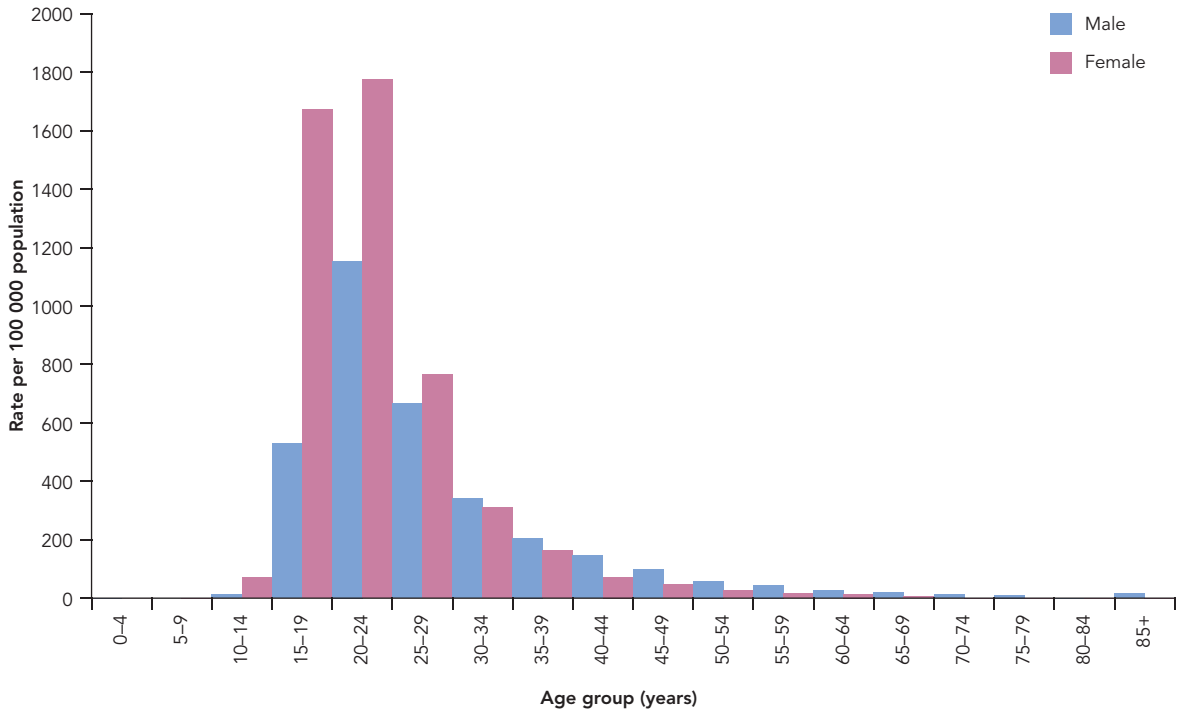


FIGURE 21.9

Notification rates of *Chlamydia* infection in Australia, 2009, by age group and sex

Source: *Communicable Diseases Intelligence* 2011, 35(2): 92. © Commonwealth of Australia. Reproduced by permission.

It is a notifiable disease in only some Australian states. Fewer than five cases a year are notified and they are generally acquired overseas.

Pathogenesis and clinical features

Serotypes D to K probably enter through minute abrasions in the mucosal surface. The organisms grow in epithelial cells and can cause a wide spectrum of clinical diseases, determined by the site of infection. If symptoms are produced, they usually reflect local mucosal inflammation with a discharge. Infection of the cervix leads to **cervicitis**, which produces no or mild symptoms in more than 80 per cent of women. Symptomatic women may experience slight vaginal discharge or intermenstrual bleeding 1–3 weeks after exposure. The absence of symptoms means that many women are not treated and are thus at risk of the infection spreading to the fallopian tubes, resulting in pelvic inflammatory disease (see page 554). Infection can be spread from mother to baby at birth, causing an eye infection—opthalmia neonatorum.

Urethral infection (**urethritis**) in men is also often asymptomatic, with only about 20 per cent experiencing a mucoid urethral discharge. The asymptomatic person may remain infectious for months and unknowingly transmit the infection to sexual partners.

In LGV, a primary ulcer develops at the site of organism inoculation (usually on the genitals, but occasionally in the oral cavity or rectum) within four weeks of infection. The ulcer is small and painless, and often goes unnoticed, especially if located within the urethra, vagina or rectum. The lesion heals spontaneously after a few days, but draining lymph nodes become infected. Over the next 2–6 weeks, painful inguinal buboes (masses) typically form, which gradually enlarge. Abscesses may form in the nodes and eventually suppurate and discharge through the skin. The organisms may spread via the lymphatics to the rectum (proctitis) and other tissues (causing pneumonitis, meningoencephalitis or hepatitis).

Diagnosis

Chlamydial infections cannot be reliably distinguished from infections caused by other organisms by clinical examination alone, so laboratory testing is essential. A number of methods are available, with the choice determined by the clinical setting, technologies available and cost. Culture is the gold standard, but is slow and requires specialised techniques because the organism can be grown only in cell culture (it is an obligate intracellular parasite). For culture, a cervical or urethral swab is usually collected (by scraping the site with a swab in order to obtain infected cells) as well as discharge. The difficulties associated with culture means that other diagnostic methods are often used. Antigen detection methods (e.g. direct fluorescent antibody, DNA probe) are commonly used. Nucleic acid amplification tests (e.g. polymerase chain reaction, transcription mediated amplification) are also available. They have superior sensitivity over culture and antigen detection methods, especially for asymptomatic infections. They also have the major advantage of being suitable for use with less invasive and self-collected

CASE HISTORY 21.4

Urethritis

Mr K, a 25-year-old bank clerk, attended a doctor's surgery because he had been experiencing a painless discharge from his penis for the last three days. He had been sexually active with his new girlfriend for the last month. On examination, the discharge was noted as being mucopurulent and the doctor suspected gonorrhoea. Mr K was given an intramuscular injection of penicillin and advised not to have sex with anyone until after his infection cleared completely. His condition improved initially, but in 14 days he returned to the doctor because he still had a discharge. He stated that he had not had sex since his last visit. This time, the doctor collected a sample of discharge and sent it to the pathology laboratory. *Neisseria gonorrhoeae* could not be seen in a Gram stain of the smear and was not cultured.

Questions

1. What are the possible reasons for the incomplete effectiveness of the treatment?
2. What should have Mr K been treated with if he did have gonorrhoea?
3. List the possible causes of urethritis in males.
4. What does this case illustrate about the diagnosis of genital infections?

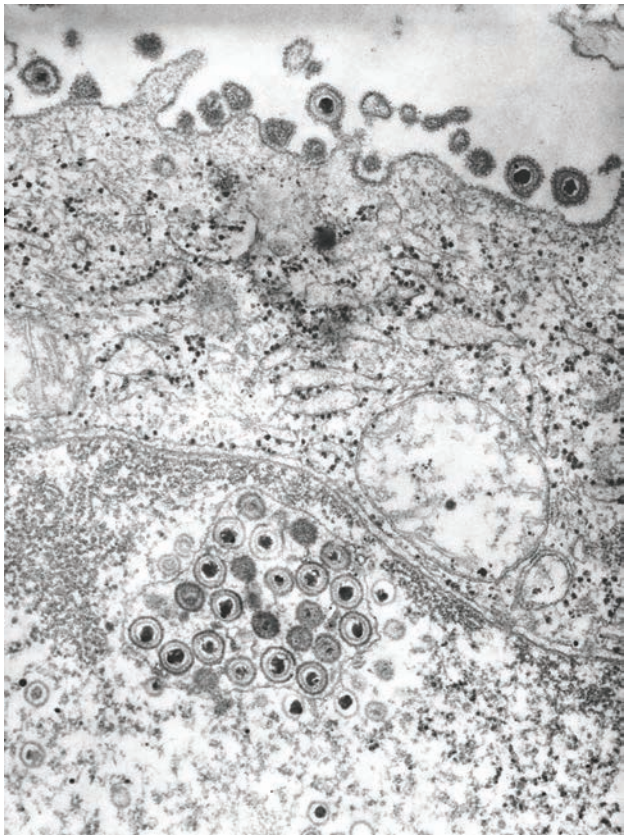


FIGURE 21.10

Herpes simplex virus

This transmission electron micrograph (TEM) shows the presence of numerous herpes simplex virions, located inside the nucleus and extracellularly.

Source: CDC/Dr Fred Murphy; Sylvia Whitfield.

specimens, such as first void urine, low vaginal swabs and tampons.

In LGV, bubo pus or tissue biopsy are the common specimens. Swabs, scrapings and small amounts of tissue should be transported in an appropriate transport medium.

Treatment and prevention

Treatment is usually with azithromycin, which can be given as a single oral dose, or doxycycline for 1–3 weeks. Sexual contacts should also be treated whether or not they are symptomatic, because of the ease of spread and the potential seriousness of fallopian tube infection. Because concurrent chlamydial and gonococcal infections are common, patients receiving treatment for gonorrhoea should also receive treatment for *Chlamydia*.

In addition to rapid diagnosis and effective treatment of cases and contacts and the promotion of safe sex practices, *Chlamydia* screening programs have been introduced in a number of countries in an attempt to reduce the incidence of these infections. These programs have generally involved the testing of young women attending family planning or sexual health clinics. The development of methods for testing of self-collected samples would enhance the reach and effectiveness of screening.

Genital herpes

Causative agents and incidence

Herpes simplex viruses are double-stranded DNA viruses that belong to the Herpesviridae family (see Figure 21.10). There are two major types of herpes simplex virus: herpes simplex virus type 1 (HSV-1) and herpes simplex type 2 (HSV-2). Both types have been found to cause genital and oral (cold sores) herpes, but type 2 predominates in the genital region while type 1 is the more common cause of oral infection. Recently, a marked increase in the number of genital infections caused by HSV-1 has been observed in North America and some European countries. It is believed that this is at least partly due to an increase in oral sex practices in young adults.

Infection with at least one of the herpes viruses seems to occur in most people, although mainly asymptomatically. Up to 75 per cent of adults have antibodies to HSV-1 and up to 50 per cent have antibodies to HSV-2. Approximately 15 per cent of women attending public antenatal clinics are found to be seropositive for HSV-2, and the prevalence is much higher in men who have sex with men, sex workers and people attending STI clinics.

Genital herpes has a worldwide distribution that appears to be increasing, as judged by seroprevalence (i.e. the numbers of people with antibodies to the virus). National notifications are not compiled in Australia.

Pathogenesis and clinical features

HSV-2 is readily inactivated by drying at room temperature. Therefore, transmission is essentially by direct contact with infected secretions or mucosal surfaces. The virus appears able to invade intact mucosal surfaces, but infects skin only through breaks or abrasions.

Initial infection in as many as 50–70 per cent of people may be completely asymptomatic or so mild that it is unrecognised. The majority of people with antibodies to HSV-2 do not have a history of clinical genital herpes. If symptomatic, the primary lesions usually occur on the penis, anus, vulva, cervix or vagina 3–14 days after exposure. The classic lesions are usually small, grouped vesicles filled with a clear fluid and surrounded by an area of inflammation (see Figure 21.11). The vesicles break down after several days to form painful, shallow ulcers. If the lesions occur over a large area of the genitalia, walking, or even the wearing of clothing, can be painful.

Local lymph nodes are usually swollen and the patient may be lethargic and feverish. Neurologic signs (headache, stiff neck, photophobia) occur in some primary infections, reflecting a viral meningitis. In males, a urethritis with a watery discharge may occur. Herpes pharyngitis can occur after oral infection.

The primary lesions usually heal within two weeks, but by that time the virus has travelled along the local sensory nerve to the sensory root ganglion where it lies dormant. Virus persistence there lasts for a lifetime. The virus can reactivate and travel back down the nerve, exit the cell and be shed asymptomatically, or spread to, and infect, mucocutaneous epithelial cells, causing new lesions.

There is considerable variation in the frequency of recurrent symptomatic attacks. In some people recurrence is rare; less than 50 per cent of infected people have a recurrence within one year. Others may have many recurrent attacks in a year. Recurrent infection may or may not be symptomatic, but an asymptomatic person can be shedding the virus. Shedding always occurs when lesions are present and for days around these symptomatic episodes. A large proportion of infections are transmitted from asymptomatic people.

Latency, reactivation and recurrent infections are typical of herpes viruses. HSV-1 undergoes a similar cycle in sensory nerves and cutaneous tissue in causing recurrent cold sores. The mechanisms involved in the establishment of latency or in the reactivation of herpes viruses are not well understood, except that reactivation can occur apparently spontaneously or in response to certain stimuli such as physical or emotional stress, ultraviolet light and fever.

HSV-2 can also be transmitted from a symptomatic or asymptomatic mother to her baby during pregnancy, at birth or postnatally. The great majority of neonatal infections are acquired at birth, especially if the mother is suffering from primary infection at that time. The virus can invade the skin, eyes, CNS and visceral organs of the infant. Many cases of neonatal infection are fatal; in a large proportion of non-fatal infections, mental retardation and defective sight and hearing are typical outcomes.

Diagnosis

Genital herpes cannot always be distinguished from other types of genital ulcer. Furthermore, many cases are asymptomatic. The virus can be cultured from vesicle fluid or ulcer

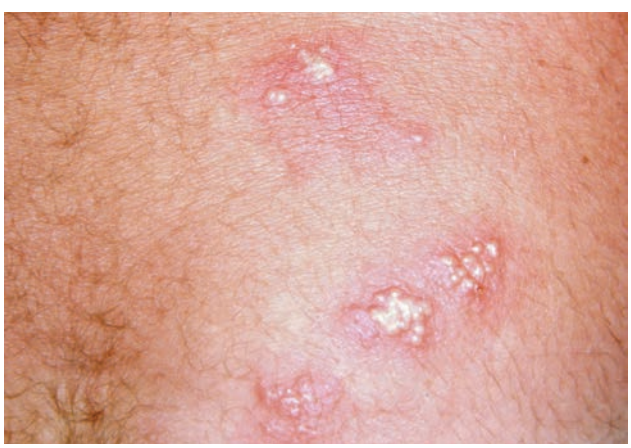


FIGURE 21.11

Vesicles of genital herpes

Source: Reproduced with permission of New Zealand Dermatological Society Incorporated at DermNetNZ.org.

CASE HISTORY 21.5

Genital herpes

A 20-year-old male presented to the sexual health clinic with a four-day history of fever, chills and myalgia. Two days prior to attending the clinic he had noted two painful lesions on his penis. He stated that his girlfriend had been diagnosed with genital herpes six months before and had been treated with aciclovir. Since that treatment she had been symptomatic only once, and they did not have sex during that time.

PCR testing of fluid from his lesions confirmed the diagnosis of genital herpes caused by HSV-2.

Questions

1. What does this case illustrate about the treatment of genital herpes?
2. What does this case illustrate about the transmissibility of genital herpes?
3. How should this man be counselled regarding his infection and preventing its spread to other people?
4. What are the other common causes of genital ulcer disease?

swabs, and this is the definitive diagnostic approach. Fluid in vesicles should be aspirated with a syringe, or the vesicles should be opened with a small gauge needle and the base of the lesion rubbed with a cotton swab. The swab should be placed in viral transport medium. If transport to the laboratory is delayed, specimens should be refrigerated.

The major disadvantages of culture are that it requires specialised laboratory techniques, and so may not be available, and it takes around 2–5 days for a result to be available. PCR is the assay with the greatest sensitivity for detecting HSV in genital lesions and can provide a result within 24 hours. Antibody detection in serum is still commonly used to diagnose HSV infection, especially when there are no lesions or other tests are negative.

Treatment

There is currently no fully effective cure for genital herpes. Treatment with aciclovir, valaciclovir or famciclovir reduces the duration of symptoms and virus shedding in clinical and subclinical cases, but does not eliminate latent infection. A continuous, low dose of one of these drugs may be used for a limited time in people with frequent recurrences to reduce the frequency and severity of recurrences and to reduce subclinical shedding. Strains of herpes simplex virus resistant to aciclovir and famciclovir have been found on rare occasions.

Prevention

Prevention of transmission of genital herpes is based on the general use of safe sex practices. Infected people should be advised to have total abstinence from sex when symptomatic because they may shed the virus from any part of the genital region. Therefore, the use of a condom does not guarantee protection against HSV infection when one partner is symptomatic.

If a pregnant woman is symptomatic near term, caesarean delivery is indicated and will often prevent neonatal infection if performed before rupture of the membranes. This does not always prevent infection of the baby, however, since *in utero* and postnatal transmission are possible. Current HSV vaccines have limited efficacy, especially in women.

Genital warts

Causative agents and transmission

More than 100 different types of human papillomaviruses (HPV) that infect the skin and mucous membranes have been identified. At least a third of these are associated with anogenital infections. Infection normally occurs through mucous membranes or traumatised skin, although vertical or oral transmission is also possible. Worldwide, it is estimated that as many as 60 per cent of sexually active people are infected with genital HPV at some stage in their lives. The exact prevalence in Australia is not known because it is not a notifiable disease.

Clinical features

Genital warts (**condyloma acuminatum**) appear as pinkish-brown masses, usually in clusters, on the penis, scrotum, vulva, cervix, or perineal or perianal regions (see Figure 21.12). They can appear at any time from 1–8 months or more after infection and then resolve spontaneously within 1–2 years.

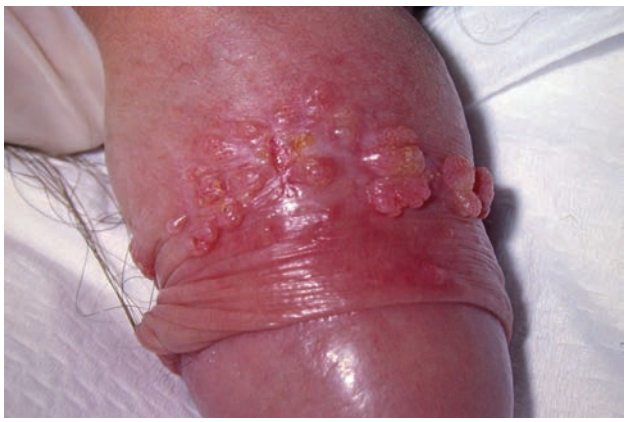


FIGURE 21.12

Genital warts on the penis

Source: Reproduced with permission of New Zealand Dermatological Society Incorporated at DermNetNZ.org.

The majority of infections are subclinical. Why some people develop warts after infection and others remain asymptomatic is not known. The importance of asymptomatically infected people is that they represent a large, unidentified reservoir of the virus. Furthermore, in a symptomatic person, areas of genital skin where warts are not present are also possibly infected.

One of the major concerns of genital warts is the epidemiological association of certain 'high-risk' virus types with carcinoma. High-risk types of HPV (e.g. types 16, 18, 31 and 45) can place women at risk of developing vulval, cervical or anal cancer. Worldwide, HPV 16 and 18 are responsible for 70 per cent of cervical carcinoma cases. There is also thought to be an association between certain virus types and anal and penile carcinoma in men. There is a relatively low incidence of cancer compared to the incidence of infection, suggesting that other factors may be important for cancer development, particularly smoking and immunosuppression (e.g. from HIV infection). Also, many strains of HPV are 'low-risk' types (e.g. HPV 6 and 11), meaning that they are not cancer-causing.

Diagnosis

Diagnosis of infection is based mainly on clinical examination; histological examination of a biopsy specimen can be used in cases of clinical uncertainty. Human papillomaviruses cannot be cultured, but the virus can be identified by DNA or RNA typing of specimens containing infected cells. Cervical infection is often initially indicated by the presence of abnormal cells in a Pap smear (see Case History 5.2: Cervical cancer, Chapter 5, page 100) and then confirmed by colposcopy. The application of 3–5 per cent acetic acid can be used to demonstrate subclinical infection of the vulva, cervix, penis and anus. Infected tissues show as areas

of whitening (called acetowhite lesions), but acetowhitening is not specific for HPV infection.

PCR methodology has been developed for detection of HPV DNA in clinical specimens. This method is increasingly being used diagnostically and in follow-up testing of women after removal of cervical tumours.

Treatment

As for warts in other areas of the body, treatment aims at removal of the warts, most often with podophyllotoxin (a plant extract) or imiquimod cream, both of which can be applied by patient self-treatment. Other possible treatments include freezing with liquid nitrogen or dry ice, laser therapy or surgical excision. These approaches do not necessarily eliminate the virus, so successful treatment most likely converts a clinical infection to a subclinical one, which is then probably lifelong.

Early identification and treatment of abnormal cervical cells may prevent the formation of cancerous lesions. Treatment of warts, however, is not always successful. After removal of warts, viruses may persist in nearby, apparently normal tissue and later cause a local recurrence.

CASE HISTORY 21.6

Genital warts

Tim, a 32-year-old chef, attended his local medical clinic, where he described seeing small bloodstains on his underpants after playing soccer and on the toilet paper after wiping himself. He said he had been aware of the bloodstaining for two weeks and could feel some small lumps around his anus. These were his only symptoms, and he felt very healthy, he said. When questioned by the doctor about his sexual activity, he admitted to having four different sexual partners over the last year, after breaking up with his girlfriend of three years. He stated that he had never had sex with another man.

On physical examination the doctor found Tim to be well apart from several pinkish warts around his anus. The doctor then ordered a full STI screen, which found that Tim also had a chlamydial infection. All other tests were negative.

Questions

1. What treatments might be offered to the patient, and what is their likely success?
2. Why did the patient not have any symptoms of chlamydial infection?
3. Could the patient suffer any complications if the two infections had not been identified and were left untreated?
4. What follow-up actions should be taken in this case?

Prevention

Papillomaviruses can exist on most areas of genital skin of an infected person even where no obvious warts are present. Thus, condom usage may reduce transmission but it does not offer complete protection.

A major advance in prevention of genital warts and their sequelae was the development of vaccines against some of the viruses. Two HPV vaccines are currently available. A bivalent vaccine provides protection against HPV types 16 and 18 infections, and a quadrivalent vaccine provides protection against HPV types 6, 11, 16 and 18. In 2006, Australia was one of the first countries to roll out an HPV vaccination program using the quadrivalent vaccine (Gardasil). This vaccine is currently provided free for 12–13-year-old girls under the national immunisation program. Initially, up to June 2009, it was also provided to older girls and young women in a catch-up program..

The vaccine protects women against HPV strains that cause 70 per cent of cervical cancers. It has also been found to provide a degree of cross-protection against some non-vaccine types in some recipients. However, because the vaccine does not protect against all papilloma virus types, and hence all cervical cancers, women still need to keep up-to-date with regular Pap smears. Also, the vaccine does not protect against pre-existing infections. It is not recommended for pregnant women or people with known hypersensitivity to yeast or other vaccine components.

Donovanosis

Also called **granuloma inguinale**, **donovanosis** is caused by the encapsulated Gram-negative rod *Klebsiella granulomatis*. It is rare in temperate climates but common in tropical and subtropical regions such as the Caribbean, New Guinea, South-East Asia and India. Currently, fewer than ten cases are reported annually in Australia. The annual notification rate has decreased substantially since the Australian government introduced the National Donovanosis Elimination Project in 2000.

After an incubation period of around 50 days, the infection is characterised by nodules, usually on the genitalia, which erode to form granulomatous ulcers. As the disease progresses, the ulcers may spread into the groin (see Figure 21.13) and anus, involving more and more tissue. In some cases, extragenital lesions may occur; these are thought to be due to auto-inoculation. Permanent genital damage and deformity can result if left untreated. The mode of transmission is predominantly by sexual contact, although repeated exposure seems to be necessary, suggesting the organism has low infectivity.

The organism is extremely fastidious and difficult to culture, so diagnosis is based on clinical appearance and microscopic examination of a stained smear, biopsy or scraping from the lesion. Organisms are typically found inside large mononuclear cells or histiocytes. PCR is highly sensitive and can provide a diagnosis, but is predominantly a research tool at the present time. Treatment

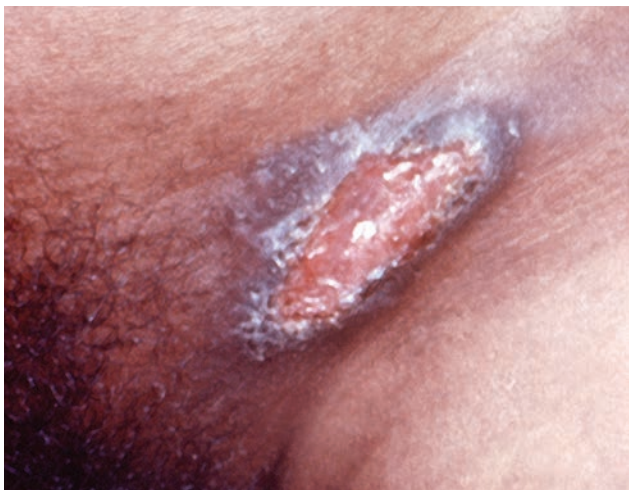


FIGURE 21.13

Donovanosis in the left inguinal lymph node

Source: CDC/Dr Pinozzi.

is usually a minimum three-week course of cotirmoxazole or doxycycline. Alternative drugs include azithromycin or ciprofloxacin.

Chancroid

Chancroid is an STI caused by a small Gram-negative rod, *Haemophilus ducreyi*. It is most frequently seen in Africa, the Caribbean and Asia, but its actual incidence is largely unknown due to lack of monitoring. The infection is notifiable in almost all Australian states and territories, but very rarely occurs. The disease is manifested by soft, painful genital ulcers 3–14 days after infection. Local lymphadenitis becomes apparent after several days. The lymph nodes gradually enlarge to such an extent that eventually they may break through the skin, discharging pus. Extragenital lesions, such as on the tongue and lip, may also be seen. The lesions can last weeks to months and are highly infectious.

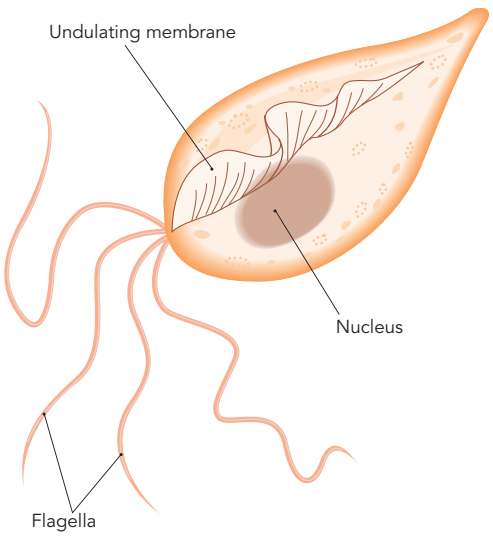
The organism is difficult to culture in the laboratory and can take up to nine days. However, culture remains the definitive diagnostic method. A Gram stain of an aspirate from the ulcer margin or enlarged lymph node may show the typical short Gram-negative rods. Co-infection with other sexually transmissible organisms is common, so assessment for other STIs is warranted. Multiplex PCR methodology allows the simultaneous detection of *Treponema pallidum*, herpes simplex virus and *H. ducreyi*. The organism is becoming increasingly resistant but is still sensitive to a number of drugs, including erythromycin, ceftriaxone and ciprofloxacin.

Trichomoniasis

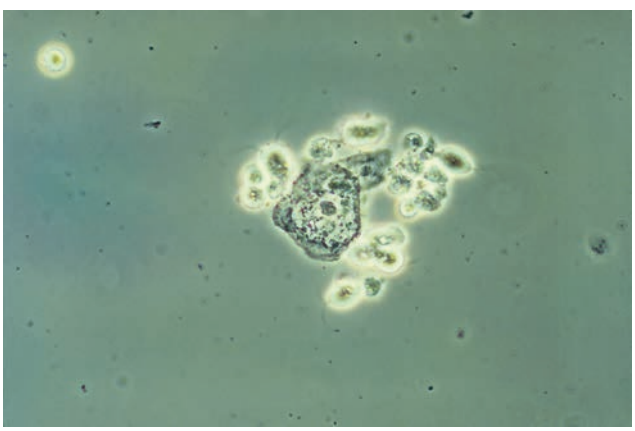
Globally, **trichomoniasis** is a very common STI with an estimated 170 million new cases a year. It is caused by the flagellated protozoan *Trichomonas vaginalis* (see Figure 21.14). The vagina is the most common site of infection in women, and the urethra is the most common site of infection in men. The organism damages epithelium in these sites, causing micro-ulcerations. After an incubation period of 4–28 days, symptomatic females develop a vaginal discharge that can range from being thin and scanty to profuse and thick. Urethritis is the most common outcome of infection in men. However, up to one-third of female infections and the majority of male infections are asymptomatic. The high incidence of trichomoniasis and its coexistence with other STIs make it a significant public health issue.

T. vaginalis infection can cause some important sequelae, including pelvic inflammatory disease, and adverse outcomes of pregnancy, such as low birth weight and prematurity. In men, complications of untreated infection include prostatitis, epididymitis and infertility. *T. vaginalis* infection is also recognised as a factor that can promote the transmission of HIV.

Microscopic examination of discharge has been the most common diagnostic method for trichomoniasis, but has low sensitivity. Culture is more sensitive and specific, but requires days to achieve growth. Latex agglutination and



(a)



(b)

FIGURE 21.14

***Trichomonas vaginalis*, the protozoan that causes trichomoniasis**

(a) Schematic drawing of the organism; (b) phase contrast wet mount of Trichomonas *in vaginal discharge.*

Source: CDC.

PCR tests have been developed for point of care use, and are likely to be used more commonly in the future. Effective treatment is achieved with metronidazole, but re-infection rates are high.

Non-specific urethritis

Non-specific urethritis (**NSU**) is inflammation of the urethra not caused by *Neisseria gonorrhoeae* or *Chlamydia trachomatis*. The organisms most often associated with NSU are *Mycoplasma genitalium*, *Trichomonas vaginalis*, herpes simplex virus and *Ureaplasma urealyticum*. However, in many cases a causative agent is not identified. Symptoms are similar to gonorrhoea, but are usually milder. They may include a watery to milky discharge, stinging or burning during urination, and itching, tingling or irritation inside the penis.

Once *N. gonorrhoea* and *C. trachomatis* have been excluded, specific tests for the other possible causes may be undertaken. *Ureaplasma urealyticum* infection can be diagnosed by culture of discharge. NSU is often treated empirically with doxycycline or azithromycin. If this treatment is ineffective in preventing recurrent episodes of urethritis, *T. vaginalis* or herpes simplex virus are likely causes.

Pelvic inflammatory disease

Pelvic inflammatory disease (**PID**) is defined as the clinical syndrome associated with infection and inflammation of the upper female reproductive tract, particularly the uterus, fallopian tubes and ovaries, and sometimes the whole of the peritoneal cavity. The most important sites of infection are the fallopian tubes (**salpingitis**), because this can lead to ectopic pregnancy or sterility. It is estimated that as many as 1 million women have PID each year in the United States and that many thousands of women have been rendered sterile as a result.

Causative agents, pathogenesis and clinical features

PID may be uni- or poly-microbial, and is initiated by microbes that ascend from the vagina and cervix. Most infections are caused by *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, or both. It is thought that in many cases *C. trachomatis* or *N. gonorrhoeae* set up infection by initially damaging the upper genital tract, and then vaginal flora (such as *Gardnerella vaginalis*, *Streptococcus agalactiae*, *Bacteroides fragilis*) ascend the tract and gradually take over. It is estimated that 10–20 per cent of untreated chlamydial or gonococcal infections progress to PID.

The symptoms associated with PID are extremely variable. Most cases of PID are asymptomatic, and only come to light during investigations for infertility. When symptomatic, the most common symptom is a dull and constant lower abdominal pain. Patients may have cervical motion tenderness (pain when the cervix is moved from side to side). Other possible manifestations include abnormal vaginal discharge, intermenstrual bleeding, fever and post-coital bleeding.

Persistent infection can lead to fibrosis and scarring of the fallopian tubes, which can block the passage of the ovum from the ovary to the uterus. This can result in sterility or the implantation of the fertilised egg in the uterine tube rather than in the uterus (called an 'ectopic' or 'tubal' pregnancy). Other possible sequelae include chronic pelvic pain and intra-abdominal adhesions.

Diagnosis and treatment

The diagnosis is usually made clinically on the findings of abnormal discharge or bleeding, together with lower abdominal tenderness, cervical motion tenderness and adnexal tenderness (tenderness around the ovaries and fallopian tubes). However, symptoms are often mild and non-specific, making a clinical diagnosis difficult. Laparoscopy is considered the gold standard method for diagnosis of PID. It enables direct visualisation of inflammation in the fallopian tubes, but is not always performed because it is invasive, requires an operating room and anaesthesia, and does not identify all cases.

Treatment of PID usually involves the use of a combination of antibiotics because of the possibility of polymicrobial infection, and should at least cover *C. trachomatis* and *N. gonorrhoea*. Several different treatment regimens exist—a typical one is metronidazole, doxycycline and ceftriaxone or ciprofloxacin. Male partners should be assessed for both gonorrhoea and chlamydial infection.

Bacterial vaginosis

Bacterial **vaginosis** is a common vaginal infection that is characterised by an abnormal vaginal discharge with an

CASE HISTORY 21.7

Pelvic inflammatory disease

A 21-year-old woman attended the casualty department of her local hospital complaining of the gradual onset of severe pain in the lower part of her abdomen. Over the last few hours she had also begun to feel feverish and nauseated. Prior to this she had noticed no other symptoms.

A pelvic examination revealed a yellowish discharge from the cervical os. A cervical swab was taken and a Gram stain showed intracellular Gram-negative diplococci. Culture results the next day confirmed infection with *Neisseria gonorrhoeae*. She stated that her two sexual partners did not have any signs of infection.

Questions

1. What organs might be involved in this infection?
2. Are any serious complications associated with this infection?
3. What possible difficulties could be experienced with the treatment of this woman?
4. Should the woman's asymptomatic sexual partners be notified and treated?

unpleasant, fish-like odour. Women may also experience burning during urination and/or itching around the outside of the vagina. However, most women are asymptomatic.

Bacterial vaginosis appears to be associated with an imbalance in the vaginal microflora. There are fewer lactobacilli and higher numbers of other microorganisms, including *Gardnerella vaginalis*, and anaerobes such as *Peptostreptococcus*, *Fusobacterium* and *Bacteroides*. The normal acidity of the vagina is decreased. What factors precipitate this change in microflora is not clear, but having a new sex partner or multiple sex partners, or douching appear, to increase the risk. Therefore, it may be sexually transmitted in some cases, but that is not the only way it is acquired or develops. *Gardnerella vaginalis* can be found in the urethra of some men, but is not associated with disease.

Apart from causing irritating symptoms, bacterial vaginosis has been associated with more serious complications in a minority of women. These include pelvic inflammatory disease, infection following surgical procedures such as hysterectomy, and pre-term birth. Bacterial vaginosis may also increase the risk of HIV transmission and increase a woman's susceptibility to other infections such as chlamydial infection and gonorrhoea.

Diagnosis is usually based on clinical observation of vaginal odour, increased vaginal pH and sometimes laboratory culture of vaginal swabs to assess the imbalance in microflora. Treatment with oral metronidazole or a clindamycin or metronidazole cream is usual, but the infection may re-occur. Natural treatments, such as probiotics, yoghurt or acid gels, have been used in an attempt to restore the lactobacillus population, but their effectiveness has not been proven.

Inflammation of the vagina, **vaginitis**, is a different condition to bacterial vaginosis. Vaginitis can be caused by a number of organisms—in particular, *Trichomonas vaginalis* (see previous section) or *Candida albicans*. *Candida albicans* is a normal inhabitant of the vagina, but in certain circumstances (e.g. during antibiotic usage, immunosuppression, pregnancy, diabetes) its numbers increase sufficiently to cause a vaginitis. It typically causes an intensely irritating (itchy) infection with a yellow, cheesy discharge.

Prevention of STIs

Since the AIDS pandemic began, the practice of 'safe sex' has become the plea of governments and health departments throughout the world. Vaccines have been heralded as the ultimate solution to the global epidemic of STIs but, despite intensive research, the only successful vaccines developed to date for STIs are for hepatitis B and some papillomaviruses. Prevention is therefore based on the following approaches.

Avoidance of high-risk encounters

Avoidance of high-risk encounters is clearly the best method for prevention of STIs. A high-risk encounter is unprotected, penetrative sex without a condom with:

- multiple partners, or with someone who has multiple partners
- someone who uses intravenous drugs
- someone other than a monogamous partner
- a prostitute.

Since HIV and some other sexually transmissible infections can be transmitted by blood, the sharing of needles and syringes in drug usage is also risky behaviour. A major reason for the high incidence of STIs throughout the world is that many infections are asymptomatic and risk of infection is not always obvious.

Barrier contraceptive devices

Barrier contraceptive devices, specifically condoms, are the best alternative to abstinence or safe sex with one partner. The success of the condom is directly related to its structural integrity. In experimental studies, intact latex condoms have been found to be impervious to bacteria and viruses. However, if the device fails (breakage, leakage or slipping off) during use, its effectiveness will clearly be reduced.

Condoms are only partially effective for protection against those diseases in which the organisms may infect all parts of the genital skin—that is, herpes simplex infections and genital warts. 'Natural' condoms made from animal membranes are less effective because they are more easily broken; also, they are not impervious to smaller particles, such as viruses. Other devices, such as the 'female condom' (a sheath or pouch), can also potentially eliminate direct contact with genital secretions.

Spermicides

Spermicides are agents that have been developed for contraceptive purposes. They do this by interfering with sperm viability. Nonoxynol-9 is the most frequently used and best-studied compound. It is a detergent that disrupts the membranes of sperm and also microorganisms. *In vitro* studies have demonstrated that most spermicides have potent antimicrobial and antiviral activity, but the clinical effectiveness of these substances has not yet been established. In addition, there is some concern that the detergent action of spermicides may cause vaginal irritation, which may enhance HIV transmission.

Contact tracing

The tracing of sexual contacts of people with STIs, and their screening and treatment, is a vital part of any prevention program. Unfortunately, this is not always possible or is not performed adequately.

Male circumcision

During the 20th century, male circumcision gained popularity for its perceived health benefits and social reasons. However, the prevalence of this practice subsequently declined markedly in many countries, including Australia. Research over the last decade has shown that removing the foreskin of males has a number of potential health benefits, including lower rates of urinary tract infections in male infants, a lower prevalence of some sexually transmitted ulcerative infections such as syphilis and chancroid, a lower

risk of penile cancer, and a lower risk of acquiring, and therefore transmitting, HIV infection. Recent randomised controlled trials in parts of Africa and South Africa have shown that, following circumcision, the incidence of HIV infection in men can be reduced by more than half. In addition, female partners of circumcised men have a lower risk of cancer of the cervix caused by high-risk oncogenic types of human papillomaviruses.

However, it should be understood that the procedure does not provide complete protection against HIV or other STIs. Indeed, the WHO recommends that male circumcision should be viewed as 'just one element of a comprehensive HIV prevention package that includes the correct and consistent use of condoms, reductions in the number of sexual partners, delaying the onset of sexual relations, avoidance of penetrative sex, and testing and counselling to know one's HIV serostatus'.

The WHO acknowledges that there are a wide range of sociocultural issues to consider in any possible introduction or expansion of male circumcision services.

SUMMARY

- The urinary tract is one of the more common sites of infection.
- In a healthy person, the upper urethra, the urinary bladder and the organs of the upper urinary tract are sterile.

URINARY TRACT INFECTIONS

- Urinary tract infections occur much more frequently in females than in males.
- UTIs represent 30–40 per cent of all hospital-acquired infections.
- The vast majority of urinary tract infections are caused by microorganisms ascending the urethra and reaching the bladder.
- Ascending infections are most often caused by the enteric bacterium, *Escherichia coli*.
- The flushing of microorganisms from the urethra during the normal passage of urine is an important defence mechanism.
- The high incidence of UTI in hospitalised people is partly due to the use of indwelling urinary catheters.
- Asymptomatic bacteriuria usually represents multiplication of bacteria in the bladder urine.
- Cystitis is an acute infection of the bladder, characterised by symptoms of dysuria, urgency and frequency.
- Pyelonephritis is an infection of the kidney(s).
- The specimen most commonly collected for the diagnosis of UTIs is a clean-catch or midstream specimen of urine (MSU).
- The incidence of community-acquired UTI in women may be reduced by promotion of good personal hygiene, adequate fluid intake and regular emptying of the bladder.
- In hospitals, prevention of UTIs is based on avoidance of catheterisation wherever possible.

INFECTIONS OF THE REPRODUCTIVE SYSTEM

- Most diseases of the reproductive (genital) system are transmitted during sexual activity and are referred to as sexually transmissible infections (STIs).
- There is a strong association between the organisms that cause genital ulcer disease and transmission of HIV.

Gonorrhoea

- Gonorrhoea is caused by the bacterium *Neisseria gonorrhoeae* (the 'gonococcus').
- The gonococcus is usually transmitted by direct person-to-person contact.
- In men, gonococcal infection is usually characterised by a urethral discharge and dysuria.
- Up to 50 per cent of infected women have very mild symptoms or are completely asymptomatic.
- Symptomatic infection in women is usually characterised by a cervico-vaginal discharge and cervical oedema.
- In untreated women, the infection may spread further up the genital tract to cause pelvic inflammatory disease (PID).
- All patients with gonococcal infection should be evaluated for other STIs.

Syphilis

- Syphilis is caused by the spirochaete *Treponema pallidum*.
- Transmission of syphilis requires close personal contact.
- An infected woman may transmit the organism to her foetus *in utero*.
- Primary syphilis is characterised by a papular lesion at the site of infection, which breaks down to form a hard-based ulcer called a chancre.
- The secondary stage of syphilis is characterised by the appearance of a red-brown, maculopapular rash.
- In tertiary syphilis, granulomatous lesions (gummas) form in various organs.
- The primary and secondary lesions of syphilis usually contain large numbers of organisms.
- Serology is the mainstay of diagnosis.
- Penicillin is the drug of choice.
- Prevention is based on the practice of safe sex, and contact tracing and screening.

Chlamydial infections

- Genital infections caused by serotypes D to K of *Chlamydia trachomatis* are the most prevalent of all STIs.
- Serotypes L1, L2 and L3 of *C. trachomatis* cause lymphogranuloma venereum (LGV).
- Many infections are asymptomatic or have only mild symptoms.
- Treatment is usually with azithromycin.

Genital herpes

- Herpes simplex virus type 2 (HSV-2) predominates in the genital region.
- Initial infection may be very mild or completely asymptomatic.
- The primary lesions are usually small, grouped vesicles filled with a clear fluid and surrounded by an area of inflammation; they break down after several days to form painful, shallow ulcers.
- The virus travels up the local sensory nerve to the sensory root ganglion where it lies dormant.
- The virus can reactivate and travel back down the nerve where it causes a recurrent attack.
- Treatment with aciclovir, valaciclovir or famciclovir reduces the duration of symptoms and virus shedding.

Genital warts

- At least 30 different types of human papillomaviruses (HPV) are associated with anogenital infections.
- Infection normally occurs through mucous membranes or traumatised skin.
- Genital warts appear as pinkish-brown masses on the penis, vulva, cervix, or perineal or perianal regions.
- Subclinical infection is extremely common.
- A major concern with genital warts is the association of certain virus types with cervical cancer.
- Treatment involves removal of warts.

Donovanosis

- Donovanosis (granuloma inguinale) is caused by the Gram-negative rod *Klebsiella granulomatis*.
- The infection is characterised by nodules, which erode to form granulomatous ulcers.
- Diagnosis of the disease involves microscopic examination of a stained smear from the lesion.
- Treatment is with azithromycin.

Chancroid

- Chancroid is an STI caused by *Haemophilus ducreyi*.
- The disease is manifested by soft, painful genital ulcers. The lymph nodes gradually enlarge and eventually break through the skin, discharging pus.
- Culture is the definitive diagnostic method.

Trichomoniasis

- Trichomoniasis is caused by the protozoan *Trichomonas vaginalis*.
- *T. vaginalis* causes vaginitis in females and urethritis in males, although many infections are asymptomatic.
- *T. vaginalis* infection can result in PID and adverse outcomes in pregnant women.

Non-specific urethritis

- Non-specific urethritis (NSU) is any inflammation of the urethra not caused by *Neisseria gonorrhoeae* or *Chlamydia trachomatis*.

Pelvic inflammatory disease

- Pelvic inflammatory disease (PID) is usually asymptomatic.
- PID is associated with infection of the pelvic organs, particularly the uterus, fallopian tubes, ovaries and peritoneal cavity.
- Most infections are caused by *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, or both.
- The symptoms are extremely variable; the most common is a dull and constant lower abdominal pain.
- Persistent infection can lead to fibrosis and scarring of the fallopian tubes which can result in sterility or ectopic pregnancy.

Bacterial vaginosis

- Bacterial vaginosis is characterised by an abnormal vaginal discharge with an unpleasant odour.
- Bacterial vaginosis is associated with an imbalance in the vaginal microflora.
- Bacterial vaginosis may cause serious complications such as PID and pre-term delivery.

Prevention of STIs

- Avoidance of high-risk encounters is the best method of preventing STIs.
- Barrier contraceptive devices are the best alternative to abstinence or safe sex with one partner.
- Tracing and screening of sexual contacts of people with STIs is vital.

STUDY QUESTIONS

1. Which parts of the urinary and reproductive systems of humans have a normal microbial flora?
2. Why are females generally more susceptible to urinary tract infections than males?
3. How does normal urine flow protect the urinary tract from infection?
4. Why do urinary catheters increase the risk of urinary tract infection?
5. Define the terms 'pyelonephritis', 'cystitis' and 'asymptomatic bacteriuria'.
6. What is an 'MSU'?
7. What is significant bacteriuria?
8. What laboratory results indicate that a urine specimen is of poor quality?
9. What are genital ulcer diseases, and why are they important?
10. How is gonorrhoea usually transmitted?
11. Why is asymptomatic gonorrhoeal infection in women so important?
12. How is syphilis transmitted?
13. What are the differences between primary and secondary syphilis?
14. What are the two main methods for diagnosing syphilis in the laboratory, and what specimens are needed for each?
15. What types of genital infections does *Chlamydia trachomatis* cause?
16. What viruses cause genital herpes?

17. How does the genital herpes virus cause recurrent infections?
18. What are the typical clinical manifestations of genital warts?
19. What important sequela is associated with genital warts in women?
20. How are genital warts prevented?
21. What is donovanosis?
22. What is vaginosis, and how does it differ from vaginitis?
23. What is non-specific urethritis, and what are the common causes?
24. What is pelvic inflammatory disease, and what are the possible serious sequelae of this disease?
25. How is pelvic inflammatory disease diagnosed?
26. List the ways by which sexually transmissible infections can be prevented.

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GLOSSARY

abscess A sac of pus walled off by a layer of fibrin.
acetyl CoA An activated compound which is an important intermediate in carbohydrate metabolism and is also able to enter other metabolic pathways.
acid-fast bacilli Bacteria belonging to the genus *Mycobacterium*, which are identifiable in an acid-fast stain.
acid-fast stain A differential stain used to identify bacteria that belong to the genus *Mycobacterium*. Also called a **Ziehl-Neelsen stain**.
acne A skin disorder that occurs when hair follicles and sebaceous glands become blocked with sebum and infected.
acquired immune system A functional system consisting of a variety of cells and organs that protect the body against pathogens which evade the non-specific defences; it also protects against future attack by the pathogen. Also called **adaptive immune system**, **specific immune system** or **specific defences**.
acquired immunity The body's third line of defence against microbes and foreign substances; this defence is based on the development of antibodies and/or cell-mediated immunity in response to the foreign material.
acquired immunodeficiency syndrome (AIDS) A disease caused by the human immunodeficiency virus which impairs the ability of the body to produce a specific immune response.
activation energy The amount of energy required for a reaction to occur.
active immunisation The use of a vaccine to prevent a specific disease in a person.
active immunity The development of antibodies or other specific defences by the acquired immune system in response to microorganisms or other foreign substances which enter the body.
active site The area of an enzyme which binds specifically to its substrate.
active transport The movement of substances across a membrane against a concentration gradient; requires energy.
acute bronchitis An inflammation of the tracheobronchial tree.
acute glomerulonephritis Inflammation of the kidneys due to the deposition of immune complexes in the glomeruli.
acute inflammatory response A non-specific defence that is the body's response to tissue injury, characterised by redness, heat, swelling and pain.
acute otitis media Acute infection and inflammation of the middle ear.
acute post-streptococcal glomerulonephritis (APSGN) A serious inflammation of the kidneys after a streptococcal infection elsewhere in the body.
adaptive immune system See **acquired immune system**.
adenine A purine base that pairs with thymine in DNA and uracil in RNA.
adenosine triphosphate (ATP) A small molecule which is important for the storage of energy released during metabolism.
adherence The attachment of microorganisms to host tissues or other surfaces.
adhesins The substances or structures on the surfaces of microorganisms that enable them to attach to cell surfaces.
adjuvant A chemical added to a vaccine to enhance its effectiveness.
aerial hyphae Long filaments of fungal cells that grow above the mycelium and bear spores.
aerobe, obligate An organism that can only grow in the presence of oxygen.
aerobic Reaction that occurs in the presence of oxygen.
aerobic respiration The process whereby cells gain energy from the breakdown of organic molecules, using the electron transport chain, with oxygen as the final electron acceptor.
aetiology The study of the cause and origin of a disease.
aflatoxin A carcinogenic toxin produced by *Aspergillus flavus*.
agammaglobulinaemia A primary immunodeficiency disease characterised by a lack of antibodies.
agar A polysaccharide derived from seaweed and used as a solidifying agent for bacterial culture media.
agar plate A solid medium contained in a petri dish for growing bacteria or fungi.
agglutination The clumping of the particles due to their cross-linking by specific antibodies.
AIDS See **acquired immunodeficiency syndrome**.
airborne precautions Precautions that prevent transmission of infectious agents disseminated through airborne droplet nuclei or small particles that remain infective over time and distance when suspended in the air.
airborne transmission The spread of infectious microorganisms through the air, usually over distances greater than one metre from the infected host.
algae A group of photosynthetic eucaryotic organisms.
algal bloom An overgrowth of algae seen on waterways.
allergen An antigen that evokes an allergic response.
allergy See **immediate hypersensitivity**.

alpha interferon (IFN-α) A cytokine secreted by virus-infected leukocytes that protects neighbouring cells from infection.
Ames test A bacterial test used to identify potential carcinogens.
aminoglycosides A group of antibiotics containing amino sugars.
amoeba A single-celled protozoan (eucaryotic organism) that moves by extending pseudopods.
amoebic dysentery A severe form of inflammatory diarrhoea caused by *Entamoeba histolytica*.
anabolism The synthesis of complex organic molecules from simple components in living cells.
anaerobe, obligate An organism that can only grow in the absence of oxygen.
anaerobic A reaction that occurs in the absence of oxygen.
analogue A compound that has a structure similar to the substrate for an enzyme and that can be bound by the enzyme.
anaphylactic shock A potentially life-threatening disorder resulting from a generalised allergic reaction throughout the body.
anaphylatoxins Substances which bind to mast cells, basophils and platelets, triggering the release of histamine and other inflammatory mediators.
anaphylaxis A systemic allergic reaction which occurs when an allergen enters the blood and circulates through the body.
Animalia A kingdom in the Linnaean classification system, containing multicellular eucaryotic organisms that do not have cell walls.
anthrax A zoonosis caused by *Bacillus anthracis* that is characterised by cutaneous, respiratory or intestinal disease.
antibacterial agent A compound that can kill bacteria or inhibit their growth.
antibiotic A chemical substance produced naturally by a microorganism that can kill or inhibit another microorganism.
antibiotic-associated colitis A serious gastrointestinal disease which can sometimes follow antibiotic therapy, caused by the establishment of *Clostridium difficile* in the intestine.
antibody A protein found in blood and other body fluids that is secreted by plasma cells in response to an antigen, and which is capable of binding specifically with that antigen. Also called **immunoglobulin**.
antibody titre The amount of antibody in a person's blood or other body fluid.
antifungal agent A compound that can kill or inhibit fungi.
antigen A foreign substance which, when introduced into the body, activates the acquired immune system and induces an immune response. Also called **immunogen**.
antigen-antibody complex See **immune complex**.
antigen-binding fragments (Fab) The end of the antibody molecule comprising the two arms responsible for binding of antigen.
antigen-binding sites The sites on an antibody molecule where binding to antigen occurs.
antigen-presenting cell A macrophage or other cell that engulfs an antigen and then presents fragments of it to lymphocytes to activate them.
antigenic Capable of inducing an immune response.
antigenic determinant The small part of the whole antigen molecule to which antibodies or activated lymphocytes bind.
antigenic drift A term used for repeated, minor mutations in the genes that code for the antigen of a microorganism.
antigenic shift A major antigenic change in a virus due to a recombination of genes when two different strains of a virus infect the same cell.
antigenic variation A phenomenon in which certain microorganisms are able to repeatedly or progressively change their cell surface antigens, enabling them to evade the immune system.
antihistamine A drug used to alleviate the redness and swelling prominent in some allergic conditions like hay fever and hives.
antimicrobial agent Any compound that kills or inhibits microorganisms. It may be synthetic or naturally occurring.
antimicrobial peptides Small peptides containing fewer than 100 amino acid residues, which protect against a broad range of microorganisms.
antimicrobial stewardship The responsible use and prescribing of antimicrobials especially in hospitals.
antiprotozoal agent A compound that can kill or inhibit protozoa.
antipyretic A drug used to reduce fever.
antiseptic A chemical agent used on skin or living tissue to kill or remove microorganisms without damaging the tissue.
antiserum A fluid derived from blood which contains antibodies.
antitoxin An antibody preparation derived from the serum of immune humans or animals which neutralises a microbial toxin.
antiviral agent A compound that can inactivate viruses.
apoptosis A process of programmed cell death in which a cell begins to kill itself.
APSGN See **acute post-streptococcal glomerulonephritis**.
arbovirus A virus which is transmitted to humans by biting arthropods (e.g. insects and ticks).
Archaea One of the three bacterial domain classifications, containing ancient bacteria such as thermophiles and halophiles.
archaebacteria Procaryotic organisms (bacteria) of ancient origin that lack peptidoglycan in their cell walls.
arthropod vector A member of the phylum Arthropoda (spiders, mites, ticks and insects) that carries an infectious agent from one host to another.

artificial active immunity Immunity produced in response to a vaccination.
artificially acquired active immunity Immunity produced following vaccination.
artificially acquired passive immunity Immunity due to the injection of preformed antibodies derived from another person.
ascariasis A gastrointestinal disease caused by the roundworm *Ascaris lumbricoides*.
aschelminth A roundworm.
asepsis The absence of disease-producing microorganisms.
aseptic meningitis A general term used for any meningitis where microorganisms are not isolated by routine bacteriological culture, especially viral meningitis.
aseptic no touch technique A special form of aseptic practice, in which key parts (of equipment) and sites (of the patient's body) are not touched by hands.
aseptic technique Procedures used to minimise the transfer of microorganisms.
asexual reproduction Reproduction that does not involve cells of different mating strains.
asymptomatic bacteriuria A condition in which a person has large numbers of bacteria in the urine without displaying any symptoms.
athlete's foot A fungal infection of the skin of the foot. Also called ***tinea pedis***.
atopy A predisposition for allergies.
attack rate A measure of the cumulative incidence of a disease among a particular population at risk.
attenuation Any process that substantially reduces or eliminates the disease-producing ability of a microorganism, while still keeping it alive.
atypical pneumonia Pneumonia characterised by diffuse, patchy lesions in the lung, an insidious onset and a non-productive cough.
Australian bat lyssavirus A virus that is related to the rabies virus and that causes a disease similar to rabies.
autoantibody An antibody produced against one's own tissue.
autoimmune disease Tissue damage and disease due to the presence of auto-antibodies.
autoimmune haemolytic anaemia A condition in which red cells are destroyed by autoantibodies in conjunction with complement.
autoclave Equipment used for sterilisation by steam under pressure.
autotroph An organism that uses carbon dioxide from the air as its principal carbon source.
avian influenza A type of influenza that mainly affects birds, but occasionally other animals, including humans.

B cell See **B lymphocyte**.
B cell receptor The antibody on the surface of a B cell that enables it to recognise a specific antigen.
B lymphocyte A cell which is a major component of the humoral immune system, giving rise to antibody-secreting plasma cells when stimulated by antigen.
bacillary dysentery Diarrhoea caused by *Shigella* spp. Also called **shigellosis**.
bacillus (pl: bacilli) A rod-shaped bacterium.
bacteraemia The presence of bacteria in the bloodstream.
bacteria Microorganisms that have a procaryotic cell structure.
bactericidal Able to kill bacteria.
bacteriophage A virus that infects bacteria.
bacteriostatic Able to inhibit bacterial growth.
Bairnsdale ulcer A cutaneous ulcer caused by *Mycobacterium ulcerans*.
basal body A structure that connects flagella to the cell wall and membrane of a bacterium.
basophil A white blood cell which contains granules of histamine and other inflammatory mediators.
BCG vaccine (Bacillus of Calmette and Guerin), a live attenuated strain of *Mycobacterium bovis* used to provide immunity to tuberculosis.
benign malaria A type of malaria in which there is a pattern of intermittent illness and wellbeing, caused by *Plasmodium vivax*, *P. ovale* and *P. malariae*.
benign tumour A mass of abnormal tissue that is not capable of indefinite growth and that does not usually kill the host.
beta interferon (IFN-β) A cytokine secreted by virus-infected fibroblasts, epithelial cells, macrophages and some other body cells that protects neighbouring cells from infection.
beta-lactamase An enzyme produced by bacteria that are resistant to antibiotics such as penicillin and cephalosporin and contain a ß-lactam ring.
beta-lactams Compound containing a five-membered betalactam ring.
binary fission The process of reproduction that involves splitting into two identical daughter cells.
biofilm A multilayer community of bacteria held together on a surface by polysaccharide secretions of some of the bacteria.
biogenesis The idea that living cells can arise only from pre-existing living cells.
biological transmission The method of transmission of a pathogen that involves replication in an insect vector.
biotechnology The industrial use of living cells to produce biological materials.
bioterrorism The fear created by the threat of biological warfare.
biotin A sulfur-containing vitamin.
blepharitis Infection and inflammation of the eyelid margin.
blepharoconjunctivitis Infection and inflammation of the eyelid.
blood agar A nutrient agar culture medium to which defibrinated horse or sheep blood is added.
blood–brain barrier The tightly joined endothelial cells of the brain's capillaries that prevent the passage of most substances and thus help to ensure that the brain's environment remains stable.

blood culture A culture of a blood sample performed in the investigation of a patient with fever or other manifestations of systemic infection.
blue-green algae See **Cyanobacteria**.
boil A large, pus-filled nodule that develops when the deeper areas of a hair follicle become infected by *Staphylococcus aureus*. Also called a **furuncle**.
booster vaccination A second or subsequent dose of a vaccine given to produce large numbers of memory cells and antibody levels in blood that remain high for many years.
botulism A disease of the nervous system caused by a neurotoxin of *Clostridium botulinum*, acquired by consumption of contaminated food.
bovine spongiform encephalopathy A prion disease of cattle that can be transmitted to humans to cause variant Creutzfeld-Jakob disease.
bright-field microscopy A type of compound light microscopy in which the specimen is fully illuminated with light.
broad spectrum antimicrobial agent A chemical which is active against a number of different microorganisms.
bronchiolitis Infection and inflammation of the bronchioles.
bronchopneumonia A diffuse, patchy inflammation of the lungs, with numerous, small discrete foci of consolidation occurring throughout the lungs. Caused by viruses and some bacteria.
brucellosis A systemic zoonosis caused by the bacteria *Brucella melitensis*, *B. abortus* and *B. suis*. Also called **undulant fever** or **Malta fever**.
Bruton's agammaglobulinaemia A primary immunodeficiency caused by incomplete maturation of B lymphocytes.
bubo An enlarged lymph node due to inflammation.
bubonic plague A systemic infection caused by the bacterium *Yersinia pestis*, characterised by large, tender buboes, most frequently in the armpit or groin.
bulla A large blister containing clear fluid, more than 0.5 cm in diameter.

campylobacteriosis A gastrointestinal infection caused by Campylobacter.
candidaemia A systemic infection caused by *Candida albicans*.
candidiasis An infection caused by the yeast *Candida albicans*. Also called **moniliasis** or **thrush**.
CAPD—continuous ambulatory peritoneal dialysis A procedure whereby a patient is able to undergo dialysis without being confined to bed.
capsid The protein coat surrounding the viral nucleic acid.
capsomere One of the protein units that make up the viral capsid.
capsule A protective outer layer present in some bacteria and composed of polysaccharide; also called **glycocalyx** or **slime layer**.
carbapenem A group of ß-lactam compounds with a broad spectrum of antibacterial activity.
carbuncle A large abscess or multiple abscesses in adjacent areas of skin caused by *Staphylococcus aureus*.
carcinogen A substance that can cause cancer.
cardiovascular syphilis A form of tertiary syphilis in which aortic aneurysm and blood vessel and valve damage may occur.
caries Tooth decay.
carrier A person who harbours and continuously sheds a pathogen without showing any symptoms of disease.
case definition The description of a syndrome—used to ensure the disease is accurately identified.
catabolism The process of breakdown of complex molecules in living cells with release of energy.
catalase An enzyme produced by some bacteria, which breaks down hydrogen peroxide.
catalyst A substance that lowers the activation energy of a reaction.
category-specific isolation precautions A method of infection control in which diseases are assigned to categories and the body substances that are considered infective within each category are specified.
CD4+ cell See **T helper cell**.
CD8+ cell See **cytotoxic T cell**.
cell culture Animal or plant cells grown in the laboratory.
cell-mediated hypersensitivity See **delayed-type hypersensitivity**.
cell-mediated immunity An immune response in which T lymphocytes act directly against target cells, release chemicals that enhance inflammation, or activate other defence cells to cause destruction of the target cells. Also called **cellular immunity**.
cell membrane See **cytoplasmic membrane**.
cell wall The outer layer of bacteria and most algal, plant and fungal cells. It provides shape and structural support for the cell.
cellular immunity See **cell-mediated immunity**.
cellulitis An infection of subcutaneous tissue.
centrioles A pair of cylindrical structures which take part in eucaryotic cell division.
cephalosporins A group of ß-lactam antibiotic compounds, derived from the fungus *Cephalosporium*, that inhibit the synthesis of peptidoglycan.
cercariae The free-swimming larval form of flukes (e.g. *Schistosoma*) which are released into the water from the intermediate snail host.
cerebrospinal fluid The fluid that circulates in the space between the inner two meningeal membranes of the brain and spinal cord.
cervicitis Infection and inflammation of the cervix.
cestode Tapeworm.
chain of transmission The route of spread of an infection.
chancre A painless, hard-based ulcer found in the primary stage of syphilis.

chancroid A sexually transmissible disease caused by *Haemophilus ducreyi*, characterised by soft, painful genital ulcers.
chemically defined medium A culture medium in which the amount of each pure chemical compound is known.
chemokine A cytokine that is responsible for the attraction, migration or homing of a certain cell in the body.
chemostat Equipment used to control the chemical composition of the culture medium during microbial growth. Can be used for the continuous culture of microorganisms.
chemotaxis The attraction of phagocytes to sites of damaged tissue or microbial invasion by chemicals released at the site.
chemotherapy The treatment of an illness or infection with a chemical substance.
chickenpox A highly infectious disease characterised by skin lesions caused by the varicella zoster virus.
chikungunya A systemic viral infection caused by the chikungunya virus transmitted by mosquitoes.
chlamydiae Very small bacteria which have a complex life cycle and can only reproduce inside a living cell.
chloramines Organic chlorine compounds used in water purification.
chloramphenicol A broad spectrum antibiotic which inhibits protein synthesis.
cholera An acute and profuse diarrhoeal disease caused by *Vibrio cholerae*.
chromosome A structure containing DNA and carrying hereditary information.
chronic fatigue syndrome (CFS) A condition in which excessive fatigue and general malaise are chronic complaints, often following an initial viral-like illness.
chronic hepatitis The persistence of hepatitis virus antigens in the bloodstream for at least six months.
chronic infection An infection that persists in the body and is accompanied by continuous shedding of the pathogen.
chronic inflammation A type of inflammation in which large numbers of lymphocytes and macrophages are involved, occurring if the acute inflammatory response is unsuccessful in clearing organisms or foreign material from the tissue.
chronic mucocutaneous candidiasis A chronic infection caused by *Candida*, usually seen in immunodeficient patients.
chronic suppurative otitis media A severe, chronic form of middle ear infection.
ciguatera A toxin produced by certain species of marine algae that can cause poisoning if it enters the food chain.
cirrhosis A disease of the liver characterised by reduced liver function and an increase in connective tissue.
clean technique Procedures designed to minimise the spread of infection.
cleaning The mechanical removal of material (visible or not) from the surfaces of objects.
clonal selection A theory proposed to explain the activation of a specific clone of lymphocytes by antigen, giving rise to a larger population of cells with the same specificity.
clone A population of cells all derived from the same parent cell.
coagulase An enzyme produced by pathogenic staphylococci that coagulates fibrinogen to form a deposit of fibrin around the bacterial cells.
coccobacilli Small rod-shaped bacteria which resemble cocci.
coccus (pl: cocci) A spherical bacterium.
codon A specific sequence of three nucleotide bases, responsible for the binding of a particular amino acid.
coenzyme An organic molecule associated with an enzyme and required for enzyme activity.
cofactor A non-protein component of an enzyme that is required for enzyme activity.
collagenase A microbial enzyme that breaks down tissue collagen.
colonisation The process in which microorganisms live and reproduce on the human body without causing disease.
colony-forming units The number of individual bacterial colonies visible on an agar plate. Each colony may have arisen from one or more cells.
colony stimulating factor A cytokine that stimulates certain cells to divide and differentiate.
commensalism A symbiotic association between two organisms in which one benefits without harming the other.
common vehicle transmission Transmission of a pathogen to a number of people from a common source such as food or water.
communicable disease A disease which is transmitted from one host to another.
community-acquired infection An infection acquired in the community, not in a healthcare facility.
community-associated MRSA (CA-MRSA) An MRSA (methicillin-resistant *Staphylococcus aureus*) that is usually acquired in the community.
community strain A strain of microorganism that originates in the community, not from a hospital. A community strain of a microorganism is usually sensitive to antimicrobial drugs.
complement A complex system of proteins circulating in the blood in an inactive state; they are involved in body defences when activated.
complement activation (or fixation) The activation of complement proteins and fixation to a receptor on antibody molecules in antigen-antibody complexes.
complete antigen A foreign substance that stimulates specific lymphocytes, inducing them to produce an immune response.
complex medium A culture medium in which the exact chemical composition of each of the nutrients is not defined.

compound light microscope A microscope with more than one lens, which uses visible light as the illumination source.
condyloma acuminatum Genital warts, appearing as pinkish-brown masses on the penis, vulva, cervix, or perineal or perianal regions.
congenital infection An infection of the foetus that occurs *in utero*.
congenital rubella syndrome A potentially severe rubella infection in the foetus due to the virus crossing the placenta from the mother's circulation.
conidia (conidiospores) Asexual fungal spores that develop on aerial hyphae.
conjugate vaccine A vaccine prepared by combining the desired antigen with another protein.
conjugation The transfer of genetic material from one bacterium to another by means of sex pili.
conjunctivitis Infection and inflammation of the conjunctiva of the eye.
contact precautions Precautions used when there is a risk of direct or indirect contact transmission of infectious agents that are not effectively contained by Standard Precautions.
contact inhibition A property of cells that regulates growth.
contact transmission Spread of a pathogen from one host to another by contact.
contagious Easily spread from one person to another.
contamination The unwanted presence of microorganisms.
continuous cell line A cell culture consisting of cells that can be propagated over many generations.
continuous culture The process of growing and harvesting microorganisms in such a way that optimal growth conditions are maintained.
coronavirus A class of virus surrounded by spikes, responsible for the SARS epidemic.
coryza See **rhinitis**.
Creutzfeldt-Jakob disease (CJD) A disease caused by a prion, characterised by fatal degeneration of brain tissue.
crossing over An event in meiosis in which part of the DNA from one chromosome is exchanged with the DNA from another.
cross-resistance Situation where an organism that has developed a mechanism of resistance to one drug will also be resistant to related drugs.
croup Inflammation of the larynx and trachea, characterised by stridor, hoarseness and a resonant cough.
crust The dried exudate from an erosion or ulcer.
cryptococcosis An infection caused by the fungus *Cryptococcus*.
cryptosporidiosis Intestinal infection caused by *Cryptosporidium parvum*.
crystallisable fragment (Fc) The tail part of an antibody molecule where complement fixation and phagocyte adherence occurs.
culture A growth of microbes in/on a culture medium.
culture medium A preparation of nutrient material for the growth of microorganisms.
cutaneous mycoses Fungal infections which affect the epidermal layers of the skin.
cyanobacteria A group of photosynthetic bacteria, formerly called blue-green algae.
cyst (i) A sac with a defined wall containing fluid or other material—for example, hydatid cyst; (ii) a form of some protozoa and helminths in which the cell is surrounded by a protective layer.
cystitis Infection and inflammation of the urinary bladder.
cytokine A soluble factor released by cells which regulates the activity of other cells involved in body defences.
cytolysis The destruction of cells by damage of their cell membranes.
cytopathic effects The microscopically observable changes to a cell caused by virus infection of the cell.
cytoplasm The contents of cells inside the plasma membrane (excluding the nucleus).
cytoplasmic membrane The membrane containing the cytoplasm of the cell. Also called plasma membrane.
cytosine A pyrimidine base that pairs with guanine in RNA and DNA.
cytotoxic hypersensitivity Destruction of body cells by the binding of antibodies to antigens on the surface of a cell, followed by complement activation and cell lysis. Also called **type II hypersensitivity**.
cytotoxic T cell A type of T lymphocyte which directly kills infected cells that display microbial antigens on their surface.

danger associated molecular pattern (DAMP) A molecule released from damaged tissue cells that enables the innate immune system to detect tissue damage.
dark-field microscopy A type of compound light microscopy using a special condenser so that objects in the specimen appear bright against a black background.
debridement Removal of necrotic tissue from a wound.
decline (or **death**) **phase** The phase of an illness when the acute symptoms have subsided.
decontamination The process of removal of undesirable substances or pathogens from an object or area by cleaning or disinfecting.
definitive host The organism that harbours the adult sexually mature form of a parasite.
degranulation The release of granules containing histamine and other inflammatory chemicals by mast cells and basophils in allergic reactions.
delayed hypersensitivity Hypersensitivity reactions that are mediated by T cells and take a day or more to appear after the introduction of antigen. Also called **cell-mediated hypersensitivity** or **type IV hypersensitivity**.

dendritic cells A group of bone marrow derived cells found in skin and lymphoid tissues that play an important role in antigen presentation to lymphocytes.
dengue fever A systemic infection caused by the dengue virus and transmitted by mosquitoes.
dengue haemorrhagic fever A serious form of dengue fever characterised by bleeding from the gums, skin and gastrointestinal tract.
deoxyribose A pentose sugar which is part of the DNA molecule.
dermatomycosis A fungal skin infection.
dermatophytes Fungi that grow on skin.
dermis The inner layer of the skin.
diapedesis The process by which neutrophils move out of the bloodstream and into the tissue space in inflammation.
diarrhoea A disruption in bowel habits characterised by more frequent passing of loose, watery stools.
differential medium A microbial growth medium that allows the differentiation of one microorganism from another.
dimorphism The ability of an organism to grow in two different forms under different environmental conditions.
diphtheria An acute infection of the upper respiratory tract caused by toxin-producing strains of *Corynebacterium diphtheriae*. The toxin can subsequently cause heart, kidney and nervous tissue disease.
diplococci Spherical bacteria that occur in pairs.
diploid cell A cell that has two sets of chromosomes.
direct contact transmission Transferral of infectious organisms directly from one person to another.
disaccharide A carbohydrate molecule composed of two sugar units.
disease A harmful alteration to the physiological or metabolic state of a host.
disease-specific isolation precautions A set of procedures to be followed to prevent the transmission of a specific disease.
disinfectant A chemical substance normally used for the disinfection of inanimate objects.
disinfection The destruction, removal or reduction in numbers of harmful microorganisms on an object to an acceptable level.
disseminated infection An infection which spreads throughout the body.
disseminated intravascular coagulation The non-specific activation of blood coagulation mechanisms by endotoxin, resulting in the blockage of small vessels by thrombi in a variety of organs.
DNA (deoxyribonucleic acid) The molecule that carries genetic information.
DNA sequencing Analysis of the order of nucleotides in a DNA molecule.
domains Part of the classification system for living organisms.
donovanosis A genital ulcer disease caused by *Calymmatobacterium granulomatis*. Also called **granuloma inguinale**.
dot blot method A method of analysing DNA.
droplet precautions Precautions intended to prevent transmission of infectious agents spread through close respiratory or mucous membrane contact with respiratory secretions.
droplet transmission The spread of infectious agents in small liquid droplets through the air.
dry heat sterilisation A method of sterilisation using dry heat at 160°C for 60 minutes.
dysentery A severe diarrhoea characterised by blood and pus in the stools.
dysuria Difficult or painful passing of urine.

Ebola A haemorrhagic fever with a high fatality rate caused by the ebola virus.
ecology The study of the relationship between organisms and their environment.
ecosystem The living and non-living components of a particular environment.
ectoparasite A parasite that lives on the surface of another organism.
elastase An enzyme that breaks down elastin in tissues.
electron micrograph A photograph of a specimen taken with an electron microscope.
electron microscope A microscope that uses electrons to produce an image of the specimen.
electron transport chain A chain of specialised compounds that can pass electrons along the chain to a final electron acceptor (molecular oxygen), releasing energy as ATP.
elephantiasis A gross enlargement of limbs, scrotum or other body parts caused by repeated infection by the worm *Wuchereria bancrofti*.
ELISA A method of detection of antibodies in blood. From Enzyme Linked Immunosorbent Assay.
Embden-Meyerhof pathway See **glycolysis**.
empirical therapy Treatment based on known or accepted principles, rather than on the results of tests.
empyema Infection of the pleural space.
encephalitis Infection and inflammation of the brain.
endemic disease A disease which is always present in a given population.
endocarditis Inflammation of the internal membrane lining of the heart.
endocytosis The process by which phagocytes take up foreign material.
endogenous infection An infection caused by microorganisms from the patient's own body.
endogenous pyrogen A fever-producing substance derived from body cells.
endophthalmitis An infection and inflammation of the fluid behind the cornea.
endoplasmic reticulum A network of membranes in eukaryotic cells connecting the nuclear membrane to

the plasma membrane and providing sites for ribosomal attachment.

endospore A resistant structure with a thick coat, formed within some Gram-positive bacterial cells.

endotoxic shock A severe, life-threatening form of septicaemia caused by the release of large amounts of endotoxin from Gram-negative bacteria.

endotoxin A lipopolysacccharide which occurs as part of the cell wall of most Gram-negative bacteria and is released when the cell dies. May produce toxic effects in the human host.

endotracheal intubation The insertion of a tube into the trachea to open the airway.

enriched medium A microbial growth medium that contains special growth factors for fastidious organisms.

enteritis Inflammation of the intestine.

Enterobacteriaceae A group of facultatively anaerobic Gram-negative bacteria, many of which are found in the human intestine.

enterotoxin An exotoxin that acts specifically on the intestine.

enzyme A protein molecule that acts as a biological catalyst.

eosinophil A blood cell present in large numbers in allergic reactions.

epidemic A sudden rapid rise in the incidence of a disease in a particular population or area.

epidemic keratoconjunctivitis An infection of the conjunctiva and cornea caused by adenoviruses.

epidemiology The study of the occurrence, spread and control of disease.

epidermis The outer layer of the skin.

epidermolysin See **exfoliatin**.

epididymitis Infection and inflammation of the epididymis.

epiglottitis Infection and inflammation of the epiglottis.

epitope The small part of a whole antigen molecule that is the immunogenic component; the site of binding of antibodies or activated lymphocytes.

ergotamine A toxin produced by the fungus *Claviceps purpurea*.

erosion A superficial, circumscribed loss of epidermis, which heals without scarring.

erysipelas A bacterial skin infection, often on the face, that typically appears as a bright red, swollen lesion with a sharply demarcated edge.

erythema Reddened skin, usually due to inflammation.

erythema migrans A unique skin lesion with a bulls-eye appearance which occurs in the early stage of Lyme disease.

ESBL (extended spectrum beta-lactamase) An enzyme produced by some Gram-negative bacteria that enable them to resist the action of a wide range of β-lactam antibiotics.

eschar The thick crust or scab that forms over a burn.

essential nutrients Substances that are essential for growth.

etiology See **aetiology**.

eubacteria All procaryotes (bacteria) containing peptidoglycan cell walls. Also called **true bacteria**.

Eucarya One of the domains used for classification of living organisms.

eucaryotic Describes organisms that have their DNA enclosed in a nucleus and contain other membrane-bound organelles. Includes animals, plants and fungi.

evidence-based practice The use of research evidence to support changes in clinical procedures.

excoriation An area of skin denuded of epidermis by scratching.

exfoliatin An exotoxin possessed by some strains of *Staphylococcus aureus* that causes layers of cells in the epidermis to separate and sheets of skin to be shed (scalded skin syndrome).

exogenous infection An infection caused by microorganisms from a source external to the patient.

exogenous pyrogen A fever-producing substance derived from outside the body, often components of bacteria, and especially lipopolysaccharides.

exotoxin A toxin secreted by a bacterium into its environment.

exponential growth Growth of a bacterial culture characterised by a doubling of cell numbers in each time period.

exudate An accumulation of plasma fluid and proteins in a tissue during inflammation.

F plasmid Extrachromosomal DNA found in F+ donor cells capable of conjugation.

facilitated diffusion The movement of substances across a membrane from a region of high concentration to a region of lower concentration using carrier proteins.

facultative anaerobes Organisms which can grow in the presence or absence of oxygen.

faecal–oral transmission The mode of transmission of pathogens that enter the body via the gastrointestinal tract and are excreted in faeces.

falciparum malaria The most severe form of malaria, caused by *Plasmodium falciparum*.

false negative A negative test result in a person who actually has the disease.

false positive A positive test result in a person who does not have the disease.

fastidious microorganism A microorganism that requires special growth factors.

fatty acid Long chain aliphatic compound containing a terminal carboxyl group.

fermentation The enzymic breakdown of pyruvate in the absence of oxygen.

fever (pyrexia) Abnormally high body temperature, representing severe or systemic infection and/or inflammation.

fibroblast A cell which gives rise to collagen and connective tissue.

fifth disease A common infection of children characterised by a rash on the cheeks, caused by parvovirus B19.
filamentous fungi Fungi consisting of thin filaments or hyphae which form a mat called a mycelium.
filariasis A systemic infection caused by several different roundworms, most commonly by *Wuchereria bancrofti*.
filtration A method for separation of solids from liquids or gases by passage through special filters which retain the solids. Used to remove bacteria from solutions and air.
fimbriae Hair-like appendages on bacteria, used for attachment to surfaces.
flagella Long thin appendages that are found on some organisms and that enable them to move.
flatworms A primitive group of worms, the main parasitic ones being flukes and tapeworms.
fluid-mosaic model The model proposed for the structure of the cell membrane, consisting of proteins embedded in a phospholipid bilayer.
flukes Flatworms with complex life cycles.
fluorescence microscopy The use of ultraviolet light in a microscope to visualise objects that fluoresce.
follicular T helper cell (fTh) Found closely associated with B lymphocytes in follicles in lymphoid organs and promote antibody secretion from B cells. Produce IL-21, a cytokine that promotes humoral responses.
folliculitis Infection of a hair follicle, usually caused by *Staphylococcus aureus*. Also called a **pimple** or **pustule**.
fomite Any inanimate object that can be involved in the spread of infection.
food biosecurity The prevention of the intentional contamination of food and water with hazardous agents, including pathogens and toxins.
food intoxication A gastrointestinal disease caused by the presence of preformed toxins (microbial or non-microbial) in food.
food poisoning A gastrointestinal disease related to the consumption of food, including microbial and non-microbial causes. Also called **food-borne illness**.
food-borne illness See **food poisoning**.
frame-shift mutation A mutation involving the deletion or insertion of one or more bases, resulting in a change in the codon and the insertion of a different amino acid into the protein.
free radical A very reactive particle containing an unpaired electron.
frequency The need to urinate more frequently than usual.
fulminant hepatitis A clinical syndrome in which there is severe impairment or necrosis of liver cells, often resulting in liver failure and death.
fulminating disease A sudden very severe disease, often with a fatal outcome.
fungaemia The presence of fungi in the bloodstream.
fungi Eucaryotic organisms with cell walls that are not capable of photosynthesis.
furuncle See **boil**.
fusidic acid A bacteriostatic agent that inhibits protein synthesis.
gammaglobulin (i) An antibody preparation extracted from the pooled serum of large numbers of blood donors; (ii) the fraction of blood proteins that contains antibodies.
gamma interferon (IFN-γ) A cytokine secreted by activated lymphocytes and NK cells that is involved in the development of acquired immune responses.
gangrenous infection An infection of the soft tissue below the dermis.
gas gangrene A type of necrotising fasciitis caused by *Clostridium* species.
gastritis Inflammation of the internal lining of the stomach.
gastroenteritis Inflammation of the gastrointestinal tract, including the stomach and intestine.
gastrointestinal infection A disease of the gastrointestinal tract caused by the establishment and multiplication of microorganisms in the gastrointestinal tract.
gene A linear segment of DNA which codes for a particular hereditary characteristic.
generalised transduction The transfer of genetic material from one bacterial cell to another by a lytic phage genital ulcer disease.
generation time The time taken for a bacterial cell to reproduce itself.
genetic engineering Manipulation of the genetic material of an organism in order to alter the characteristics of the organism in a particular way.
genetic recombination Formation of DNA involving the reciprocal exchange of homologous (paired) segments of DNA at any place on the chromosome.
genital ulcer disease Any infection characterised by ulceration of the genital region.
genital warts See **condyloma acuminatum**.
genus Part of the binomial classification system above the level of species—for example, *Staphylococcus* in *Staphylococcus aureus*.
germ theory of disease The principle proposed by Koch that microorganisms can cause disease.
German measles See **rubella**.
germicide A substance that kills microorganisms (germs).
Ghon complex The characteristic finding in tuberculosis of tubercles (small granulomas) in the lung plus enlarged lymph nodes.
giardiasis A gastrointestinal infection caused by the protozoan *Giardia intestinalis*.
gingivitis A periodontal disease involving inflammation or infection of the gums.
glandular fever A systemic infection caused by the Epstein-Barr virus. Also called **infectious mononucleosis**.
glomerular haematuria The presence of blood in the urine due to inflammation or infection of the glomeruli of the kidneys.
glomerulonephritis Inflammation of the glomeruli of the kidneys.
glucan A polysaccharide made up of glucose molecules.
glycerol An organic alcohol containing three carbon atoms. It combines with fatty acids to form triglycerides.

glycocalyx A structure consisting of polysaccharides found outside the cell wall of some bacteria. Also called **capsule** or **slime layer**.
glycogen A branched polysaccharide containing glucose units which acts as a carbon storage molecule.
glycolysis The anaerobic metabolic pathway in which glucose is converted to pyruvate.
Golgi complex A membranous organelle in eucaryotic cells involved in the secretion of proteins.
gonorrhoea A sexually transmissible disease caused by the bacterium *Neisseria gonorrhoeae*.
Gram-negative Describes a bacterium that stains red with the Gram stain.
Gram-positive Describes a bacterium that retains the blue colour of the Gram stain.
Gram stain A standard procedure for staining bacteria which divides almost all bacteria into two groups, and serves as the first step in classifying and identifying them.
granuloma A lesion associated with chronic inflammation that typically consists of a mass of different types of cells arranged in fairly discrete layers, and completely walled off by collagen.
granuloma inguinale See **donovanosis**.
guanine A purine nucleotide base that pairs with cytosine in RNA and DNA.
gumma A soft, granulomatous lesion in the skin, bones, liver or other organs, characteristic of tertiary syphilis.
gut-associated lymphoid tissue (GALT) Clusters of lymphoid cells lining the mucosal surface of the intestinal tract.

HAART (highly active antiretroviral therapy) Treatment of HIV infection with two or more drugs at once in order to limit the development of resistance.
haemagglutination Aggregation (agglutination) of red blood cells.
haematuria The presence of blood in urine.
haemoflagellates A flagellated protozoan usually found in the bloodstream of its host.
haemolysin A toxin that breaks down red cells.
haemolytic uraemic syndrome (HUS) A possible complication of some infections caused by toxigenic strains of *E. coli*, characterised by haemolytic anaemia, uraemia, thrombocytopaenia and renal failure.
haemorrhagic fever A disease characterised by fever, malaise, myalgia, prostration, multisystem involvement and widespread haemorrhage, and caused by a number of different viruses.
half-life The time taken for half of a substance to be used or destroyed.
Hansen's disease An alternative name for leprosy.
hapten A small molecule that cannot initiate an immune response on its own, but which reacts with antibodies or activated T cells produced against it.
healthcare-associated infection (HAI) An infection occurring in a patient in a hospital or other healthcare facility in whom the infection was not present or incubating at the time of admission.
heavy (H) chain The longer polypeptide chain of an antibody molecule.
helminths Worms.
HEPA (high-efficiency particulate air) filter A filter that removes a minimum of 99.97 per cent of particles equal to, or larger than, 0.3 mm in diameter.
hepatitis Injury and inflammation of the liver.
hepatitis A Hepatitis caused by the hepatitis A virus.
hepatitis B Hepatitis caused by the hepatitis B virus.
hepatitis C Hepatitis caused by the hepatitis C virus.
hepatitis D Hepatitis caused by the hepatitis D virus.
hepatitis E Hepatitis caused by the hepatitis E virus.
herd immunity The principle that individuals who are immune to an infectious disease will not be carriers of the organism, reducing the reservoir of that microbe in the community, and therefore the number of susceptible people who will encounter it.
herpes encephalitis Infection of the brain caused by the herpes virus, especially serious in neonates.
heterotroph An organism which requires an organic source of carbon for energy.
hexose A sugar molecule containing six carbon atoms.
Hfr strain A bacterial strain which exhibits a high frequency of recombination due to the insertion of an F+ plasmid into the chromosomal DNA.
histamine A substance present in many body cell types, especially mast cells, basophils and platelets, that causes vasodilation and increased capillary permeability.
histones Proteins associated with DNA in eukaryotic chromosomes.
hookworm A type of roundworm which enters the body by burrowing through the skin and travels via the bloodstream and attaches to the lungs and intestines.
horizontal transmission Transmission of a disease from one person to another, usually by physical contact.
hospital-acquired infection An infection acquired in a hospital. Also called a **nosocomial infection**.
hospital-associated MRSA (HA-MRSA) An MRSA (methicillin-resistant *Staphylococcus aureus*) that is usually acquired in hospitals.
hospital strain A strain of microorganism that originates in hospitals. This organism is likely to be resistant to some antimicrobial drugs.
human leucocyte antigen A major histocompatibility (MHC) protein that is found on the surface of leucocytes.
human metapneumovirus A recently identified virus that is a significant cause of severe respiratory infections worldwide.
humoral immune response An immune response to foreign antigen characterised by the activation of B lymphocytes and the production of specific antibodies against the antigen.
humoral immunity Immunity provided by antibodies present in the body's fluids.

hyaluronidase An enzyme produced by some bacteria which breaks down the hyaluronic acid of connective tissue.
hydatid disease A disease characterised by the production of fluid-filled sacs in the body, containing developing forms of the tapeworm *Echinococcus granulosis*.
hydrophilic Water loving.
hydrophobic Water hating; does not mix with water.
hypersensitivity An over-reaction of the immune system to an antigen which it has previously encountered.
hypertonic A solution with a higher concentration of dissolved substances than is present in a cell.
hyphae Long filaments of cells found in fungi.
hypotonic A solution with a lower concentration of dissolved substances than is present in a cell.

iatrogenic infection An infection resulting from a medical procedure or treatment.
IgA A class of antibody molecules found mainly in mucosal secretions and breast milk.
IgD A class of antibody molecules found on the surface of B lymphocytes.
IgE The class of antibody molecules responsible for allergic reactions.
IgG The major class of antibody molecules in blood, and the class which crosses the placenta from mother to foetus.
IgM The class of antibody molecules in blood that are produced early in response to infection and that are indicative of recent infection.
imidazoles A group of antifungal drugs that inhibit sterol synthesis.
immediate hypersensitivity A hypersensitive reaction that occurs within minutes to hours after a sensitised person comes in contact with a foreign antigen. Also called **allergy** or **type I hypersensitvity**.
immune complex An antibody molecule bound specifically to its antigen.
immune complex hypersensitivity A hypersensitive reaction that results when antigen-antibody complexes are not cleared quickly from the body, instead being deposited in tissues. Also called **type III hypersensitivity**.
immune response The specific response to the presence of a microorganism or foreign substance in the body.
immune surveillance The mechanisms of the immune system that enable it to seek out and destroy tumour cells.
immune system The system of specific and non-specific defences the body has to eliminate foreign microorganisms and substances, and to provide long-term immunity to them.
immunisation The exposure of a person to material that is antigenic but not pathogenic to make them immune to a certain microorganism. Also called **vaccination**.
immunity The capacity of the immune system to successfully defend the body against a potentially infectious agent.
immunocompromised Having a defect in one or more of the key components of the immune system (lymphocytes, phagocytes or complement).
immunodeficiency A condition in which the production or function of one of the key components of the immune system (lymphocytes, phagocytes or complement) is abnormal.
immunodeficiency disease A disease which causes immunodeficiency.
immunofluorescence A method of diagnosis involving labelling with fluorescent antibodies.
immunogen See **antigen**.
immunogenic Capable of inducing an immune response.
immunoglobulin See **antibody**.
immunological memory The property of the immune system which enables it to react more rapidly and vigorously on subsequent exposures to the same antigen.
immunology The study of the body's immune system.
immunosuppression A depletion or reduction of the immune defences of the host.
impetigo A highly infectious skin infection caused by staphylococci, streptococci or both.
in vitro Outside a living organism; in the laboratory.
in vivo In a living organism.
incidence rate The proportion of a population that contracts a disease within a given time period.
inclusions Small deposits found inside cells (e.g. storage granules).
incubation period The period of time which elapses between exposure to a pathogen and the appearance of disease symptoms.
index case The first case of a disease in an epidemic.
indirect contact transmission Transferral of organisms via an intermediate object (fomite) or person.
inducer A substance which stimulates the expression of a gene.
inducible enzyme An enzyme which is synthesised under the influence of an inducer.
induration A raised red area on the skin resulting from tuberculin sensitivity.
infection The growth of pathogenic microorganisms in the body.
infectious disease A disease which is caused by a pathogenic microorganism or its products.
infectious mononucleosis See **glandular fever**.
infective dose The number of microorganisms which must gain entry to the body in order to establish an infection.
infective endocarditis An inflammation of the endocardium caused by a microorganism.
inflammation The body's response to tissue injury, characterised by redness, swelling, heat and pain.
inflammatory diarrhoea Diarrhoea characterised by stools containing blood, mucus and pus.

influenza A respiratory infection caused by influenza A and B viruses.
innate immunity See **non-specific immunity**.
integrase An enzyme that integrates viral DNA into the host cell genome. The human immunodeficiency virus (HIV) has an integrase.
interferon (IFN) (i) A protein secreted by virus-infected cells which helps neighbouring cells to resist infection; (ii) a lymphokine secreted by activated T lymphocytes.
interleukin A cytokine that acts as a chemical messenger between leukocytes.
interleukin-1 (IL-1) A cytokine released by macrophages and other cells that stimulates activated T cells.
interleukin-2 (IL-2) A cytokine that encourages activated T cells to proliferate and differentiate.
intermediate host The host which harbours the immature or larval form of a parasite.
intoxication A disease due to a microbial toxin.
invasive phase The stage in a disease when the pathogen is invading the host and causing severe symptoms.
iodophor An antiseptic preparation containing iodine and an organic compound.
ionising radiation A form of radiation that damages or kills microorganisms by causing disruption to their DNA molecules.
ischaemia Loss of blood supply to a tissue.
isomers Organic compounds with the same chemical formula but different molecular structures.
isotonic A solution containing the same concentration of dissolved substances as is found in a cell.

Japanese encephalitis A type of encephalitis with a high fatality rate caused by the Japanese encephalitis virus spread by mosquitoes.

keratin A tough protein found in skin, nails and hair that is resistant to most weak acids, bases, and bacterial enzymes and toxins.
keratinase A microbial enzyme that degrades keratin.
keratitis Infection and inflammation of the cornea.
keratoconjunctivitis Infection and inflammation of the conjunctiva and cornea.
kinases A group of enzymes produced by certain bacteria that enable them to dissolve fibrin clots.
kinins Small peptides present in blood and other body fluids in an inactive form which, when activated, cause vasodilation, increased vascular permeability and pain.
Koch's Postulates A set of criteria proposed by Robert Koch, which should be met in order to determine whether an organism is responsible for causing a particular disease.
Koplik's spots Found in measles, they are areas of white necrosis on a reddened mucosa in the mouth.
Krebs cycle See **TCA cycle**.
kuru A human prion disease which has been found in people of the Fore tribes in Papua New Guinea.

lactoferrin A substance, present in a number of body secretions, that inhibits the growth of some microorganisms by binding the growth factor iron.
lag phase The period of bacterial growth during which bacteria are exposed to fresh medium but do not increase in number.
Langerhans cell A type of dendritic cell found in skin.
laryngitis Infection and inflammation of the larynx.
laryngotracheitis Infection and inflammation of the larynx and trachea.
lassa fever A haemorrhagic fever caused by the lassa fever virus which is transmitted to humans from rodents.
latent Refers to the presence of a pathogen in the body without replicating.
latent syphilis An asymptomatic period of syphilis infection occurring between the symptomatic primary and secondary stages.
latent viral infection An infection in which the virus remains dormant in the host for long periods without replicating or producing disease symptoms.
legionellosis See **Legionnaires' disease**.
Legionnaires' disease A pneumonia caused by *Legionella pneumophila* or other species of Legionella. Also called **legionellosis**.
leishmaniasis Also called kala azar, a disease transmitted by sandflies, resulting in visceral or skin lesions, caused by species of *Leishmania*.
lepromatous leprosy A form of leprosy in which there is uncontrolled proliferation of the bacterium resulting in extensive skin lesions and nerve involvement.
leprosy An infection of skin and nerves caused by *Mycobacterium leprae*.
leptospirosis A systemic bacterial infection caused by *Leptospira interrogans*.
lesion An area of damaged tissue.
leukocidin A toxin that kills phagocytic leukocytes (neutrophils and macrophages).
leukocyte A white blood cell.
leukocytosis A significant increase in the number of white cells in blood.
leukopenia A decrease below normal numbers of white cells in the blood.
leukotriene A mediator of inflammation synthesised from arachidonic acid, derived from mast cell membranes.
ligand A carbohydrate-specific binding protein involved in adherence.
ligase chain reaction A combined nucleic acid probe and amplification technique for diagnosis of some microbial diseases.
light (L) chain The shorter polypeptide chain of an antibody molecule.
light microscopy The use of a microscope which uses white light to visualise the specimen.
lincosamides A group of antibacterial agents which inhibit protein synthesis.
lipase An enzyme which breaks down fats into glycerol and the component fatty acids.

lipid A An endotoxin which occurs as part of the cell wall of Gram-negative bacteria.
lipopolysaccharide A kind of molecule composed of lipid and polysaccharide units. The lipopolysaccharides which occur as part of the wall of Gram-negative bacteria are called **endotoxins**.
lipoproteins Molecules composed of lipid and protein units.
listeriosis A systemic bacterial infection caused by *Listeria monocytogenes*.
lobar pneumonia An infection and consolidation of the lung confined to certain lobes.
localised infection An infection which is confined to one area of the body.
log phase The period of bacterial growth when the bacteria are dividing at their maximum rate and cell numbers are increasing exponentially.
logarithmic growth Growth of a bacterial culture during which there is a doubling of cell numbers in each specified time interval.
Lyme disease A systemic infection caused by the spirochaete *Borrelia burgdorferi*.
lymph nodes Small, bean-shaped organs occurring throughout the lymphatic system and important in immune responses.
lymphadenopathy A swelling of the lymph nodes.
lymphocyte A white blood cell that plays a critical role in immune responses.
lymphogranuloma venereum A sexually transmissible disease caused by *Chlamydia trachomatis*.
lymphokines Cytokines secreted by activated T helper cells.
lyophilisation Removal of water under controlled conditions.
lysis The rupture of a cell due to osmotic pressure or viral infection.
lysogeny A state in which phage DNA is incorporated into the DNA of the bacterial cell that it has infected, but does not replicate or cause cell lysis.
lysosomes Organelles that contain digestive enzymes; particularly prominent in phagocytes.
lysozyme An enzyme found in body secretions that breaks down the cell wall of many bacteria.
lytic cycle Infection of a cell by a virus resulting in lysis (destruction) of the host cell.

macrolides A group of bacteriostatic antibiotics which affect protein synthesis; used as an alternative for people who are allergic to penicillin.
macrophage A large phagocytic cell found in most tissues and organs of the body.
macule A circumscribed, flat area of altered skin colour.
mad cow disease See **bovine spongiform encephalopathy**.
major histocompatibility complex (MHC) The gene complex which codes for proteins that are used as recognition molecules by lymphocytes. The proteins are important antigens in transplant rejection.
malaise A general feeling of being unwell.
malaria A systemic infection caused by protozoa of the genus *Plasmodium*.
malignant tumour A mass of abnormal tissue that grows rapidly and metastasises, and that usually kills the host.
Malta fever See **brucellosis**.
Mantoux skin test A skin test for immunity to tuberculosis. Also called the tuberculin skin test.
Marburg A haemorrhagic fever with a high fatality rate caused by the Marburg virus.
margination The process by which neutrophils stick to the inner walls of the capillaries prior to their movement through the capillary wall and into the tissues.
mast cell A cell found throughout the body that contains histamine and other inflammatory mediators.
MBL metallo beta-lactamase An enzyme produce by some Gram-negative bacteria which provides the organism with resistance to β-lactam antibiotics.
measles A systemic disease characterised by a skin rash caused by the rubeola virus. Also called **rubeola**.
measles encephalitis A rare complication of measles infection in which the virus persists in brain tissue.
mechanical transmission The transmission of an infectious agent on the outside of an insect's body or on a person's hands.
mediators of inflammation The chemicals that are responsible for the physiological events that occur in inflammation.
medical asepsis Procedures that limit the number, growth and spread of microorganisms by practices such as good hygiene, handwashing and disinfection.
meiosis The process in reproduction that leads to a halving of the number of chromosomes in a eucaryotic cell, from diploid to haploid.
melioidosis An infection caused by the bacterium *Burkholderia pseudomallei*. Usually a pneumonia, but the organism can form abscesses in many body organs.
membrane attack complex The components of the complement system that damage membranes of cells, resulting in their lysis.
memory cell A lymphocyte that persists in the body for months to years and provides long-term immunity.
meninges The three membranes that surround the brain and the spinal cord.
meningitis Infection and inflammation of the meninges.
meningococcaemia The presence of *Neisseria meningitidis* in the bloodstream.
meningococcal meningitis Meningitis caused by *Neisseria meningitidis*.
meningococcus Another name for *Neisseria meningitidis*.
merozoite A form of the malarial parasite found in the liver or infected blood cells.
merozoites A stage in the life cycle of the malaria parasite. Merozoites are released from the liver into

the bloodstream and produce the typical symptoms of malaria at regular intervals.

mesophile An organism which grows in the temperature range between about 10°C and 50°C.

messenger RNA (mRNA) The type of RNA that carries the genetic message from DNA to the ribosome where it acts as a template to direct the incorporation of the correct amino acids into protein.

metabolic pathway A sequence of enzymically directed reactions in which the product of one reaction serves as the substrate for the next reaction.

metabolism The sum of all the chemical reactions which occur in a living cell.

methicillin-resistant *Staphylococcus aureus* (MRSA) A strain of *Staphylococcus aureus* that is resistant to methicillin and usually a number of other antimicrobial drugs.

metronidazole A widely used antiprotozoal drug.

microbiota Normal microorganisms present on the human body.

microfilaria A microscopic roundworm larva which may ultimately block the lymph capillaries and cause elephantiasis.

mid-stream specimen of urine (MSU) A specimen of urine that is collected after the first few millilitres is allowed to pass.

miliary tuberculosis A serious form of tuberculosis in which the bacterium spreads throughout the body.

minimum inhibitory concentration (MIC) The lowest concentration of an antimicrobial agent which will prevent the growth of a particular microorganism.

mitochondria Membrane-rich organelles found in eukaryotic cells; the site of oxidative phosphorylation reactions which produce ATP.

mitosis The process by which eucaryotic nuclei divide, maintaining the same number of chromosomes.

MMR vaccine A vaccine to protect against measles, mumps and rubella.

molluscum contagiosum A skin infection caused by viruses of the pox group.

moniliasis See **candidiasis**.

monocyte A phagocytic blood cell that is the precursor of the tissue macrophage.

monosaccharide A simple carbohydrate consisting of a single sugar unit containing five or six carbon atoms.

morbidity (i) The incidence of disease in the total population; (ii) the state of being diseased.

morphology The external appearance.

mortality The number of deaths that result from a particular disease.

moulds Filamentous fungi that are capable of growth in many different habitats.

mucinase An enzyme produced by some bacteria which digests a component of mucus, enhancing their ability to colonise mucous membranes.

mucociliary escalator The ciliated epithelium of the upper respiratory tract which moves mucus and anything trapped in it upwards away from the lungs.

mucocutaneous Relating to the mucous membranes and the epithelium.

multi-drug resistant organisms Microorganisms that are unaffected by multiple types of antibiotics.

multi-resistant *Staphylococcus aureus* (MRSA) A strain of *Staphylococcus aureus* that is resistant to a number of antimicrobial drugs. These strains are responsible for many infections in hospitals.

mumps A systemic viral infection in which swelling of the parotid glands is the most characteristic clinical feature.

Murray Valley encephalitis A severe, epidemic type of encephalitis caused by the Murray Valley encephalitis virus spread by mosquitoes.

mutagen An agent which can cause a mutation.

mutation A permanent change in the structure of DNA.

mutualism A symbiotic relationship between two organisms in which both organisms benefit.

mycelium A mass of fungal hyphae which intertwine forming a mat.

mycology The study of fungi.

mycoplasmas Very small bacteria which lack a cell wall.

mycosis A fungal infection.

naive lymphocyte A mature lymphocyte that has not encountered its specific antigen.

naked virus A virus that is not surrounded by an envelope.

narrow spectrum antimicrobial agent A drug which is active against a limited number of microrganisms.

narrow spectrum drug An antimicrobial agent which is active against only a few species of organisms.

natural immunity See **non-specific immunity**.

natural killer (NK) cells A group of non-specific lymphoid cells that destroy cancer cells and virus-infected cells.

naturally acquired active immunity Immunity that develops after an infection.

naturally acquired passive immunity Immunity due to the natural transfer of antibodies—for example, immunity acquired by the foetus via transplacental transfer of antibodies.

necrosis Tissue death.

necrotising fasciitis Infection and inflammation in the soft tissue below the dermis (the superficial fascia).

necrotising infection Infection of the soft tissue below the dermis.

nematode A roundworm.

neoplasia The alteration of cellular DNA in such a way that the cell multiplies uncontrollably.

neoplasm The tumour formed when cellular DNA is altered by an external agent.

nephrotoxicity Able to cause damage to the kidneys.

neurosyphilis A form of tertiary syphilis in which lesions form in the central nervous system.

neurotoxin An exotoxin which acts predominantly on nervous tissue.

neutralisation A process in which antibodies bind to and block specific attachment sites on viruses or bacterial exotoxins.
neutropenia A decrease in the number of neutrophils in the blood.
neutrophil The most abundant type of white blood cell. It is highly phagocytic and actively motile. Also called a **polymorphonuclear leukocyte (PMN)**, **polymorph** or **pus cell**.
neutrophilia A significant increase in the number of neutrophils in blood. It is a characteristic sign of severe bacterial infection.
Nipah encephalitis A type of encephalitis caused by the Nipah virus spread from bats to pigs to humans.
nitrogen fixation The ability of some microorganisms to use nitrogen from the air to grow.
nodule A circumscribed, elevated area of skin, larger than 1 cm in diameter.
non-communicable diseases Diseases which are not usually spread from person to person.
non-inflammatory diarrhoea A diarrhoea characterised by watery stools without blood, mucus or pus.
non-polar Describes a substance which does not carry any positive or negative charge on its molecules.
non-specific defences Defences that offer general protection against all potentially harmful agents. These defences include the skin and mucous membranes, cellular defences, inflammation, antimicrobial proteins and fever.
non-specific immunity Immunity provided by the non-specific defences to prevent the entry of pathogens into the body, or to immediately destroy them if they do manage to enter. Also called **innate immunity** and **natural immunity**.
non-specific urethritis Any infection of the urethra not caused by *Neisseria gonorrhoeae* or *Chlamydia trachomatis*.
nonsense codon A codon that does not code for an amino acid.
normal flora The microorganisms that inhabit the human body without causing disease.
normal immunoglobulin An immunoglobulin preparation containing a mixture of antibodies that is extracted from the serum of large numbers of blood donors.
nosocomial infection An infection that is acquired in a hospital or other healthcare facility.
nuclear membrane The membrane enclosing the nucleus.
nucleocapsid Refers to the protein-nucleic acid complex of the virus.
nucleoside A compound consisting of a purine or pyrimidine base joined to a pentose sugar molecule.
nucleotide A nucleoside joined to one or more phosphate groups.
nucleus An organelle in eucaryotic cells where chromosomes are located.
obligate aerobe See **aerobe, obligate**.
obligate anaerobe See **anaerobe, obligate**.
oedema Swelling of a tissue due to an accumulation of fluid.
oncogene A gene which, when activated in certain conditions, leads to malignant transformation of the cell.
oncogenic Cancer-causing.
onychomycosis See **paronychia**.
oophoritis Inflammation of the ovaries.
ophthalmia neonatorum An eye infection in neonates caused by *Neisseria gonorrhoeae*, acquired from the mother during passage through the birth canal.
opportunism The occurrence of an action that would not usually occur unless under favourable conditions.
opportunistic infections Infections caused by organisms that do not usually cause disease, but can become pathogenic under certain conditions, such as depression of the immune system.
opsonin A serum protein (antibody or complement) that enhances phagocyte activity.
opsonisation The enhancement of phagocyte activity with opsonins.
orchitis Infection and inflammation of the testicles**.**
organelle A structure bounded by a membrane and found within eucaryotic cells.
organic Describes a compound containing carbon.
ornithosis See **psittacosis**.
osmosis The movement of water from an area of high water concentration to one of low concentration across a selectively permeable membrane.
osmotic pressure The pressure required to prevent the movement of water by osmosis.
osteomyelitis Infection and inflammation of a bone.
otitis media Infection and inflammation of the middle ear.
ototoxicity Able to cause damage to the ears or hearing.
outbreak of infection The occurrence of a number of cases of the disease in excess of the number expected in a given time or place.
oxidation The loss of electrons from a molecule.
oxidative phosphorylation The process whereby the energy of electrons from oxidation reactions is coupled to the synthesis of ATP.

pandemic An epidemic that spreads worldwide.
papule A circumscribed, elevated area of skin, less than 1 cm in diameter.
parasitaemia The presence of protozoa or helminths in the blood.
parasite An organism that derives its nutrients from another living organism (the host).
parasitism A symbiotic relationship in which one organism benefits while the other, the host, is harmed.
parenteral Entering the body through a route that breaches the skin, such as subcutaneous or intramuscular, injection, intravenous infusion.
paronychia An infection of the finger or toe nail.

passive immunisation Immunisation involving the transfer of preformed antibodies to an individual.
passive immunity The immunity resulting from the transfer of pre-made antibodies or immune cells from an immune person to a non-immune person.
passive transport The movement of substances across a membrane without expenditure of energy.
pasteurisation A method of mild heat treatment developed by Pasteur to destroy microorganisms responsible for food spoilage and some pathogens.
pathogen An organism that is able to produce disease.
pathogen associated molecular patterns Molecules found on microorganisms, but not on human cells.
pathogenicity The ability of a microorganism to cause disease.
pattern recognition receptor A receptor on phagocytes that enables them to detect and attach to microorganisms.
pediculosis A lice infestation usually involving the scalp, pubic area or trunk.
pelvic inflammatory disease A clinical syndrome due to infection of the pelvic organs, particularly the uterus, fallopian tubes, ovaries or the whole peritoneal cavity, which may lead to scarring and blockage of the fallopian tubes.
penicillin The first antibiotic to be discovered; one of a group of bacteriocidal compounds which inhibit cell wall synthesis.
pentose A sugar molecule containing five carbon atoms.
peptide A chain of amino acids joined by peptide bonds.
peptidoglycan A major structural component of bacterial cell walls.
perforins Protein molecules which are inserted into the plasma membrane of a target cell bringing about its death.
perinatal infection An infection acquired at birth.
peritonitis Infection and inflammation of the peritoneal cavity.
personal protective equipment The barriers used to protect the mucous membranes, airways, skin and clothing of the healthcare worker and/or patient from contamination with infectious microorganisms.
pertussis A potentially severe infection of the respiratory tract caused by *Bordetella pertussis*. Also called **whooping cough**.
petechia A small flat haemorrhage.
phage See **bacteriophage**.
phage typing A method of identification of different strains of bacteria by determining their susceptibility to infection by specific bacteriophage.
phagocyte A cell that actively ingests and digests foreign material in the body.
phagolysosome A structure in a phagocyte derived from the fusion of a phagosome with lysosomes and in which lysosomal enzymes break down foreign material.
phagosome A phagocytic vacuole which contains ingested foreign material formed within the cytoplasm of a phagocyte.
pharyngitis Infection and inflammation of the oropharynx.
pharyngoconjunctival fever A syndrome of sore throat and conjunctival infection caused by adenoviruses.
phospholipid A compound consisting of glycerol, two fatty acids, phosphate and various bases.
phospholipid bilayer A double layer of phospholipid molecules which form the basic structure (matrix) of cell membranes.
photosynthesis The process occurring in green plants in which energy from the sun is used to synthesise carbohydrate (glucose) from carbon dioxide and water.
pili Appendages on a bacterial cell some of which are used to transfer DNA during conjugation and some of which are for attachment to surfaces.
pinworm A tiny roundworm that lives in the human intestine. Also called threadworm.
pityriasis versicolor A skin infection caused by the yeast *Malassezia furfur*. Also called tinea versicolor.
plague An infection of lymph nodes and other organs caused by the bacterium *Yersinia pestis*.
Plantae Plants, one of the original Linnaean classifications.
plaque A coating of microorganisms and organic matter on tooth enamel.
plasma The fluid part of blood without the cells and platelets.
plasma cell The cell responsible for synthesis and secretion of antibodies in humoral immunity.
plasma membrane See **cytoplasmic membrane**.
plasmid A small piece of cyclic DNA found in some bacteria which replicates independently of the chromosomal DNA.
plate count A method of determining the number of bacteria in a sample.
platyhelminthes Flatworms.
pleomorphic Occurring in more than one form.
pleural effusion The entry of an inflammatory exudate into the space between the lung tissue and pleural membranes.
pleurisy Inflammation of the pleural membranes.
pneumonia Infection and inflammation of the lung(s).
pneumonic plague Infection of the lungs by the plague bacterium, *Yersinia pestis*.
point mutation The substitution of one base for another in DNA, causing mutation.
polar Refers to a molecule which carries a positive or negative charge.
polio (poliomyelitis) A systemic infection caused by the polio virus in which infection of the central nervous system may occur and may result in paralysis.
polyenes A group of antifungal compounds which interfere with sterol synthesis.
polymerase chain reaction A technique used to copy a specific nucleotide sequence contained within a

sample of DNA, thus increasing the quantity of DNA to detectable levels.

polymorph See **neutrophil**.

polymorphonuclear leukocyte (PMN) See **neutrophil**.

polypeptide A long chain of amino acids, joined by peptide bonds.

polyribosome A ribosome complex where a number of ribosomes are bound along a strand of mRNA.

polysaccharide A polymer of sugar units.

Pontiac fever A mild form of *Legionella* infection, characterised by myalgia, fever, malaise and headache.

portal of entry The point of entry of a pathogen into the body.

portal of exit The point at which a pathogen exits from the body.

post-operative infection Any infection that occurs as a result of surgery.

post-polio syndrome A syndrome in which symptoms of muscular weakness occur late in life in people who have recovered from childhood polio.

post-streptococcal glomerulonephritis Infection of the glomeruli of the kidneys, following a streptococcal infection elsewhere in the body.

PPD (purified protein derivative) The antigen used in the Mantoux skin test for immunity to tuberculosis.

precipitation The cross-linking of soluble antigens by antibody to form large complexes which precipitate out of solution.

prevalence rate The proportion of people with a particular disease at a given point in time.

primary cell line Animal cells extracted from live tissue which are grown in tissue culture in the laboratory.

primary immune response The response of the acquired immune system the first time it is exposed to a particular antigen.

primary immunodeficiency Abnormal production or function of phagocytes, lymphocytes or complement due to a genetic or developmental defect.

primary infection An acute infection which is the cause of the initial illness.

primary lymphoid organs The thymus and the bone marrow which are the sites of lymphocyte maturation.

primary metabolite A compound produced during the active growth phase of a microorganism.

primary prevention In public health, the maintenance of health by good hygiene, nutrition and vaccination.

primary syphilis The first stage of syphilis infection characterised by the development of a papular lesion at the site of infection.

prion An infectious protein agent that causes fatal, neurodegenerative diseases in humans or animals.

probe A small fragment of DNA containing a nucleotide sequence which is specific to the particular organism being looked at.

probiotics Formulations of microorganisms that, when ingested or applied to the body in some other way, may be beneficial to one's health.

Procaryota The classification kingdom which contains all the bacteria.

procaryotic Having characteristics of the procaryotes, including a lack of subcellular organelles.

prodromal period The period in the course of a disease when non-specific symptoms are present before the onset of specific signs or symptoms.

proglottid Segment of a tapeworm.

prophage Bacteriophage DNA which has been integrated into the bacterial host DNA.

prophylactic antibiotic An antibiotic administered in order to prevent the occurrence of an infection.

prophylaxis The administration of a drug to prevent a disease.

prostaglandins A group of biologically active substances, some of which are mediators of inflammation.

prosthetic group A molecule bound to an enzyme that is necessary for enzyme activity.

protease An enzyme that breaks down proteins. The human immunodeficiency virus (HIV) has a protease that is crucial in the formation of new virus particles.

protective asepsis Infection control procedures designed to prevent the transmission of an infection to or from a patient.

proteinuria The presence of protein in the urine.

Protista The biological kingdom comprising unicellular eucaryotic organisms.

protoplast A bacterium without its cell wall, enclosed by the cell membrane.

protozoa Single-celled eucaryotic organisms belonging to the kingdom Protista.

pseudopod 'False foot'—an extension of the cell membrane allowing the cytoplasm to flow into it.

psittacosis A pneumonia caused by *Chlamydia psittaci*, usually acquired from birds. Also called **ornithosis**.

psychrophile An organism which grows best in cool temperatures (15–20°C).

puerperal fever An acute streptococcal infection of the uterus and surrounding area, associated with childbirth, also known as childbed fever.

pulse field gel electrophoresis A technique for analysis of DNA composition.

PUO—pyrexia of unknown origin The presence of a fever without any obvious infection.

pure culture A culture consisting of only one species of organism.

purpura Numerous petechiae; small flat haemorrhages on the skin.

pus A thick, creamy-coloured fluid comprising a mixture of dead or dying neutrophils, tissue debris and remaining pathogens.

pus cell See **neutrophil**.

pustule See **folliculitis**.

PVL Panton-Valentine leukocidin A toxin produced by some strains of community-acquired MRSA that attacks neutrophils and promotes inflammation.

pyelonephritis Inflammation of one or both kidneys.

pyogenic Pus forming.
pyrexia Fever.
pyrogen A substance which produces fever.
pyruvic acid A compound containing three carbon atoms, the main product of the metabolism of glucose via the glycolytic pathway.
pyuria The presence of large numbers of leucocytes (pus) in urine.

Q fever A pneumonia caused by the bacterium *Coxiella burnetii*.
quinine An antimicrobial drug extracted from the bark of the chinchona tree.
quinolones A group of antibacterials which interfere with DNA synthesis.
quorum sensing A mechanism, using chemical signals, with which bacterial cells communicate with each other to regulate activities such as biofilm production and toxin secretion.

rabies An infection of the central nervous system caused by the rabies virus.
recombinant DNA DNA formed from two different species.
regeneration The restoration of a tissue's orginal structure and function after damage by the replacement of cells by mitosis.
repression The process whereby a repressor molecule prevents the synthesis of a protein.
repressor A protein that interferes with the expression of a gene.
reservoir of infection A site where microorganisms persist and which acts as a continual source of infectious agents.
resident flora See **normal flora**.
resistance The ability of the body to prevent the occurrence of an infection.
resistance factors (R) Genes which bestow antimicrobial resistance on bacteria, usually carried on plasmids.
respiration In metabolism, the aerobic breakdown of carbohydrates with release of energy.
restriction endonucleases Enzymes derived from bacteria which are able to cut double-stranded DNA at specific sites on the DNA molecule. Used in genetic engineering.
Restriction Fragment Length Polymorphisms (RFLPs) Fragments of DNA produced by digestion with restriction endonucleases and used to characterise the DNA by electrophoresis.
retinitis Inflammation of the retina.
retroviruses A class of single-stranded RNA viruses which replicate by first synthesising DNA from RNA.
reverse barrier nursing See **protective asepsis**.
reverse transcriptase A viral enzyme that transcribes RNA into DNA.
Reye's syndrome A sometimes fatal disease occasionally seen in children after a viral infection which was treated with aspirin.
rheumatic fever A non-infectious complication of *Streptococcus pyogenes* sore throat in which immune damage to heart and other tissues occurs.
rheumatic heart disease Scarring and deformation of the heart valves due to rheumatic fever.
rheumatoid arthritis A combination autoimmune disease and immune-complex hypersensitivity which causes chronic inflammation and joint damage.
rheumatoid factor An anti-IgG antibody found in the blood of people with rheumatoid arthritis.
rhinitis Infection and inflammation of the nasal passages. Also called coryza.
ribose A pentose sugar which occurs in ribonucleic acid.
ribosomal RNA (rRNA) A large molecule that combines with protein to form particles called ribosomes, which act as a support framework or site of protein synthesis in all cells.
ribosomes Particles containing RNA and protein found in the cytoplasm and acting as the site of protein synthesis.
rickettsiae Small intracellular bacteria.
rifampicin An antibiotic which inhibits RNA synthesis, used to treat TB.
Rift Valley fever A haemorrhagic fever caused by the Rift Valley fever virus, which is transmitted to humans by mosquitoes and aerosols from infected animals.
ringworm A skin infection caused by several different fungi. Also called **tinea**.
RNA (ribonucleic acid) Nucleic acid which takes part in protein synthesis.
Ross River fever A systemic infection caused by the Ross River virus which is transmitted by mosquitoes.
rubella A systemic disease characterised by a skin rash and caused by the rubella virus. Also called **German measles**.
rubeola See **measles**.

Sabin vaccine The oral poliomyelitis vaccine containing an attenuated virus.
Salk vaccine The inactivated poliomyelitis vaccine administered by intramuscular injection.
salmonellosis A mainly gastrointestinal infection caused by species of Salmonella.
salpingitis Inflammation of the fallopian tubes.
sanitisation Thorough cleaning of an object to remove most microorganisms.
SARS Severe acute respiratory syndrome; a type of pneumonia caused by a coronavirus.
scabies A skin infestation caused by the mite *Sarcoptes scabiei* var. *hominis*.
scalded skin syndrome A skin disorder caused by strains of *Staphylococcus aureus* which produce the exfoliatin toxin.
scale An abnormal accumulation of keratin in skin.

scanning electron microscope An electron microscope that provides a three-dimensional image of a specimen at very high magnifications.
scar tissue replacement Replacement of damaged tissue with scar tissue, which does not have the physiological functions of the original tissue.
scarlet fever An erythematous skin rash caused by some strains of *Streptococcus pyogenes* which produce an erythrogenic toxin.
schistosomiasis A debilitating systemic disease caused by flukes of the genus Schistosoma.
schizogony The process of multiple fission in which one cell divides many times to produce multiple daughter cells.
scolex The head of a tapeworm.
secondary immune response The immune response resulting from exposure to the same antigen for the second time. The response is faster, stronger and longer lasting than a primary response.
secondary immunodeficiency Immunodeficiency resulting from damage or suppression of otherwise normal components of the immune system by infection, cancer, malnutrition, drugs or other therapies.
secondary infection An infection that occurs following a primary infection, when the patient is in a weakened state.
secondary lymphoid organs The collection of lymphoid organs and tissues that are the sites in the body where immune reactions occur.
secondary metabolites Products of microbial metabolism, usually accumulating in the stationary phase.
secondary prevention In public health, the development of tests and screening programs to promote good health.
secondary syphilis The secondary stage of syphilis characterised by a skin rash or lesions on mucous membranes.
selective medium A microbial culture medium that allows some organisms to grow but not others.
selective toxicity The ability of an antimicrobial agent to inactivate or destroy a microorganism without harming the host cell.
semi-permeable membrane The membrane surrounding most living cells that controls the passage of substances in and out of the cell. Also called selectively permeable membrane.
sepsis Poisoning due to infection by microorganisms.
septic shock A life-threatening disorder due to the presence of large amounts of endotoxin in the body.
septicaemia A serious clinical syndrome resulting from the presence of bacteria in the bloodstream.
serial dilution A method used to dilute a sample so that the number of bacteria present can be counted accurately.
seroconversion The development of antibodies to an antigen in a person's blood.
serology The detection of antibodies or antigens in a sample of blood or other body fluid.
seroprevalence The prevalence of a disease in the community as determined by the number of people who are carrying antibodies to the disease at any one time.
serotypes Different antigenic strains of a microorganism.
serum The liquid part of blood after the cells and clotting factors have been removed.
sex pilus See **pili**.
sexually transmissible infection An infection acquired during sexual activity.
shigellosis See **bacillary dysentery**.
shingles A disease caused by the reactivation of the varicella zoster (chickenpox) virus characterised by painful, vesicular lesions on the skin.
significant bacteriuria A bacterial count of 108/L or more in a properly collected mid-stream specimen of urine.
signs of disease Measurable changes which can be observed in the patient.
sinusitis Inflammation of the sinus cavities.
slime layer See **glycocalyx**.
smallpox A serious systemic disease that has been eradicated from the world.
source of infection The individual or object from which an infection is acquired.
southern blot A technique for DNA analysis.
specialised transduction The accidental inclusion of a piece of chromosomal DNA adjacent to a prophage when the prophage is transferred to another cell.
species A group of organisms with similar properties; the second name in bacterial classification.
specific defences See **acquired immune system**.
specific immune system See **acquired immune system**.
specific immunoglobulin (SIG) An antibody preparation obtained from the serum of people who are convalescing from a particular infection and have a large amount of a specific antibody.
spermine An antibacterial substance found in semen.
spirillum A spiral-shaped bacterium.
spirochaete A spiral-shaped bacterium with an axial filament.
spongiform encephalopathy A prion disease producing microscopic vacuoles in the brain, giving it a spongy appearance.
spontaneous generation The theory that life can arise spontaneously from non-living matter.
sporadic disease Occasional or random outbreak of disease.
sporangiophore An aerial hypha which carries a sporangium.
sporangiospores Fungal spores produced in a sporangium.
sporangium A sac containing spores.
spore An asexual reproductive structure formed by fungi and actinomycetes. See also **endospore**.
spore coat The thick protective layer formed around a bacterial endospore.
sporozoan A kind of non-motile protozoan.

sporozoite A trophozoite of malaria which occurs in the salivary glands of mosquitoes.
sputum A specimen from the lungs produced from a deep cough.
staining The application of a stain or dye to microorganisms prior to examination under a microscope.
Standard Precautions The work practices that should be applied to all people, regardless of their infection status.
staphylococci A genus of Gram-positive bacteria characterised by small round cocci which occur in clusters.
staphylokinase An enzyme produced by *Staphylococcus aureus* which breaks down fibrin clots.
starch A carbohydrate polymer consisting of branched chains of glucose units.
stationary phase A stage in growth of a bacterial culture when the number of new cells being produced equals the number of cells which die.
stereospecificity The way in which the spatial configuration (shape) of a molecule determines its ability to bind to another molecule.
stereoisomers Two molecules which have the same chemical structure, but differ in shape; mirror images of each other.
sterile technique Methods used to ensure that an object or area is free of all microorganisms.
sterilisation The complete destruction or removal of all microorganisms, including viruses and endospores, from an object.
sterility The complete absence of any microorganisms.
strain Genetically different cells in a species.
streak plate method The method by which microorganisms are isolated as separate colonies on an agar plate.
streptococci A genus of Gram-positive bacteria which occur as chains of cocci.
streptokinase An enzyme produced by some streptococci which breaks down fibrin and which is used therapeutically as a thrombolytic agent.
subacute sclerosing panencephalitis A rare and serious complication of measles due to viral persistence in brain tissue.
subclinical infection An infection which does not produce any recognisable signs or symptoms, but elicits an immune response.
subcutaneous infection An infection involving tissue beneath the skin.
substrate The molecule which an enzyme reacts with.
subunit vaccine A vaccine based on a part of a microorganism.
sulfa drug Synthetic drug containing sulfur.
sulfonamides A group of bacteriostatic agents which interfere with folic acid synthesis.
superantigens Substances produced by some microbes that activate large numbers of T cells, resulting in a massive release of cytokines and tissue injury.
superbug A microorganism that is unaffected by multiple types of antibiotics.
superficial mycosis A fungal infection located on the skin or hair shaft.
superinfection An overgrowth of a resistant microbial species, usually after a period of antimicrobial therapy.
superoxide The free radical O_2^-.
superoxide dismutase An enzyme that destroys the free radical O_2^-.
surgical asepsis The procedures followed to maintain sterility.
surgical site infection A post-operative infection involving the operative field.
surveillance The collection of data on all aspects of the occurrence and spread of a disease.
susceptibility The lack of resistance or a vulnerability to an infection.
symbiosis Two different organisms living together.
symptoms of disease Changes felt and reported by the patient as a result of disease.
syncytia A multi-nucleate mass of fused body cells.
syndrome A group of signs and symptoms which are characteristic of a particular disease.
synergism A situation where the effect of two drugs working together is greater than the action of the drugs working alone.
syphilis A sexually transmissible disease caused by the spirochaete *Treponema pallidum*.
systemic candidiasis See **candidaemia**.
systemic disease A disease which affects the whole body.
systemic infection An infection in which microorganisms enter the bloodstream and are disseminated through the body.
systemic inflammatory response An immune response to an infection that enters the bloodstream and circulates through the body.
systemic mycosis A fungal infection which occurs in deep tissue or involves various organs in the body.

T4 cell A T lymphocyte that possesses the CD4 receptor on its surface; it displays helper activity. Also called a **T helper cell**.
T8 cell A T lymphocyte that possesses the CD8 receptor on its surface; it may display cytotoxic or suppressor activity. Those with cytotoxic activity are called **cytotoxic T cells**.
T cell See **T lymphocyte**.
T dependent antigen An antigen that requires the involvement of T cells in order for activation of B cells to occur.
T helper cell (Th) A T lymphocyte that helps cells to produce an immune response.
T lymphocyte A type of lymphocyte which matures in the thymus gland; responsible for cell-mediated immunity and regulation of immune responses.
T regulatory cell (Treg) A T lymphocyte that inhibits some immune responses.

tapeworm A segmented flatworm, the adult stage of which lives in the intestine.
TCA (tricarboxylic acid) cycle A pathway in carbohydrate metabolism whereby pyruvic acid is converted to carbon dioxide and water with the release of large amounts of energy stored as ATP.
teichoic acid A polysaccharide which occurs in the walls of Gram-positive bacteria.
temperate phage A bacteriophage which exists in a lysogenic state in the host cell.
template A pattern for the replication of a certain structure, as in DNA or RNA.
teratogen A substance causing abnormalities to the foetus *in utero*.
tertiary prevention In public health, actions to prevent deterioration in health.
tertiary syphilis The third stage of syphilis infection in which bacterial multiplication and dissemination and a cell-mediated hypersensitivity response of the immune system combine to form lesions in various organs.
tetanus A disease characterised by severe muscle spasm caused by the exotoxin of *Clostridium tetani*.
tetracyclines A group of bacteriostatic drugs which inhibit protein synthesis.
therapeutic index Ratio of the effective dose of a drug to the dose that will be lethal for the host (patient).
thermal death point The lowest temperature at which all the microorganisms present will be killed in a given time.
thermophile An organism which grows well at high temperatures (50–60°C).
threadworm See **pinworm**.
thrush A superficial infection of mucous membranes characterised by milky-white patches of inflammation.
thymine A pyrimidine base in DNA which pairs with adenine.
thymine dimer A compound produced by the formation of bonds between adjacent thymine molecules in DNA chains exposed to UV radiation.
thymus An organ found in the upper thoracic region beneath the sternum. Immature lymphocytes develop into mature T cells under the influence of thymic hormones.
tincture of iodine A solution of iodine in alcohol.
tinea See **ringworm**.
tinea capitis Tinea of the scalp.
tinea corporis Tinea of the body (trunk).
tinea cruris Tinea of the groin.
tinea pedis See **athlete's foot**.
tinea unguium Tinea of the nails.
tinea versicolor See **pityriasis versicolor**.
tissue tropism The ability of a virus to attach to specific receptor molecules on the cell surface.
titre A measure of the amount of specific antibody in blood or other body fluid. The reciprocal of the greatest dilution of the fluid that shows a positive reaction in an antibody–antigen reaction.
tolerance A non-responsiveness of the immune system to a given antigen.
toll-like receptor A type of non-specific receptor for a foreign particle or microorganism found on the surface of phagocytes and certain other cell types. It recognises molecules called pathogen-associated molecular patterns.
tonsillitis Infection and inflammation of the tonsils.
total parenteral nutrition (TPN) A procedure whereby all nutrition and drugs are administered via a central catheter.
toxaemia The presence of toxins in the bloodstream.
toxic shock syndrome A systemic disease caused by certain strains of *Staphylococcus aureus* that produce the toxic shock syndrome toxin.
toxin A substance produced by microorganisms that interferes with the normal functioning of host cells or tissues.
toxoid A bacterial exotoxin that has been modified to remove its toxicity while still retaining its ability to elicit an immune response.
toxoplasmosis A systemic infection caused by the protozoan *Toxoplasma gondii*.
tracheitis Infection and inflammmation of the trachea.
trachoma An eye infection which can lead to blindness, caused by *Chlamydia trachomatis*.
transcription The process of synthesis of messenger RNA using DNA as a template.
transduction Transfer of DNA from one bacterial cell to another by a bacteriophage.
transfer RNA (tRNA) A small RNA molecule responsible for transferring the correct amino acid to the growing peptide chain in protein synthesis.
transformation The process in which naked DNA passes from one bacterial cell to another resulting in a change in the properties of the recipient cell.
transient flora Microorganisms which are found on the human body for only a short time and do not cause disease.
translation The formation of a polypeptide on a messenger RNA template.
transmissible spongiform encaphalopathy A group of progressive, fatal, neurodegenerative diseases (e.g. Creutzfeld-Jacob disease) caused by prions.
Transmission-based Precautions Precautions applied in addition to Standard Precautions to patients infected (suspected or confirmed) with agents transmitted by the contact, droplet or airborne routes.
transmission electron microscope An electron microscope used to study the internal structures of cells at high magnification.
transport medium A liquid or semi-liquid medium into which some specimens are placed to prevent them from drying and to maintain microorganism viability.
transport proteins Proteins located in the cell membrane and responsible for the transport of substances into and out of the cell.

transposon A fragment of cellular DNA in which genes for resistance to a number of different antimicrobials are located beside each other and so can be transferred to other cells during conjugation.
traveller's diarrhoea The acute diarrhoea that frequently occurs in people visiting a foreign country. Often caused by *E. coli*.
trematodes See **flukes**.
trichinosis A disease caused by the nematode *Trichinella spiralis* which is acquired by eating poorly cooked meat (mainly pork).
trichomoniasis An infection caused by *Trichomonas vaginalis*.
triclosan A chlorophenol used as a broad spectrum antibacterial.
triple antigen vaccine (DTP) A vaccine used to immunise against diphtheria, pertussis and tetanus.
trophozoite A vegetative form of a protozoan.
true bacteria The largest group of bacteria in the three-domain classification scheme.
trypanosomes A group of flagellated protozoa.
tubercle A granuloma lesion characteristic of tuberculosis infection.
tuberculin A protein of the *Mycobacterium* cell used in the Mantoux skin test.
tuberculin skin test See **Mantoux skin test**.
tuberculoid leprosy The form of leprosy in which a strong cell-mediated immune response limits the multiplication of the organism and the disease is confined to patches of skin and certain nerve trunks.
tuberculosis (TB) An infection of the lungs or other organs caused by species of *Mycobacteria*.
tumour A mass of abnormal tissue due to uncontrolled replication of tissue cells.
tumour-associated antigen A normal cell antigen that has been altered and is present on the surface of a tumour cell.
tumour necrosis factor A cytokine which kills virus-infected cells as well as being important in inflammation and lymphocyte proliferation.
tumour-specific antigen A newly formed antigen on the surface of a tumour cell.
turbidity Cloudiness of a liquid.
type I hypersensitivity See **immediate hypersensitivity**.
type II hypersensitivity See **cytotoxic hypersensitivity**.
type III hypersensitivity See **immune complex hypersensitivity**.
type IV hypersensitivity See **delayed type hypersensitivity**.
type 1 T helper cell (Th1) A T lymphocyte that helps to activate other T cells and phagocytes to produce a cell-mediated immune response.
type 2 T helper cell (Th2) A T lymphocyte type that helps to activate eosinophils and mast cells and that is also involved in production of allergic reactions.
ulcer An area of tissue loss, varying in depth. An ulcer may involve skin only, or may extend more deeply into subcutaneous tissue.
ultraviolet radiation Low-energy short-wavelength radiation which can disrupt chemical bonds—for example, in DNA—causing mutation or cell death.
undulant fever See **brucellosis**.
Universal Precautions A set of infection control procedures designed to prevent transmission of blood-borne pathogens.
urethritis Infection and inflammation of the urethra.
urgency The urgent need to urinate; symptomatic of urinary tract infection.

vaccination See **immunisation**.
vaccine A preparation containing one or more antigens that is used to immunise a person against a specific disease.
vacuole A membrane-bound structure in the cytoplasm of cells, usually containing gas or storage molecules.
vaginal candiasis An infection of the vagina due to *Candida albicans*.
vaginitis Inflammation of the vagina.
vaginosis A vaginal infection characterised by an abnormal vaginal discharge associated with an imbalance of the vaginal microflora.
variant Creutzfeldt-Jakob disease A prion disease acquired by consumption of contaminated beef from a cow with mad cow disease.
varicella Chickenpox.
vasoconstriction A decrease in the diameter of blood vessels.
vasodilation Dilation of blood vessels.
vegetation A clump of bacteria, fibrin and platelets on the endocardium, characteristic of infective endocarditis.
vegetative cells Actively growing cells.
vertical transmission Transmission of an infection from mother to foetus *in utero*.
vesicle A small blister containing clear fluid, less than 0.5 cm in diameter.
vibrio A small curved (comma-shaped) bacterium.
viraemia The presence of viruses in the blood.
viral attachment proteins Proteins on the surface of the virus which attach to specific receptors on the surface of the target cell.
viral genome The genetic material of a virus.
virion An entire mature virus particle, occurring outside a host cell and consisting of nucleic acid, protein capsid and sometimes an envelope.
viroid An infectious particle consisting only of circular RNA and lacking a capsid.
virulence The degree of pathogenicity of an organism.
virulence factors The factors which contribute to an organism's ability to cause disease.
virus A tiny infectious particle consisting of nucleic acid and a protein coat.
virustatic Able to inhibit viral replication.

vitamin An essential growth factor—often a co-enzyme in a metabolic reaction.
volutin Granules of polyphosphate in bacterial cells.

wart A benign mass of tissue on the skin or mucous membrane caused by a papilloma virus.
water-borne transmission Transmission of an infectious agent in contaminated water.
Weil's disease See **leptospirosis**.
West Nile encephalitis A type of encephalitis caused by the West Nile virus spread by mosquitoes.
wet preparation A suspension of microorganisms in a drop of fluid for microscopic examination.
wheal A raised, often itchy erythematous lesion.
whooping cough See **pertussis**.

X-linked agammaglobulinaemia A primary immunodeficiency disease caused by incomplete maturation of B lymphocytes.

yeast A unicellular fungus.
yellow fever A systemic infection caused by the yellow fever virus and transmitted by mosquitoes.

Ziehl-Neelsen stain See **acid-fast stain**.
zone of inhibition A clear area corresponding to inhibition of bacterial growth which appears on an agar plate around an antimicrobial disk.
zoonosis An infection of animals that can be transmitted to humans.
zoster Shingles.

INDEX

Page numbers in *italics* indicate figures.