PART

III

Bioenergetics and Metabolism

Prodigious feats of endurance exhibited by hummingbirds

The tiny ruby-throated hummingbird can store enough fuel to fly across the Gulf of Mexico, a distance of some 800 kilometres, without resting. This achievement is possible because of the ability to convert fuels into the cellular energy currency, ATP, represented by the model at the right.

(Courtesy: (Left) K.D.McGraw)

"All the vital mechanisms, varied as they are, have only one object, that of preserving constant the conditions of life in the internal environment."

– ***Claude Bernard, Memoirs, 1855***

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Like motor traffic, metabolic pathways flow more efficiently when regulated by signals.

CHAPTER

19

Metabolic Concepts

DEFINITION OF METABOLISM

The term metabolism applies to the assembly of biochemical reactions which are employed by the organisms for the synthesis of cell materials and for the utilization of energy from their environments. In other words, the metabolism of an organism (or of a cell) may be defined as 'the sum total of all the enzyme-catalyzed reactions that occur in an organism (or in a cell)'. The large number of reactions in a cell are organized into a relatively small number of sequences or pathways. It is a highly coordinated and purposeful cell activity, in which multienzyme systems cooperate. This obviously points out to the fact that the metabolism of even a simple unicellular organism is time-variant, *i.e.*, all its aspects are not actually expressed at any given point.

The magnitude of metabolism may be appreciated by taking the examples of microorganisms and the human beings :

(*a*) Microorganisms like bacteria (*e.g.*, *Escherichia coli*) can double in number every 40 minutes in a culture medium containing only glucose and inorganic salts, or in 20 minutes in a rich broth. The components of the medium are depleted and very little is added to the medium by the cells. Each cell contains hundreds to thousands of molecules of each of about 2,500 different proteins, about 1,000 types of organic compounds and a variety of nucleic acids. It is, thus, apparent that the bacterial cells participate in a variety of metabolic activities in a remarkable way.

(*b*) Human adults maintain a constant weight for about 40 years, during which period a total of about 60 quintals of solid food and 45,000 litres of water are metabolized. And yet both body weight and body composition remain almost constant.

TERMINOLOGY OF METABOLISM

The various processes constituting metabolism may be divided, somewhat arbitrarily, into catabolism and anabolism. Those processes, whose major function is the generation of chemical energy in forms suitable for the mechanical and chemical processes of the cells, are termed as catabolism (*cata*^{G} = down ; *ballein*^{G} = to throw) ; whereas those processes, which utilize the energy generated by catabolism for the biosynthesis of cell components, are termed as *anabolism* (ana^{G} = up). The various activities powered by catabolism include mechanical movement, growth, reproduction, accumulation of foods, elimination of wastes, generation of electricity, maintenance of temperature etc. The various anabolic activities may be exemplified by food manufacture etc.

The terms **catabolism** and **anabolism** were coined by the physiologist Gasket in 1886.

Some processes can be either catabolic or anabolic, depending on the energy conditions in the cell. These are referred to as **amphibolism.**

$$\text{Fuels (carbohydrates, fats)} \xrightarrow{\text{Catabolism}} CO_2 + H_2O + \text{Useful energy}$$

$$\text{Useful energy + Small molecules} \xrightarrow{\text{Anabolism}} \text{Complex molecules}$$

The relationship among catabolism, anabolism and other terms of metabolic importance is summarized in Table 19–1.

Table 19–1. Classification of general metabolic terms

Processes are	degradative :	biosynthetic :
	Catabolism	Anabolism
	Dissimilation	Assiilation
Energy	yielding :	consuming :
	Exergonic	Endothermic
	Exothermic	Endergonic
Terminal electron acceptor is	oxygen :	not oxygen :
	Aerobic	Anaerobic
	Respiration	Fermentation

(Adapted from MF Mallette, CO Clagett, AT Phillips and RL McCarl, 1979)

In all cells, metabolism enables the cell to perform its vital functions. Metabolism performs following 4 specific functions :

(*a*) to obtain chemical energy from the dfegradation of energy-rich nutrients or from captured solar energy.

(*b*) to convert nutrient molecules into precursors of cell macromolecules.

(*c*) to assemble these precursors into proteins, lipids, polysaccharides, nucleic acids and other cell components.

(*d*) to form and degrade biomolecules required in specialized functions of cells.

These features of metabolism are closely inter related since the synthesis of the molecules, that are a component of cell, requires an input of energy, while at the same time it is obvious that the cell components (such as those that make up the cell membrane and its constituent transport proteins) are

needed to provide the energy supply and to control intracellular solute concentrations. The specialized functions (such as movement, the secretion of a particular type of molecule or the stimulation of an adjacent cell) also require biosynthetic processes as well as a supply of energy.

Terms *catabolism* and *dissimilation* are synonyms and refer to the pathways or routes breaking down food materials into simpler compounds and resulting in the release of energy contained in them. The processes of *anabolism* or *assimilation* (also synonyms) utilize food materials (or intermediates from catabolism) and energy to synthesize cell components.

In dealing with the energy relations of the biological processes, the term *exergonic* is used to denote a chemical reaction which liberates chemical-free energy. The term *exothermic* refers to the total energy liberated including heat. As the magnitude of heat energy is small and also that it cannot drive biological reactions, the biochemists are more interested in free energy changes and often use the term exergonic. The corresponding energy-consuming term *endergonic* refers to the processes which require an input of free energy while the term *endothermic* denotes a total energy requirement including heat.

The organisms which reduce oxygen are said to be *aerobic*. The route or pathway by which this reaction is accomplished (*esp*., its terminal steps) is called *respiration*. The organisms which reduce not oxygen but other compounds are said to be anaerobic. The term *fermentation* is complementary to respiration. It is to be noted that the term respiration is included twice in the above table. According to the modern trend among the boichemists, respiration is defined as the terminal processes involved in the reduction of molecular oxygen. The previous trend, as depicted in the older literature, used the term respiration for any process transferring electrons and releasing energy, whether terminating at molecular oxygen or not.

FUNCTIONS OF METABOLISM

An arrays of enzyme-catalyzed chemical reactions, that bring about transformations of certain organic compounds vital to the organism, constitute *metabolic pathways* or *metabolic routes*. The molecules or the compounds which participate in these reactions are called as *metabolic intermediates* or *metabolites*. The synthesis of cell constituents begins with the metabolic pathways which supply the building blocks. The major cell constituents are complex carbohydrates, the proteins, the lipids, and the nucleic acids. They are formed from relatively simple starting materials (Fig. 19–1). In mammalian cells, these key metabolites come from one of the following 3 sources.

(*a*) Absorption of the metabolites *from outside the cell* and by implication, if we consider the organism as a whole, by absorption of the metabolites as dietary constituents from the alimentary tract.

(*b*) Release of the metabolite *from a source stored with the cell*. The metabolite may be released either from a molecule used solely for storage (such as glycogen or triacylglycerol), or from a molecule that has another function within the cell (such as an enzyme or a membrane lipid).

(*c*) The metabolite may be formed *by the metabolism of a simple precursor*. However, the precursor must be absrobed by the cell or derived from a source stored within the cell.

Following are the key functions of metabolism :

A. Metabolism enables the cell to convert some of the energy found in nutrients into a form which will support biosynthesis, the maintenance of homeostasis and the cell's other energy-requiring processes.

Biosynthetic reaction sequences or pathways in the cell require an input of energy and this will normally be in the form of ATP or a reduced coenzyme (NADH, NADPH). It is noteworthy that the usefulness of ATP as an energy source is not a consequence of any special or 'high energy' form of the phosphate bond, but is merely a function of how far the hydrolysis of ATP is displaced from equilibrium.

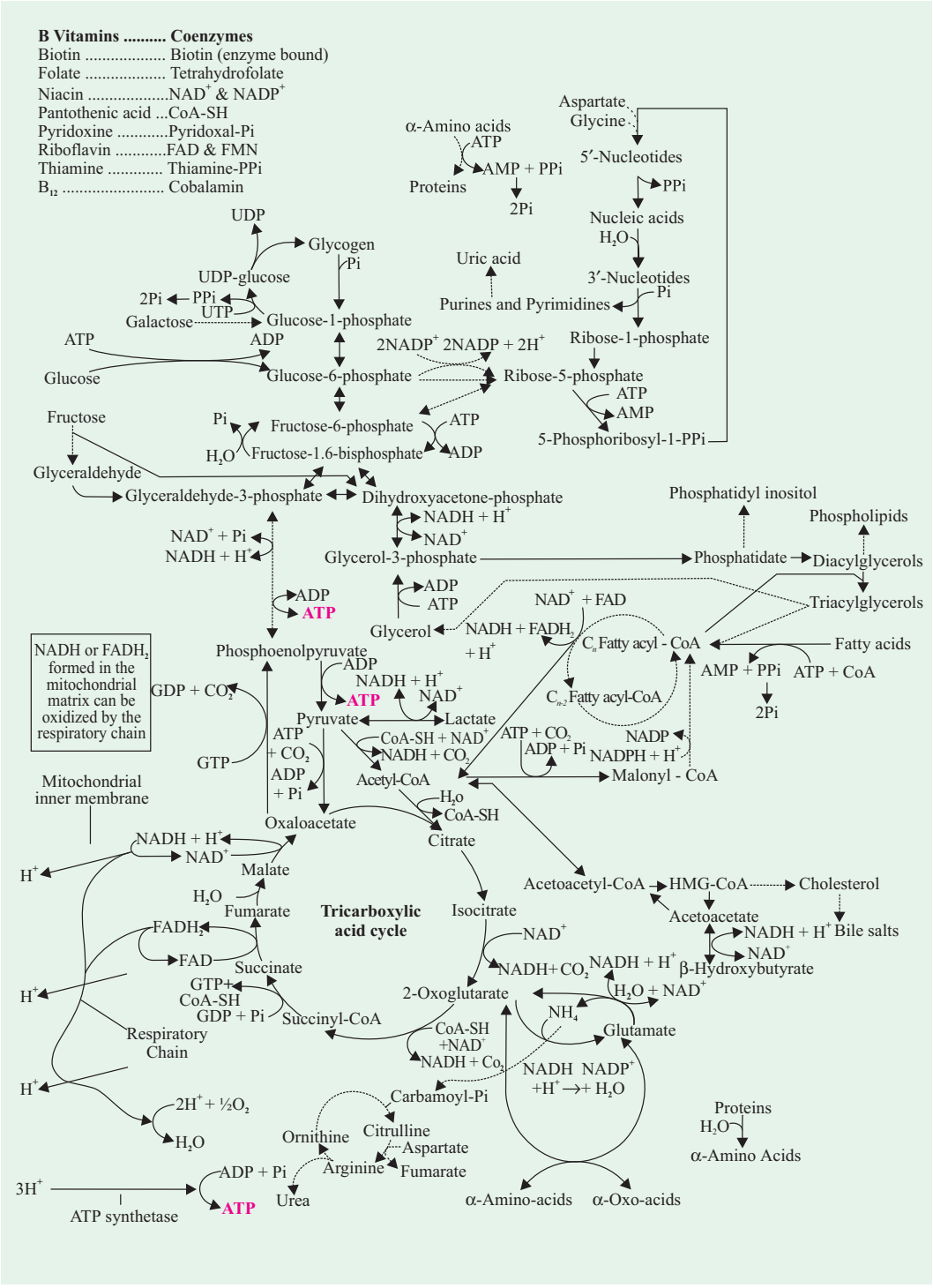


Fig. 19–1. Overview of metabolism

The points of ATP formation are in red.

Mammalian cells have 5 reaction schemes which are capable of supplying cells with adequate amounts of energy in one of these forms. These energy conserving pathways (or energy-supplying processes) are listed below :

1. The ***glycolytic pathway*** **:** This converts glucose into 3-c compounds, pyruvate or lactate.
2. The ***citric acid cycle (CAC)*** **:** This converts 2-c acetate units into carbon dioxide.
3. The ***pentose phosphate pathway (PPP)*** **:** This converts glucose-6-phosphate into pentose phosphate, and reduces the $NADP^+$ coenzyme.
4. The ***β oxidation of fatty acids*** **:** This converts fatty acids into 2-c units, and reduces the coenzymes FAD and NAD^+.
5. ***Oxidative phosphorylation*** **:** This is the electron transport phosphorylation process in which molecular oxygen is used to oxidize the coenzymes, which are reduced in the other 4 pathways, with the production of water and the conversion of ADP plus phosphate into ATP.

One of the 5 energy-supplying processes, the glycolytic pathway, can be distinguished from the others because it is capable of operating independently of a supply of oxygen. Unluckily, this has led people to believe that it is an anaerobic pathway, which is misleading since the pathway can (and does!) operate in an oxygen-independent mode even oxygen is plentifully available. The other 4 energy-supplying pathways lack this flexibility since they can only function in the presence of oxygen.

B. Metabolism provides key metabolites which are required for the synthesis of many essential cell components.

The central metabolic pathways (Fig. 19–1) include all 5 of the energy-conserving pathways listed in the preceding section. In addition to supplying the cell with energy, these central pathways provide precursors required for the formation of the carbohydrates, proteins, lipids and nucleic acids which the cell is composed of. Most of the pathways, shown in Fig. 19–1, are found in almost all mammalian cells, although some reactions which occur only in specialized cells have been incorporated *for the sake of completeness* (for example, the metabolism of galactose and fructose, the formation of urea and glycogen and the hydrolysis of glucose-6-phosphate.

Glycolysis, which is responsible for the conversion of glucose into 3-C compounds, illustrates the dual role of these central metabolic pathways. The glycolytic pathway supplies the cell with ATP and also provides the acetyl-coenzyme A (=acetyl-CoA) and glycerol-3-phosphate required to form the phospholipids needed for the cell membranes.

The pentose phsophate pathway provides a different sort of example since it is responsible for supplying energy (as NADPH) for fatty acid synthesis, but it also supplies the phosphoribosyl-1-pyrophosphate needed for the formation of nucleotides, the precursors of the nucleic acids.

CLASSICAL SUBDIVISIONS OF METABOLISM

The concept of metabolism must be viewed as an integrated set of pathways within the cell, rather than emphasizing the artificial subdivisions between the different areas of metabolism. However, the metabolism of the cell is too complex to consider all at once and there are some advantages in using the 3 classical subdivisions of metabolism: the carbohydrate, lipid and nitrogen metabolisms.

Fig. 19–2 shows how the metabolic pathways depicted in Fig. 19–1 are divided into the 3 main areas of metabolism.

Nitrogen metabolism and lipid metabolism are shown. The remaining areas show carbohydrate metabolism plus the final common pathways of the citric acid cycle and oxidative phosphorylation.

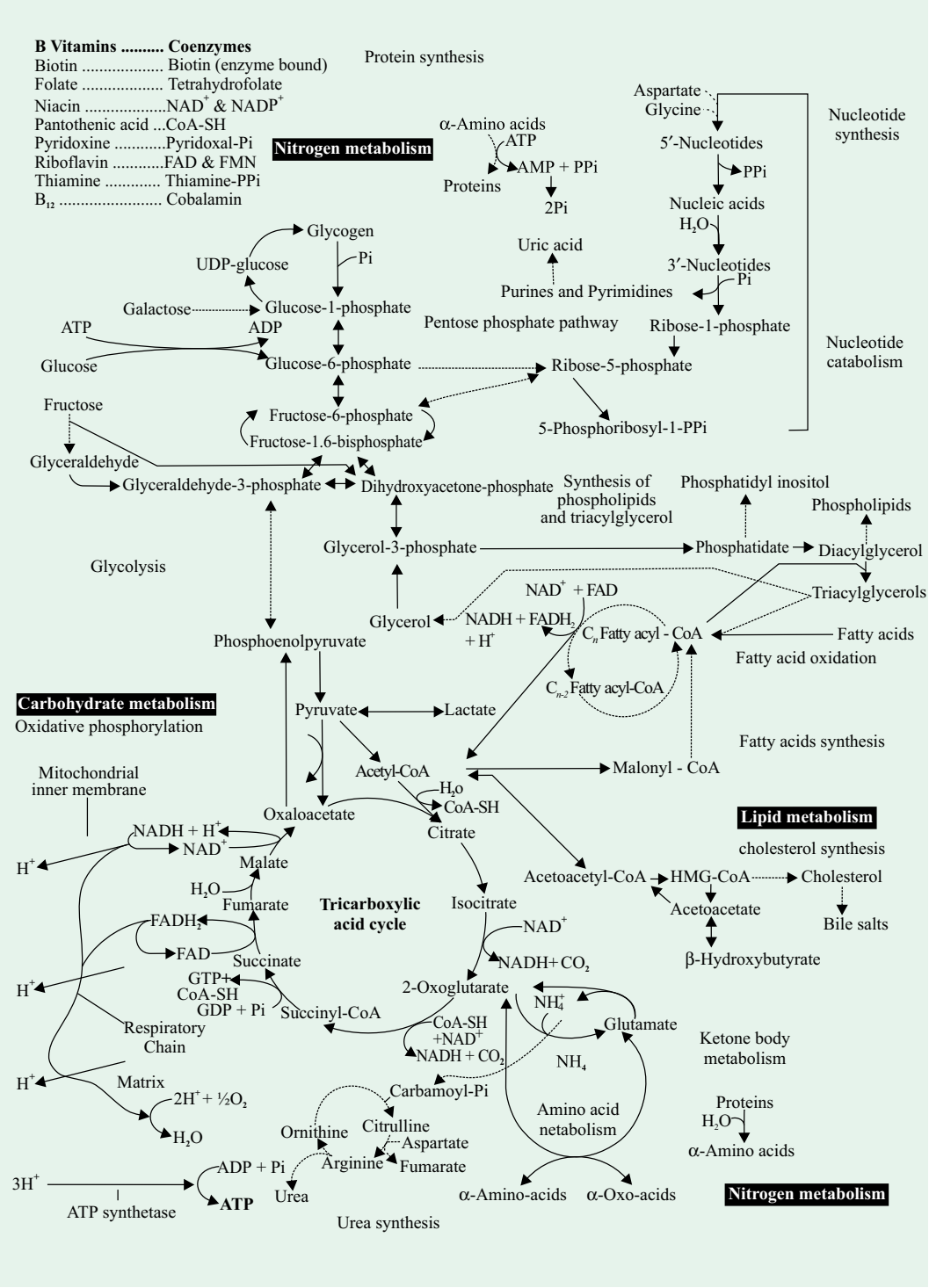


Fig. 19–2. Classical subdivisions of metabolism

Nitrogen metabolism and lipid metabolism are shown. The remaining areas show carbohydrate metabolism plus the final common pathways of citric acid cycle and oxidative phosphorylation.

Within each of these major areas, Fig. 19–2 also identifies the figure that gives details of particular pathways with well-defined functions, such as the energy-supplying pathways mentioned earlier. This is done for carbohydrate metabolism in Fig. 19–3, but a comparison of Figs. 19.2 and 19.3. illustrates the artificiality of the 3 classical subdivisions. Most of the energy conserving pathways fall within carbohydrate metabolism, except the β oxidation scheme from lipid metabolism which is not included in Fig. 19–3. Furthermore, the carbon skeletons of amino acids provide an important source of substrate for CAC, although they are part of nitrogen metabolism.

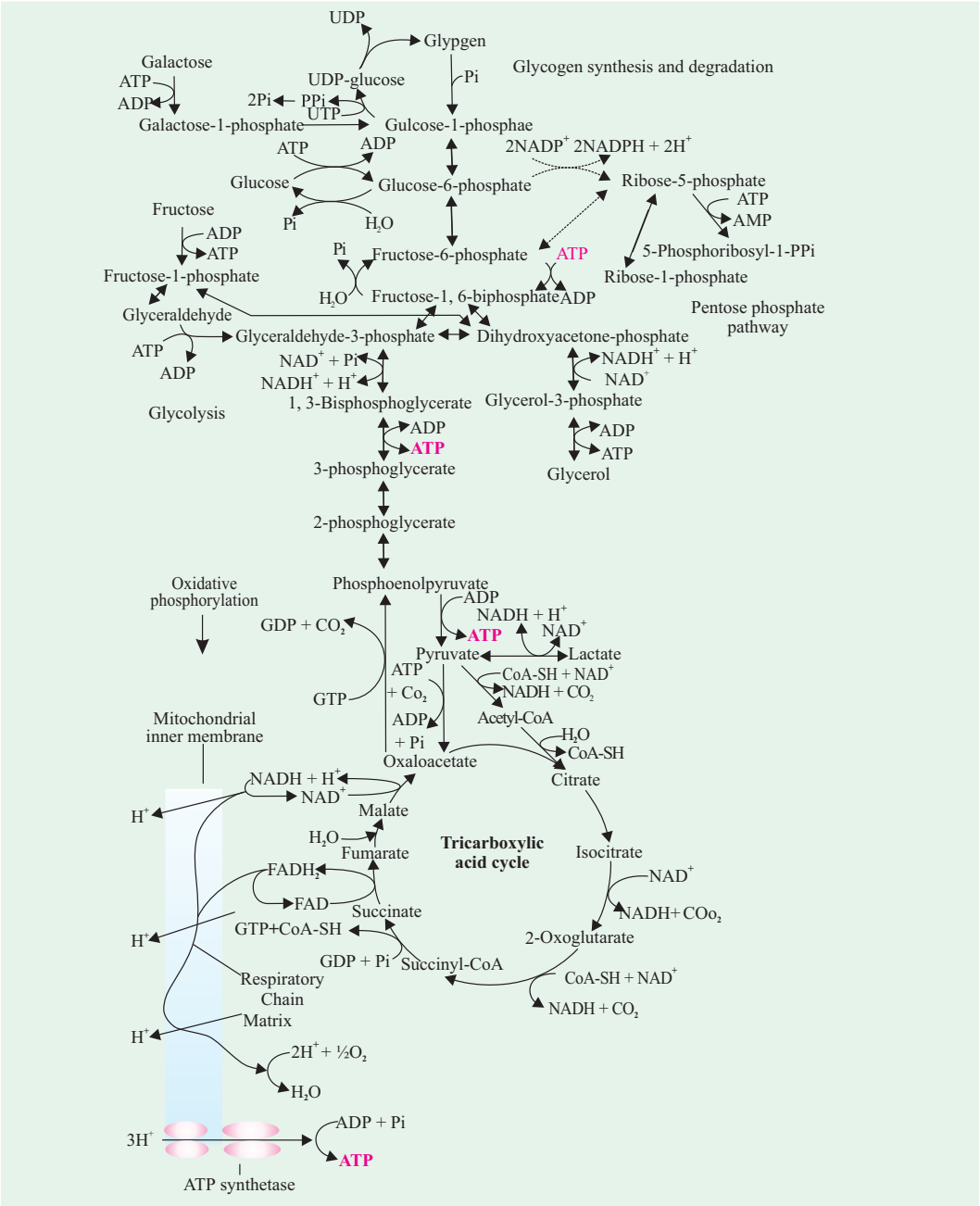


Fig. 19–3. Carbohydrate metabolism

The points of ATP formation are in red.

METABOLIC PATHWAYS

Since metabolic processes take place in a series of progressive, stepwise individual reactions, they can be described conveniently in the form of metabolic pathways, as shown in Fig. 19–4.

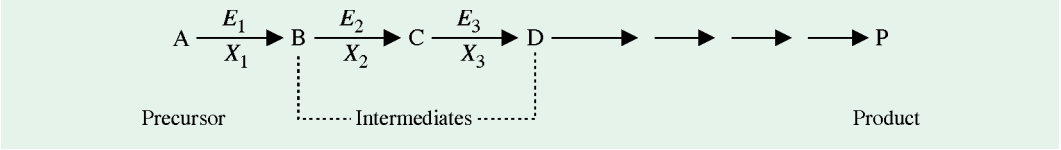


Fig. 19–4. Schematic representation of a typical linear metabolic pathway

[The specific enzymes required for each step are E_1, E_2 and E_3. The cofactors, if required, are X_1, X_2 and X_3.]

The starting material or precursor used in the anabolic pathways is invariably some rather simple, plentiful substance. For example, the biosynthesis of cholesterol in the animal organism starts with acetic acid as its coenzyme A derivative, acetyl CoA. The latter is abundant since it is an end product in the metabolism of both carbohydrate oxidation and fatty acid oxidation. The biosynthesis of heme in some 9 or 10 separate steps from glycine, succinyl CoA and iron is another excellent example.

To obtain the product, a metabolic sequence should be essentially irreversible. Reversibility, however, occurs at one or, sometimes, few steps by a separate reaction and its corresponding enzyme, as shown in Fig. 19–5. The important examples are glycolysis and the synthesis and degradation of liver glycogen from or to glucose.

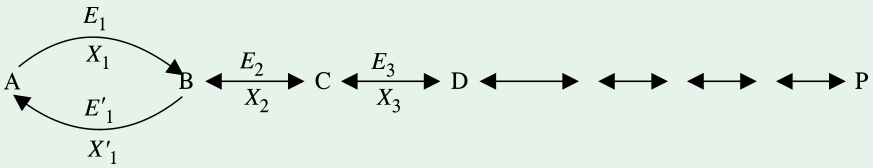


Fig. 19–5. Schematic representation of a typical irreversible metabolic pathway, showing the point of irreversibility, A to B

[For reversion, different enzymes E_1 and E_1', and usually different cofactors X_1 and X_1' are required for the forward and backward reactions, respectively.]

Some metabolic sequences may have a common path for many steps and then branch into two or more separate paths as shown in Fig. 19–6. For example, in carbohydrate metabolism, glucose-6-phosphate, derived from free glucose, serves as a starting point for the biosynthesis of glycogen, the

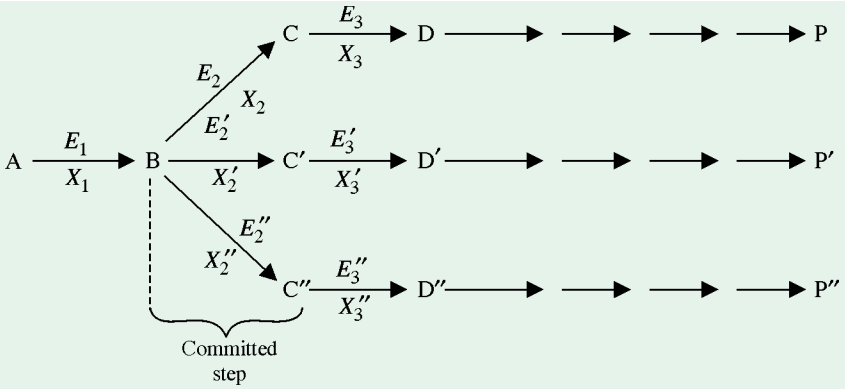


Fig. 19–6. Schematic representation of a branched metabolic pathway, using B as a common starting point

[Deficiency of an enzyme E_2, E_3 etc., or a cofactor X_2, X_3 etc., required for any of the path shown may result in a diversion of B into one or both of the other paths]

Embden-Meyerhof-Parnas (E.M.P.) pathway and the pentose phosphate pathway. The first step after such a branch point is often termed a **committed step** since its metabolite and succeeding ones have no role in metabolism other than the formation of the specific product of that path. This step often involves a great loss of free energy and hence is essentially irreversible. In case of blockage of one branch due to hereditary deficiency of an enzyme, the metabolism of the initial metabolite will be diverted into one or more of the `alternate' pathways at the branching point. Phenylketonuria (PKU) affords a good example. The diversion of phenylalanine (Phe) into other pathways occurs and there is formation of excessive amounts of phenylpyruvic acid and other related metabolites. Their increased amounts are toxic and apparently cause abnormalities in the affected children.

The metabolic pathways have been classically divided into two types, *catobolic* and *anabolic*. Both these pathways take place simultaneously in cells and their rates are regulated independently.

(A) Catabolic (= Degradative) Pathways

These comprise pathways in which large organic nutrient molecules (*e.g.*, carbohydrates, proteins and lipids) are broken down to smaller simpler compounds (*e.g.*, CO_2, NH_3 and lactic acid), frequently involving the participation of oxidation reactions, and result in the release of chemical free energy contained in the large organic molecules. This energy is then utilized by the organism for growth, movement, replication and also for transduction into other forms of energy, such as mechanical, thermal or electrical. At certain steps in the catabolic pathway, much of the free energy is conserved in the form of energy-carrying molecule adenosine triphosphate (ATP). Some may be conserved as energy-rich hydrogen atoms carried by the coezyme, NADP in its reduced form, NADPH (refer Fig. 19–7).

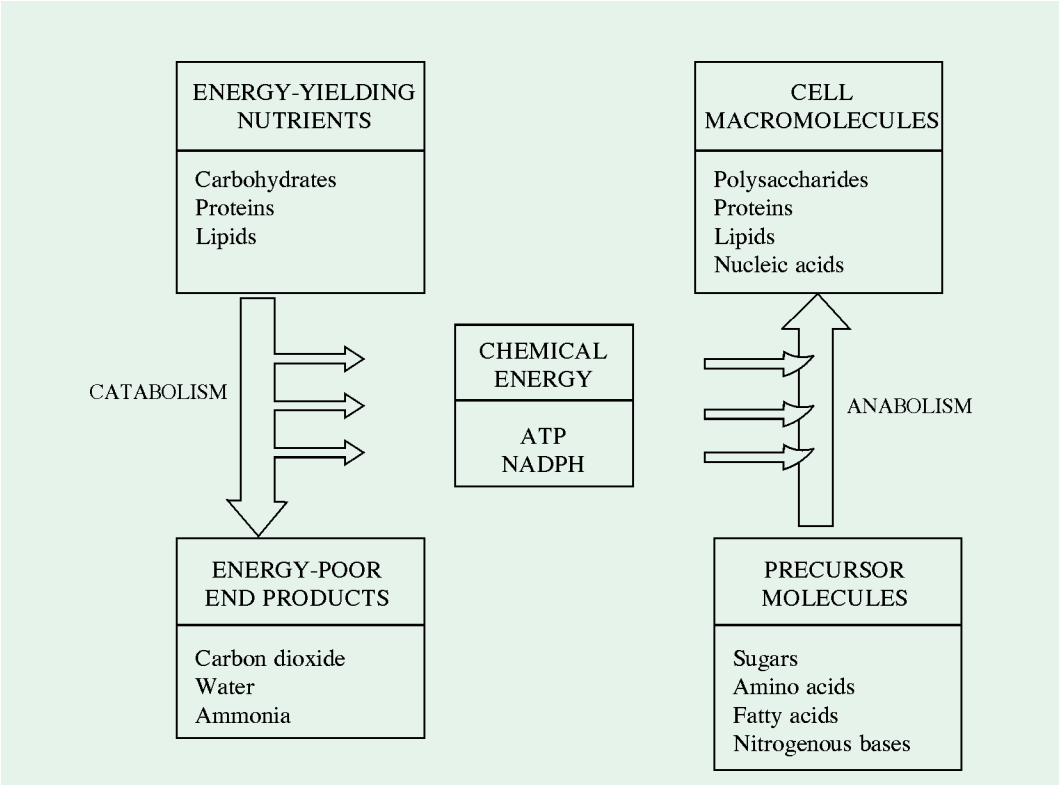


Fig. 19–7. Energy relations between catabolic and anabolic pathways

(B) Anabolic (= Biosynthetic) Pathways

These include pathways in which complex organic compounds are produced from simpler precursors, usually involving the participation of reduction reactions, and require an input of chemical free energy which is furnished by the breakdown of ATP to ADP and phosphate. Biosynthesis of some cell components also requires high-energy hydrogen atoms, which are donated by NADPH (Fig. 19–7).

CENTRAL PATHWAYS

In spite of much diversity in the structure and function of the metabolites, there is a remarkable order and simplicity in various metabolic routes. This can be well illustrated by describing a central area of metabolism which is common to the two pathways (catabolic and anabolic) and also provides a direct link between them (refer Fig. 19–8).

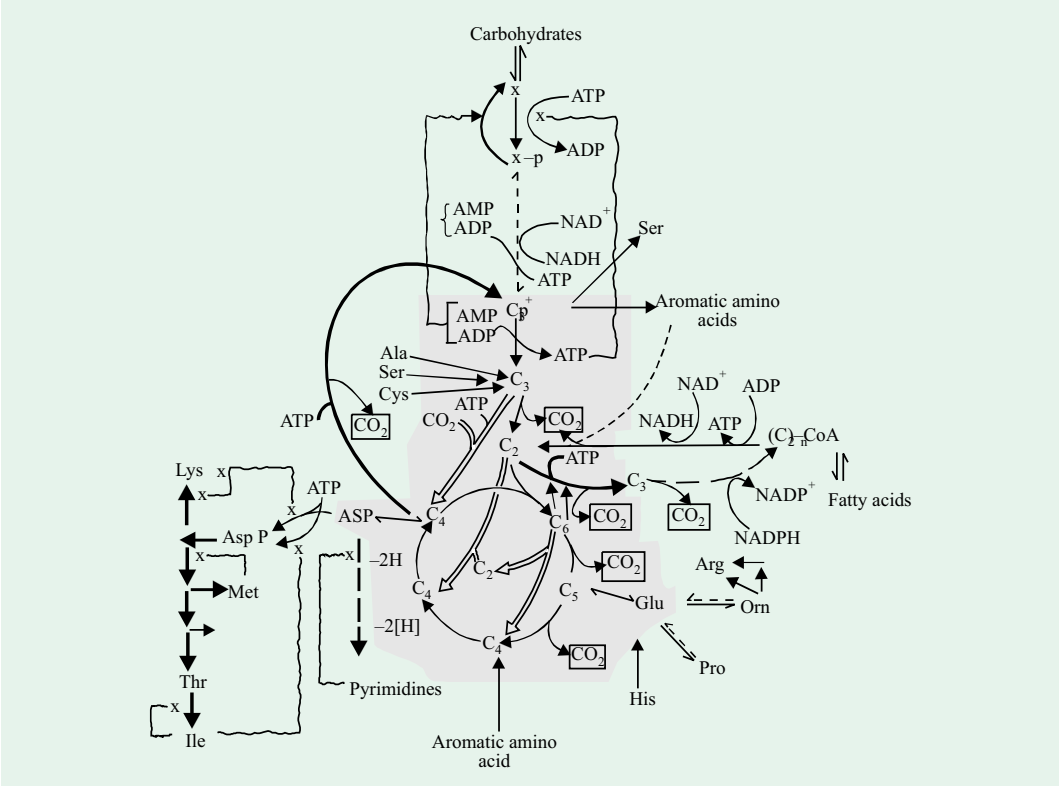


Fig. 19–8. Metabolic principles

Central area of metabolism is indicated by shading.

Thin arrows	=	Catabolic reactions
Thick solid arrows	=	Anabolic reactions
Thick hollow arrows	=	Anaplerotic reactions
Wavy arrows	=	Activation by end products
Wavy crosses	=	Inhibition by end products

During early stages of catabolism, large molecules are broken down to yield CO_2 and H_2O and also a 'restricted group' of small organic molecules, liberating about 1/3rd of the available free energy. These organic molecules are as follows :

(*a*) for carbohydrates—triose phosphates and/or pyruvate

(*b*) for proteins—acetyl-CoA, oxaloacetate, α-ketoglutarate, fumarate and succinate

(*c*) for fats—acetyl-CoA, propionyl-CoA and glycerol

The same compounds are also produced even when organisms like bacteria utilize exotic carbon sources such as aliphatic (*e.g.*, γ-aminobutyric or itaconic acids), aromatic (*e.g.*, benzoic or mandelic acids) or heterocyclic (*e.g.*, purines or uric acid) compounds.

It is interesting to note that one and the same set of reactions is involved in 3 crucial phases of metabolism :

(*a*) the *interconversion* of the various products of catabolism, mentioned above.

(*b*) their *complete combustion* to CO_2 and H_2O, thus providing the organism with the remaining 2/3rd of its energy supply.

(*c*) the supply of *crucial intermediates* for the anabolic processes.

The central pathways are composed of relatively few reactions. In nutshell, they consist of following steps :

1. triose phosphate $\rightleftharpoons$ pyruvate
2. pyruvate $\longrightarrow$ acetyl-CoA
3. oxaloacetate $\rightleftharpoons$ aspartate
4. α-keto- (or o × o) glutarate $\rightleftharpoons$ glutamate
5. cyclic set of reactions catalyzing the complete combustion of acetyl-CoA (a C_2 compound) to CO_2 plus H_2O.

CATABOLISM VERSUS ANABOLISM

There are some fundamental differences between the catabolic and anabolic processes which have been shown in Fig. 19–9.

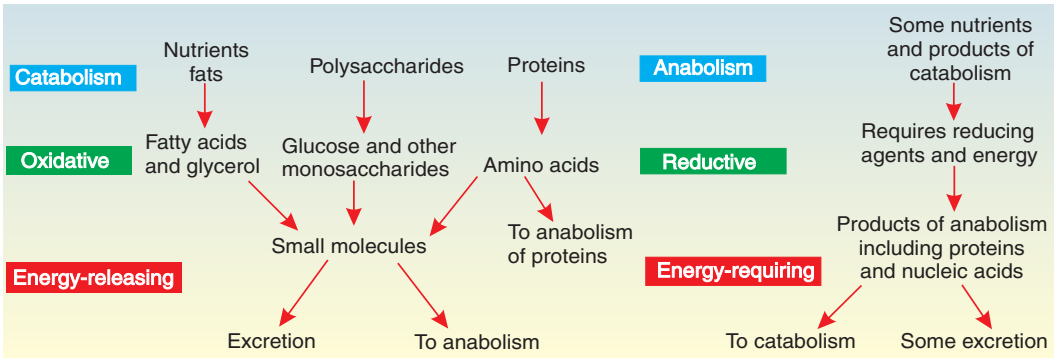


Fig. 19–9. A comparison of catabolism and anabolism

A. The catabolic pathways have clearly defined beginnings but no clearly distinguishable end products. On the contrary, the anabolic pathways lead to clearly identifiable end products from diffuse beginnings. The catabolic routes affect the conversion of nutrient carbon sources into the intermediates of central pathways ; the anabolic routes comprise of various enzymatic steps which result in synthesizing the macromolecules from these intermediates.

B. Catabolism is converging process since it begins with many diverse cell components but ends in a final common pathway with only a few end products. Anabolism, on the other hand, is a diverging process since it begins with a few simple precursor molecules from which a large number of different macromolecules are made.

C. The catabolic and anabolic sequences or routes mostly follow altogether different pathways in detail. This is evident from the fact that the product of catabolism is quite different from the carbon source in anabolism. Following examples support this statement :

1. The amino acids, serine and cysteine are catabolized to pyruvate but their biosynthesis begins with glycerophosphate and aspartate respectively.
2. The aromatic amino acids are catabolically degraded to acetyl-CoA plus fumarate (or succinate) and their synthesis begins with phosphoenolpyruvate and an aldotetrose phosphate.
3. The catabolism of fatty acids terminates in the production of acetyl-CoA and its biosynthesis although commences with acetyl-CoA but follows a different path. It is first converted to the more reactive malonyl-CoA, which is not an intermediate in the catabolic chain, and then to other long-chain acetyl-CoAs.
4. In glucose metabolism, which is characterized by a reversal of enzymatic reactions, biosynthetic and degradative steps differ at the two most critical points, i.e., at either end. For instance, glucose is converted to glucose-6-phosphate during catabolism using ATP ; yet it is formed in anabolism from phosphate ester by hydrolysis. Pyruvate is produced catabolically from its enol phosphate by transphosphorylation to ADP ; it is, however, used anabolically by virtue of two linked reactions (carboxylation to oxaloacetate and then its transformation to enolphosphate).

The above-mentioned examples point out to a generalization that the initial and final reactions in most catabolic and anabolic pathways are virtually irreversible thermodynamically, *i.e.*, they have $\Delta G^{\circ\prime}$ values equal to ≤ -4 kcal/mole.

The free energy change of a reaction occurring when reactants and products are at unit activity is called the standard free energy change and is denoted by the symbol ΔG°. However, biochemists usually use $\Delta G^{\circ\prime}$, which is the **standard free energy change at pH 7.**

D. The catabolic and the corresponding anabolic sequences between a given precursor and a given product are usually not identical. They may use different enzymatic reactions in the intermediate steps. For instance, in the breakdown of glucose to pyruvic acid in the liver, 11 specific enzymes in a definite sequence catalyze the successive steps. But glucose synthesis from pyruvic acid does not occur by merely a reversal of all the enzymatic steps in glucose degradation. Instead, it proceeds differently and uses only 9 of the 11 enzymatic steps used in glucose degradation, the other 2 steps being replaced by an entirely different set of enzyme-catalyzed reactions. Likewise, the corresponding catabolic and anabolic pathways between proteins and amino acids or between fatty acids and acetyl-CoA are also unidentical.

Biosynthetic and degradative pathways are rearely, if ever, simple reversals of one another, even though they often begin and end with the same metabolites. Though they may share some common intermediates or enzymatic steps, the two pathways are separate reaction sequences, regulated by distinct mechanisms and with different enzymes catalyzing their regulated reactions. Although seemingly, it appears wasteful to have different catabolic and anabolic pathways between a substrate and the product, there are at least two weighty reasons for the different corresponding catabolic and anabolic pathways :

I. *A pathway can be exergonic in only one direction.* Firstly, to proceed in a particular direction. If pathway must be exergonic in that direction. If a pathway was strongly exergonic then reversal of that pathway is just as strongly endergonic under the same conditions. Thus, the pathway adopted for breakdown of a biomolecule may not be possible for its synthesis because of high energy requirements. This can be easily explained by the famous hill-and-boulder example (Fig. 19–10). A boulder dislodged at the top of a hill will roll downhill, losing energy as it descends. At many points, the boulder may lose a large amount of energy as it falls over highly steep places. A tractor, however, may not be able to haul the boulder back up the hill by the path of its descent, but may succeed if it bypasses the steep slopes by taking a gradual zigzag path. Thus,

A **boulder** is a large stone, rounded by action of water.

degradation of a complex organic molecule is essentially a 'downhill' process, resulting in a loss of free energy; whereas biosynthesis of organic molecule is an 'uphill' process, requiring an input of energy.

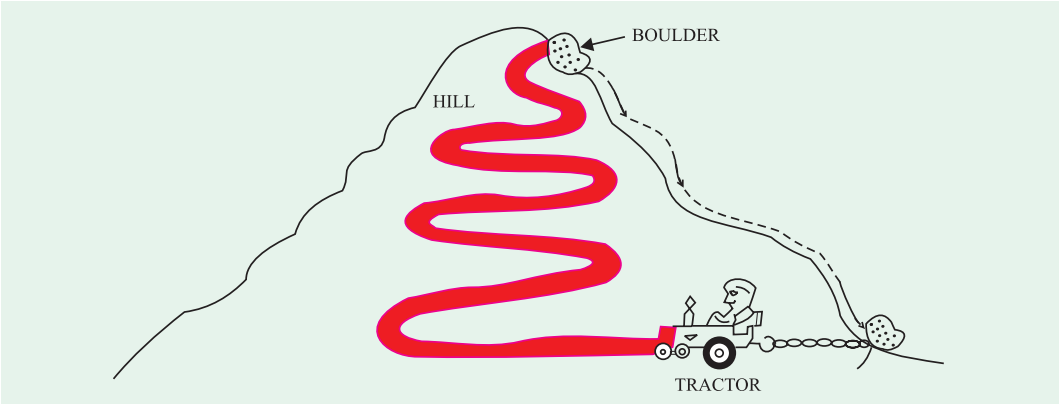


Fig. 19–10. The hill-and-boulder analogy

[Dashed lines indicate gradual fall while the solid arrows represent abrupt fall of the boulder.]

II. *Pathways must be separately regulated to avoid futile cycles.* The catabolic and anabolic pathways must be independently regulated. If only one pathway were used reversibly for both degradation and biosynthesis, slowing down the degradative pathway by inhibiting one of its enzymes would also slow down the corresponding biosynthetic pathway. Independent regulation is possible only when the two pathways are either entirely different or at least the rate-controlling step(s) should have different enzymes (refer Fig. 19–11). An important aspect of the metabolic pathways is the need to control the flow of metabolites in relation to the bioenergetic status of a cell. When ATP is in plenty, there is less need for carbon to be oxidized in the citric acid cycle. At such times, the cell can store carbon as carbohydrates and fats, so that gluconeogenesis, fatty acid synthesis and related pathways come into play. When ATP levels lower down, the cell must mobilize stored carbon to generate substrates for the citric acid cycle, so carbohydrate and fat breakdown must occur. Using distinct pathways for the biosynthetic and degradative processes is crucial for control, so conditions that activate one pathway tend to inhibit the opposed pathway and *vice versa*.

Sometimes, the degradative and biosynthetic pathways occur simultaneously in different parts of the cell. For instance, the oxidation of fatty acids to acetyl-CoA in the liver takes place in mitochondria where different enzymes catalyzing the various steps are present ; whereas the synthesis of fatty

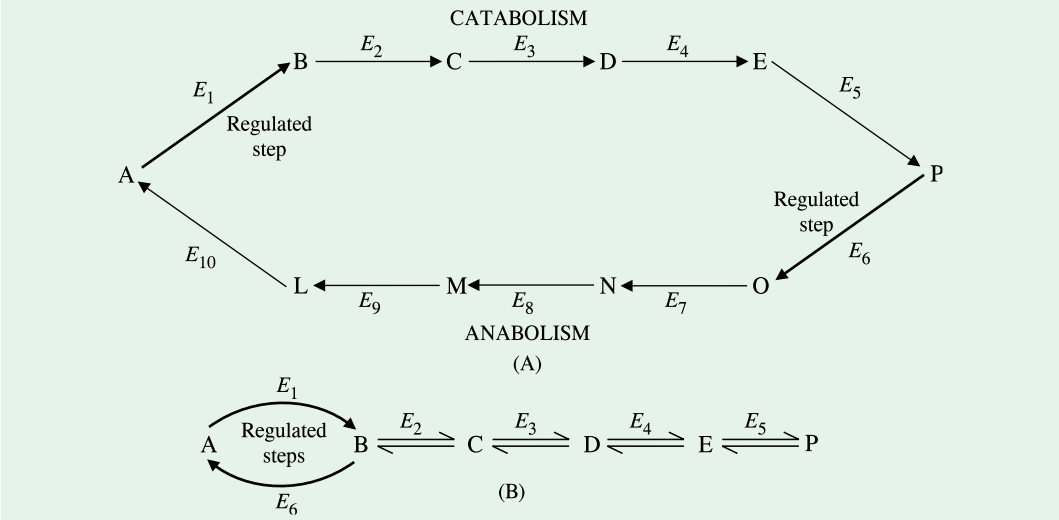


Fig. 19–11. Two examples of independent regulation of catabolic and anabolic pathways

(A) Parallel routes proceed *via* an entirely different set of enzymes.

(B) The two pathways differ in only one enzyme.

[Regulated steps are designated by thick arrows.]

acids from acetyl-CoA takes place in cytosol where a different set of enzymes catalyzing these steps are localized. Thus, *oxidative events (i.e., fatty acid oxidation) are favoured in mitochondria and reductive events (i.e., fatty acid synthesis) in cytosol.*

Consider what would happen, for example, if fatty acid synthesis and oxidation took place in the same cell compartment and in an unregulated way. Two-carbon fragments released by oxidation would be immediately utilized for resynthesis, a situation called the **futile cycle**. No useful work is done, and the net result is simply consumption of the ATP used in the endergonic reactions of fatty acid synthesis.

A similar futile cycle could result from the interconversion of fructose-6-phosphate with fructose-1,6- bisphosphate in carbohydrate metabolism.

$$\text{Fructose-6-phosphate} + \text{ATP} \longrightarrow \text{Fructose-1-6-bisphosphate} + \text{ADP}$$

$$\text{Fructose-1,6-bisphosphate} + H_2O \longrightarrow \text{Fructose-6-phosphate} + P_i$$

$$\text{Net :} \qquad \text{ATP} + H_2O \longrightarrow \text{ADP} + P_i$$

The first reaction occurs in glycolysis, and the second participates in a biosynthetic pathway, glyconeogenesis. Both processes occur in the cytosol. The net effect of carrying out both reactions simultaneously would be the wasteful hydrolysis of ATP to ADP and P*i*. However, enzymes catalyzing both of the above reactions respond to allosteric effectors, such that one enzyme is inhibited by conditions that activate the other. Thus, effective control prevents the futile cycle from operating, even though the two enzymes occupy the same cell compartment. Henceforth, it is more appropriate to call this situation– two seemingly opposed cellular reactions that are independently controlled– a **substrate cycle**. Evidences suggest that a substrate cycle represents an efficient regulatory mechanism, because a small change in the activity of either or both enzymes can have immense effect on the flux of metabolites in one direction or the other.

As stated earlier, metabolism is essentially a series of coupled, interconnecting chemical reactions that begins with a particular molecule and converts it into some other molecule(s) in a carefully defined manner. Fig. 19–12 is a highly simplified view of various processes occurring in living body. This figure is a sort of metabolic chart, similar to one that adorns a wall, like a giant road map, in biochemistry laboratories. The figure illustrates two important principles :

(*a*) Metabolism can be subdivided into 2 major categories– catabolism and anabolism.

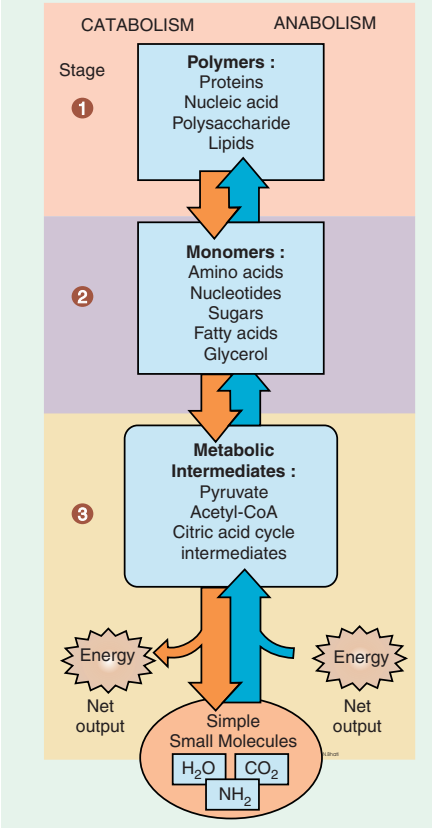


Fig. 19–12. A brief overview of metabolism

In this and the subsequent Fig. 19–12, the catabolic pathways (shown in red) proceed downward and anabolic pathways (shown in blue) proceed upward. Electron flow is shown by narrow arrows. Note the 3 stages of metabolism.

(*b*) Both catabolic and anabolic pathways occur in 3 stages of complexity. These are :

Stage 1 : the interconversion of polymers and complex lipids with monomeric intermediates

Stage 2 : the interconversion of monomeric sugars, amino acids, and lipids will still simpler organic compounds

Stage 3 : the ultimate degradation to, or synthesis from, inorganic compounds, including CO_2, H_2O and NH_3

Fig. 19–13 presents metabolism in more detail. Shown in this figure are the central metabolic pathways and some key intermediates. It may, however, appear from Figs. 19–11 and 19–12 that

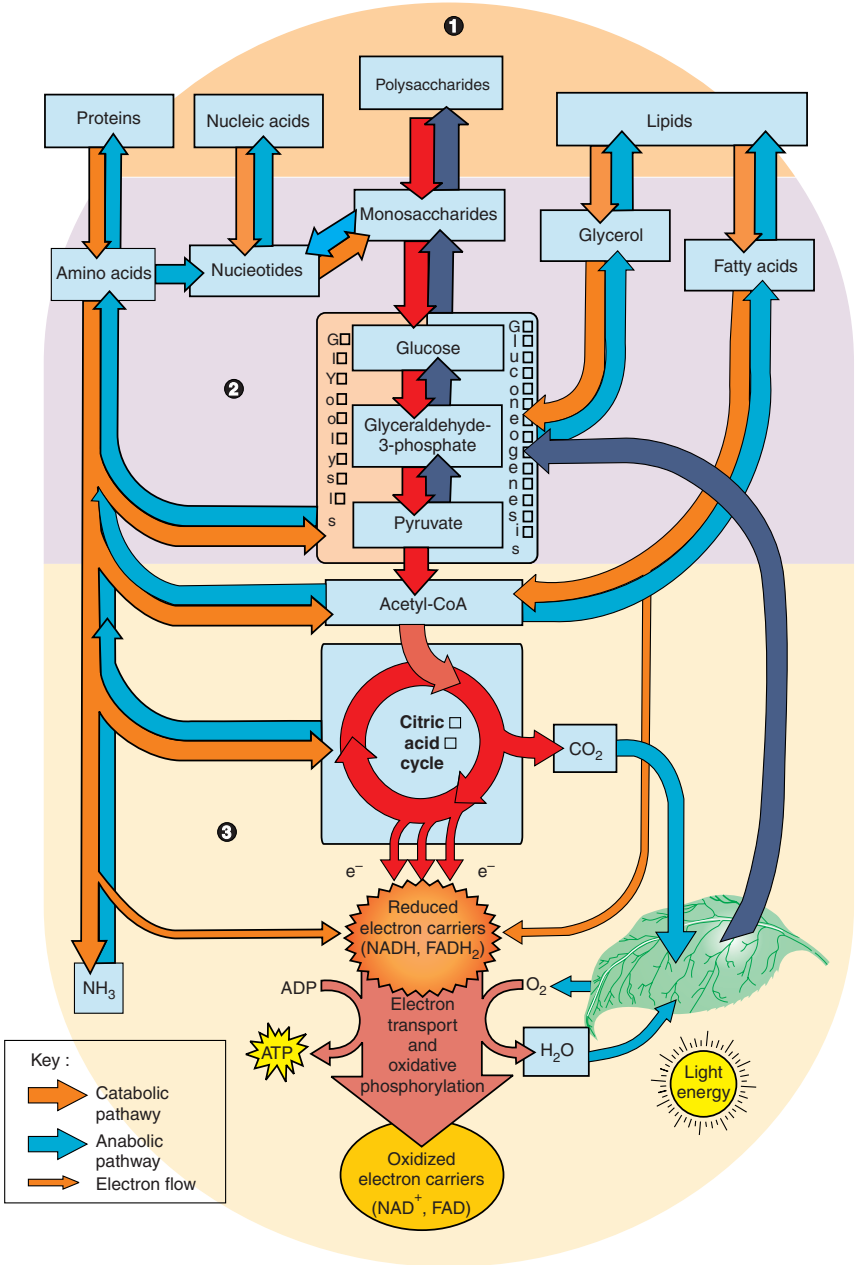


Fig. 19–13. A detailed overview of metabolism

some pathways operate simply as the reversal of other pathways. For example, fatty acids are synthesized from acetyl-CoA, but they are also converted to acetyl-CoA by β oxidation. Similarly, glucose-6-phosphate is synthesized from pyruvate in gluconeogenesis, which looks at first glance like a simple reversal of glycolysis. It is important to realize that in these cases, the opposed pathways are quite distinct from one another.

In spite of these well-marked differences, there is a close connection between catabolism and anabolism at 3 levels :

(*a*) *On that of carbon sources* : The products of catabolism, through the intervention of central pathways, become the substrates of anabolism.

(*b*) *On that of energy supply* : The catabolic processes usually produce energy in the form of ATP which is consumed by the energy-requiring anabolic processes.

(*c*) *On that of reducing power*: Catabolism is mainly an oxidative process and hence consumes oxidizing power and generates reducing power. Anabolism, on the other hand, is mainly a reductive process and hence consumes reducing power and produces oxidizing power. Much of the reducing power, either generated or consumed, is provided by the pyridine (= nicotinamide) nucleotides, NAD^+/NADH and $NADP^+$/NADPH. However, an important difference is : catabolism produces both NADH and NADPH while anabolism requires and consumes NADPH almost exclusively.

ANAPLEROTIC PATHWAYS

The terminal stages of catabolism usually lead to complete removal of most metabolties from the common pathways in the form of CO_2 plus H_2O plus NH_4^+ (or urea) and some other nitrogenous bases. Anabolism also provides a constant drain on the pools of the intermediates of the common pathways. Thus, provision must be made for their replenishment by some subordinate or ancillary routes which are termed anaplerotic. These routes involve the insertion of 1-carbon (such as CO_2) or a 2-carbon (such as acetyl-CoA) fragment into the common pool.

SECONDARY PATHWAYS

Till now a discussion of *primary* (or *central*) *pathways* has figured in which relatively large quantities of nutrients of the cells (*i.e*., carbohydrates, proteins and lipids) are metabolized. As an instance, hundreds of grams of glucose are oxidized to CO_2 and H_2O each day by a human adult. But there are other routes also where the flow of metabolites is in meagre quantities (say only few milligrams per day), involving both degradation and biosynthesis of substances. These pathways constitute *secondary pathways* and are usually involved in the biosynthesis of specialized products such as coenzymes, hormones etc., which are needed in traces only. Certain other specialized biomolecules such as pigments, toxins, antibiotics, alkaloids etc., are also made by specialized secondary pathways whose details are not yet well understood.

UNIFYING THEMES OF METABOLIC PATHWAYS

More than a thousand chemical reactions take place in even as simple an organism as *Escherichia coli.* In higher multicellular organisms, the situation seems to be more intimidating because of the sheer number of reactants, resultants, and the reactions, of course. However, a closure scrutiny reveals that metabolism has a coherent design containing many common motifs.It is, in fact, these unifying themes that make the comprehension of this complexity more manageable. These unifying themes (or the motifs) inlcude : (*a*) common activated carriers and substrates, (*b*) common reaction types and their mechanism, and (*c*) common regulatory schemes. All these motifs stem from a common evolutionary heritage.

1. Activated Carriers

There is repeated appearance of a limited number of activated intermediates in all metabolic process. Table 19–2 lists the names of some activated carriers used in metabolism. The phosphoryl transfer can be used to drive otherwise endergonic reactions. The phosphoryl-group donor in all such reactions is ATP. Many such activated carriers are described below :

Table 19–2. Some activated carriers in metabolism

Carrier molecule in activated form	*Group carried*	*Vitamin precursor*
ATP	Phosphoryl	
NADH and NADPH	Electrons	Niacin (vitamin B_5)
$FADH_2$	Electrons	Riboflavin (vitamin B_2)
$FMNH_2$	Electrons	Riboflavin (vitamin B_2)
Coenzyme A	Acyl	Pantothenate (vitamin B_3)
Lipoamide	Acyl	
Thiamine pyrophosphate	Aldehyde	Thiamine (vitamin B_1)
Biotin	CO_2	Biotin
Tetrahydrofolate	One-carbon units	Folate
S-adenosylmethionine	Methyl	
Uridine diphosphate glucose	Glucose	
Cytidine diphosphate diacylglycerol	Phosphatidate	
Nucleoside triphosphates	Nucleotides	

Note that many of the activated carriers are coenzymes that are derived from water-soluble vitamins.

A. Activated carriers of electrons for fuel oxidation. In aerobic organisms, the ultimate electron acceptor in the oxidation of fuel molecules is O_2. As electrons are not transferred directly to O_2, the fuel molecules transfer them to special carriers, which are either pyridine nucleotides or flavins. The reduced forms of thse carriers then transfer their high potential electrons to O_2.

Nicotinamide adenine dinucleotide, NAD^+ (Fig. 19–14) is a major electron carrier in the oxidation of fuel molecules. The reactive part of NAD^+ is its nicotinamide ring, a pyridine derivative

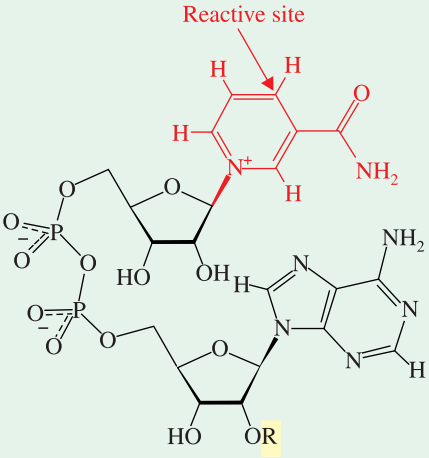
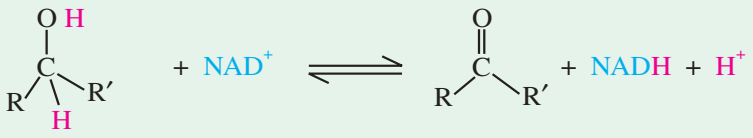


Fig. 19–14. Structure of the oxidized form of nicotinamide-derived electron carrier

Nicotinamide adenine dinucleotode (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$) are main carriers of high-energy electrons.

(In NAD^+, R = H; in $NADP^+$, R = PO_3^{2-})

synthesized from the vitamin B_5 (niacin). During the oxidation of a substrate, the nicotinamide ring of NAD^+ accepts a hydrogen ion and two electrons, which are equivalent to a hydride ion. The reduced form of NAD^+ is called NADH. In the oxidized form, the nitrogen atom carries a positive charge, as indicated by NAD^+. NAD^+ is the electron acceptor in following type of reactions :



In this dehydrogenation, one H atom of the substrate is directly transferred to NAD^+, while the other appears in the solvent as a proton.

The other major electron carrier in the oxidation of fuel molecules is the coenzyme **flavin adenine dinucleotide, FAD** (Fig. 19–15). FAD is the oxidized form of this carrier and $FADH_2$, the reduced form.

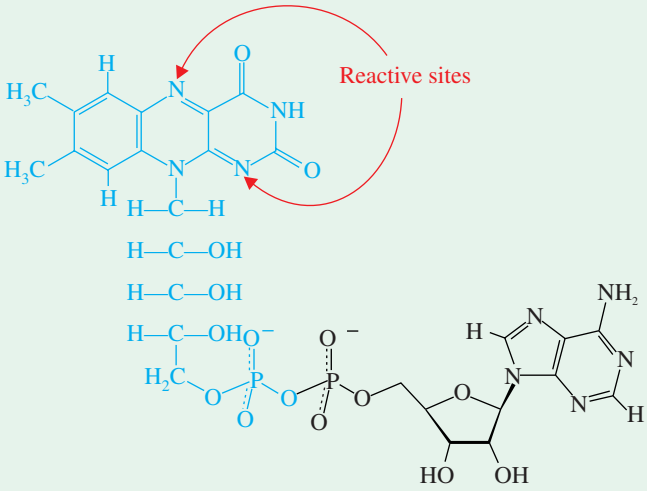
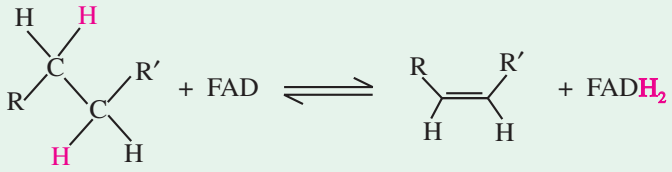


Fig. 19–15. Structure of the oxidized form of flavin adenine dinucleotide

This electron carrier consists of a flavin mononucleotide (FMN) unit (shown in blue) and an AMP unit (shown in black).

FAD is the electron acceptor in following type of reactions :



The reactive part of FAD is its isoalloxazine ring, a derivative of the vitamin B_2 riboflavin (Fig. 19–16). FAD, like NAD^+, can accept two electrons. In doing so, FAD, unlike NAD^+, takes up two protons.

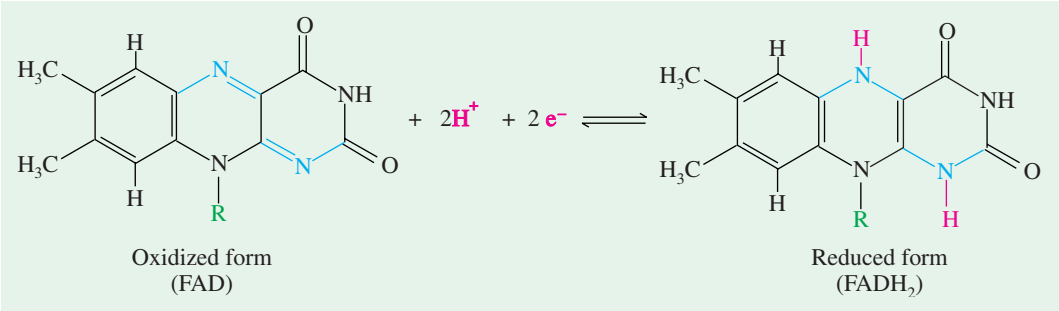
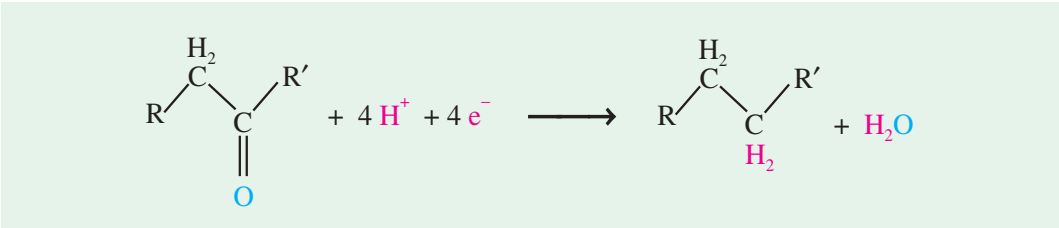


Fig. 19–16. Structures of reactive parts of FAD and $FADH_2$

The electrons and protons are carried by the isoalloxazine ring component of FAD and $FADH_2$.

B. Activated carriers of electrons for reductive biosynthesis. High potential electrons are required in most biosyntheses because the precursors are more oxidized than the products. Hence, reducing power is needed besides ATP. As an illustration, in the biosynthesis of fatty acids, the keto group of an added 2-C unit is reduced to a methylene (—CH_2) group in many steps. This requires an input of 4 electrons.



The electron donor in most reductive biosyntheses is NADPH, the reduced form of nicotinamide adenine dinucleotide phosphate ($NADP^+$; see Fig. 19–17). NADPH differs from NADH in that the 2'-hydroxyl group of its adenosine moiety is esterified with phosphate. NADPH carries electrons in the same way as NADH. *Whereas NADPH is used almost exclusively for reductive biosyntheses, NADH is used primarily for ATP production.*

C. An activated carrier of 2-carbon fragments. Coenzyme A is a carrier of acyl groups (Fig. 19– 16). Acyl groups are important constituents both in catabolism (as in the oxidation of fatty acids)

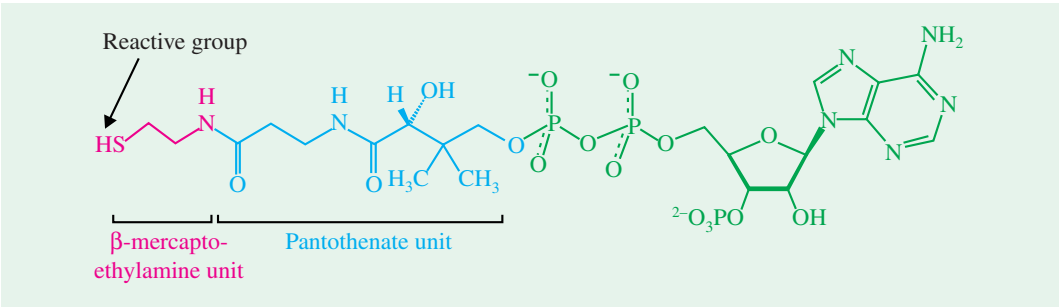
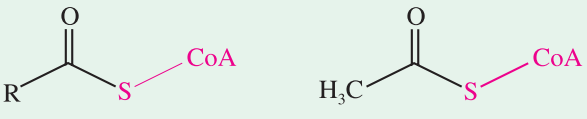


Fig. 19–17. Structure of coenzyme A (CoA)

and in anabolism (as in the synthesis of membrane lipids). The terminal sulfhydryl (—SH) group in CoA is the reactive site. Acyl groups are linked to CoA by thioester bonds. The resultant derivative is called an acyl CoA. An acyl group often linked to CoA is the acetyl unit and the resultant derivative is called *acetyl CoA*. The hydrolysis of acetyl CoA proceeds as follows :



Acyl CoA Acetyl CoA

$$\text{Acetyl CoA} + H_2O \rightleftharpoons \text{Acetate} + \text{CoA} + H^+$$

Acetyl CoA has a high acetyl potential (acetyl group-transfer potential) because transfer of the acetyl group is exergonic. Acetyl CoA carries an activated acetyl group, just as ATP carries an activated phosphoryl group.

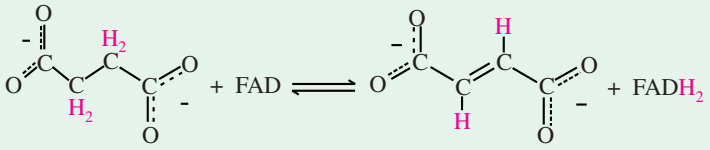
2. Key Reactions

Just as there is an economy of design in the use of activated carriers, so is there an economy of design in biochemical reactions. Although the number of reactions in metabolism is bewilderingly large, the number of *kinds* of reactions is amazingly small. There are only 6 types of key reactions which are reiterated throughout the metabolism (Table 19–3). These reaction types are discussed below :

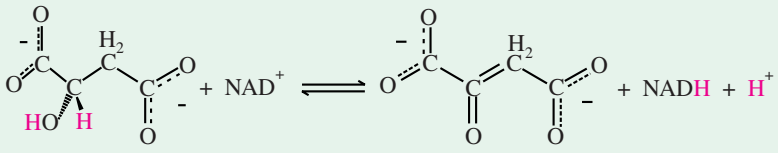
Table 19–3. Types of chemical reactions in metabolism

Type of reaction	*Characteristics*
1. Oxidation-reduction reactions	Electron transfer ...(i)
2. Ligation reactions	Formation of covalent bonds (*i.e*, C-C bonds)
3. Isomerization reactions	Rearrangement of atoms to form isomers
4. Group-transfer reactions	Transfer of a functional group from one molecule to another
5. Hydrolytic reactions	Cleavage of bonds by the addition of water
6. Addition or removal of functional groups	Addition of functional groups to double bonds or their removal to form double bonds ...(iii)

1. Oxidation-reduction reactions. These are essential components of many pathways and the energy is often derived from the oxidation of carbon compounds. Following 2 reactions may be considered :



Succinate Fumarate ... (1)

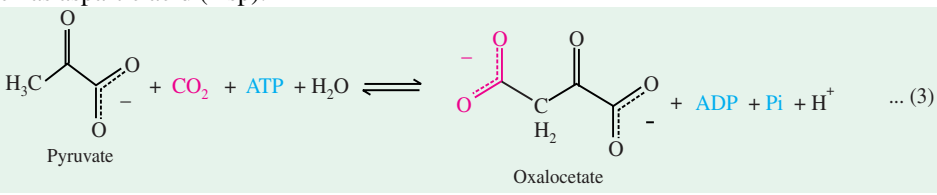


Malate Oxaloacetate ... (2)

These 2 oxidation-reduction reactions are components of the citric acid cycle, which completely oxidizes the activated 2-C fragment of acetyl-CoA to two molecules of CO_2. In reaction 1, $FADH_2$ carries the electrons whereas in Reaction 2, NADH acts as electron carrier. In biosynthetic oxidation-reduction reactions, NADPH is the reductant.

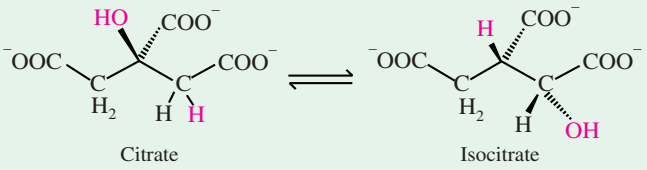
2. Ligation reactions. These reactions form bonds by using free energy from cleavage of ATP molecule. Reaction 3 illustrates the ATP-dependent formation of a C—C bond, required to

combine smaller molecules to form larger molecules. Oxaloacetate (OAA) is formed from pyruvate and CO_2. OAA can be used in the citric acid cycle or converted into amino acids, such as aspartic acid (Asp).



... (3)

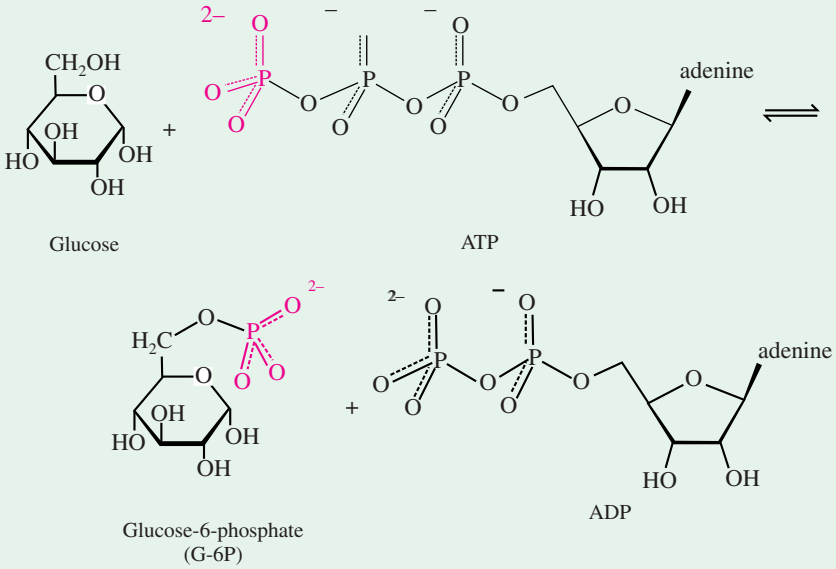
3. **Isomerization reactions.** These rearrange particular atoms within the molecule. Their role is often to prepare a molecule for subsequent reactions.



...(4)

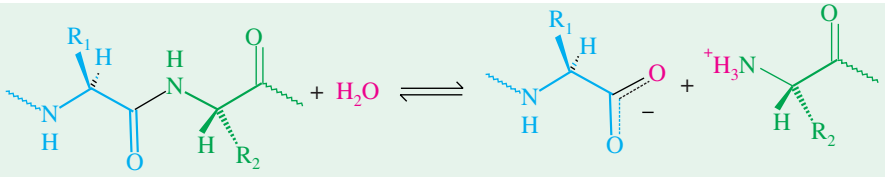
Reaction 4 is again a component of the citric acid cycle. This isomerization prepares the molecule for subsequent oxidation and decarboxylation by removing the OH group of citrate from a tertiary to a secondary position.

4. **Group-transfer reactions.** These reactions play a variety of roles. In one such reaction (Reaction 5), a phosphoryl group is transferred from the activated phosphoryl-group carrier, ATP, to glucose. This reaction traps glucose in the cell so that further catabolism can take place.



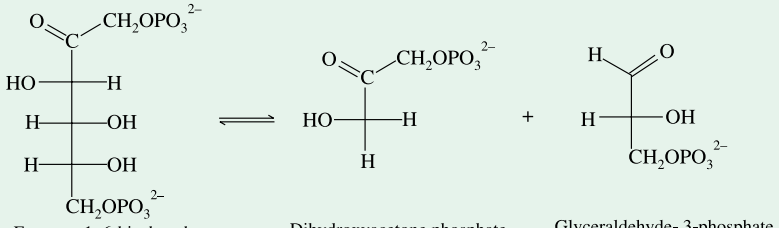
...(5)

5. **Hydrolytic reactions.** These cleave bonds by the addition of water. Hydrolysis is a common means employed to break down large molecules. Proteins are digested by hydrolytic cleavage. Reaction 6 illustrates the hydrolysis of a peptide to yield two smaller peptides.



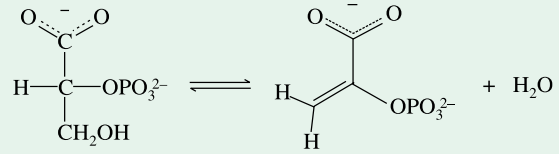
...(6)

6. Addition or removal of functional groups. These types of reactions are catalyzed by *lyases* and consists either in the addition of functional groups to double bonds or the removal of groups to form double bonds. An important example (Reaction 7) is the conversion of 6-C molecule fructose-1, 6-bisphosphate (F-1,6-BP) into two 3-C frgaments, dihydroxyacetone phosphate and glyceraldehyde-3-phosphate.



...(7)

This reaction is a key step in glycolysis, a key pathway for extracting energy from glucose. Another important reaction of this type is the formation of phosphoenolpyruvate (PEP) from 2-phosphoglycerate (2-PG).



...(8)

The dehydration sets up the next step in the glycolytic pathway, a group-transfer reaction that use the high phosphoryl-transfer potential of the product PEP to form ATP from ADP.

These 6 fundamental reaction types are the basis of metabolism. It is remarkable to note that *all 6 types can proceed in either direction,* depending on the standard free energy for the specific reaction and the concentration of the reactants and resultants inside the cell. In Fig. 19–17 reactions are shown which illustrate how simple themes are reiterated. The same sequence of reactions is employed in the citric acid cycle, fatty acid degradation, the degradation of amino acid lysine, and (in reverse) the biosynthesis of fatty acids.

3. Mode of Regulation

Metabolism is regulated in a variety of ways. But the metabolic control must be flexible, because the external environments of the cells are not constant. Metabolism is regulated by controlling following 3 parameters :

(a) ***Amounts of enzymes.*** The amount of a particular enzyme depends on both its rate of synthesis and its rate of degradation. The level of most enzymes is adjusted primarily by changing the rate of transcription of the genes encoding them.

(b) ***Catalytic activities of enzymes.*** The catalytic activities of many enzymes are regulated in many ways. In ***feedback inhibition,*** the first reaction in many biosynthetic pathways is allosterically inhibited by the ultimate product of the pathway. The inhibition of aspartate transcarbamoylase by cytidine triphosphate is a well-known example. This type of control can be almost instantaneous. Another recurring mechanism is ***reversible covalent modification.*** For example, glycogen phosphorylase, an enzyme catalyzing the breakdown of glycogen (a storage form of sugar), is activated by phosphorylation of a particular serine residue when glucose is scarce.

(c) ***Accessibility of substrates.*** Metabolism is also regulated by controlling the flux of substrates.

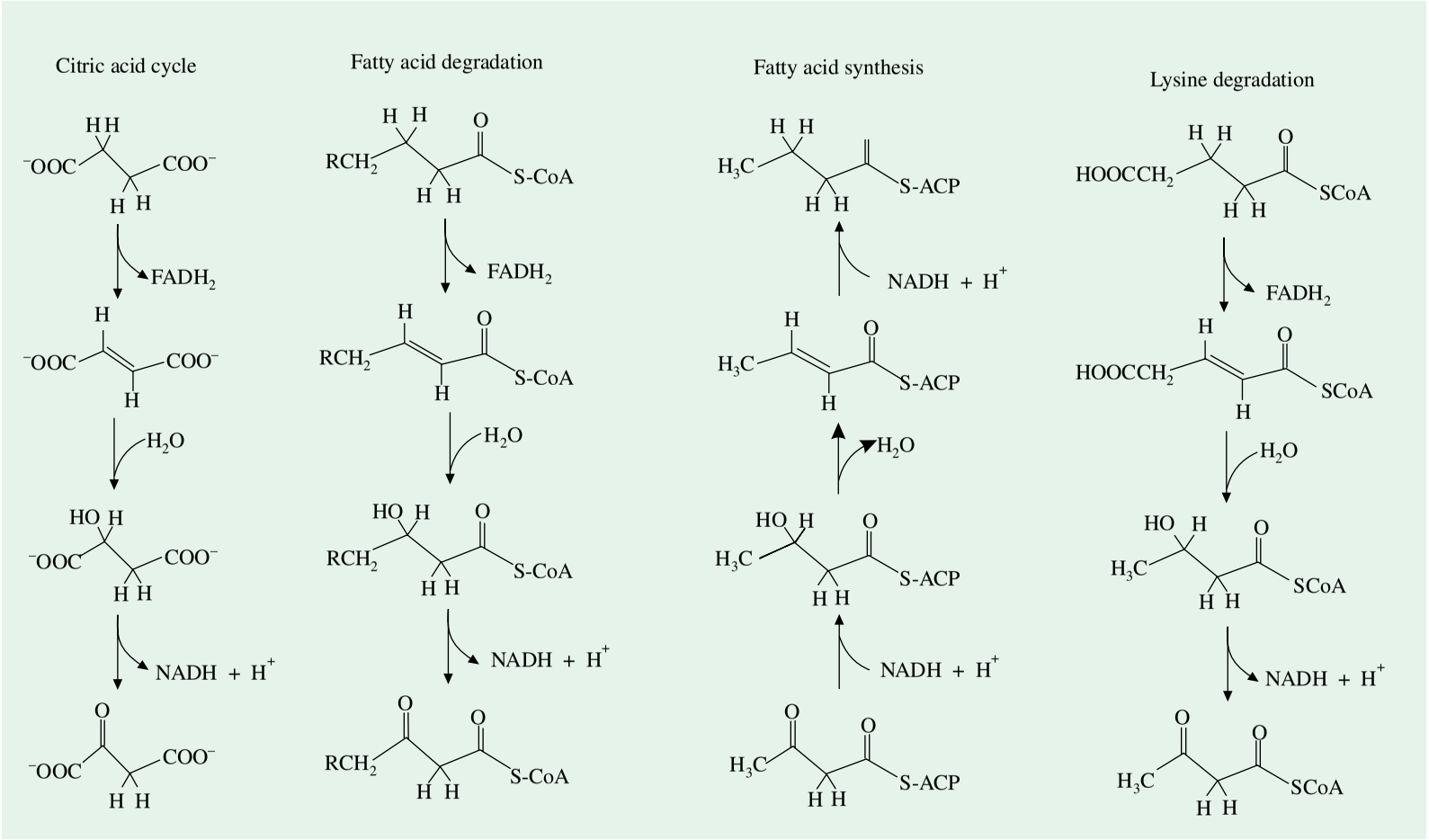


Fig. 19–18 . Metabolic motifs

Some metabolic pathways have similar sequences of reactions in common– in this case, an oxidation, the addition of a functional group (from a water molecule) to a double bond, and another oxidation.
(ACP = Acyl carrier protein)

Contents

The transfer of substrates from one compartment of a cell to another (for example, from the cytosol to mitochondria) can serve as a control point.

Distinct pathways for biosynthesis and degradation contribute to metabolic regulation. The energy charge (refer page 390), which depends on the relative amounts of ATP, ADP, and AMP, plays a role in metabolic regulation. A high energy charge inhibits ATP-generating (or catabolic) pathways, whereas it stimulates ATP-utilizing (or anabolic) pathways.

REGULATION OF METABOLIC PATHWAYS

The metabolic pathways need be regulated so that there is neither a lack nor an excess of any essential product. An excess as well as a deficiency of a metobolic product or even of its intermediate metabolites can be harmful to the cell. Thus, in the normal healthy individual, the regulatory mechanisms maintain a balance between the various anabolic and catabolic pathways. The regulation of metabolic pathways is accomplished by many control mechanisms, as depicted in Fig. 19–19. These mechanisms may act directly at a local or subcellular level or indirectly at an extracellular level. These are described below :

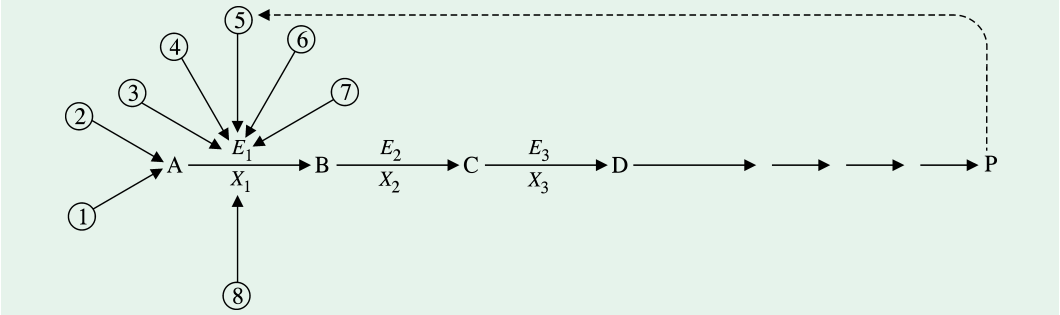


Fig. 19–19. Regulation of metabolic pathways

[Numbers refer to mechanisms of regulation that tally with the description of the text.]

1. ***Nutrient supply.*** The metabolic sequences tend to adapt quantitatively to the supply of a nutrient. This usually entails an increase (or decrease) in the amount of one or more enzymes involved in the metabolic pathway. As an example, relatively large amount of renin (a milk-coagulating enzyme) is present in the gastric mucosa and gastric secretion of young nursing animals whereas this is virtually lacking in the adults. The absence of the substrate may also lead to a lack of its enzyme.

2. ***Nutrient transport.*** The supply of nutrient (or substrate) into a cell can be regulated by controlling the transport of the nutrient across the cell membrane. As an illustratation, *insulin* regulates carbohydrate metabolism by facilitating the transport of glucose into the cells.

3. ***Enzyme amount.*** The quantity of enzyme available for a reaction may be controlled genetically by induction or repression ; or the activity of an enzyme may be affected at the cellular level either by its inherent capacity for interconversion to inactive forms or by allosteric effects or even by inhibitors. Furthermore, the synthesis of many enzymes is either induced or repressed by certain specific hormones. For example, the *adrenoglucocorticoid hormones* are inducers for the synthesis of gluconeogenic enzymes whereas insulin serves as a repressor for the formation of these same enzymes but an inducer for the synthesis of the key glycolytic enzymes.

4. ***Product need.*** A demand of a metabolic product may stimulate its increased output. This may, however, result directly from a mass action effect or indirectly by other regulatory mechanisms such as hormonal or neural control. The two typical examples of the product need are (a) the stimulation of hepatic glucogenolysis by a low blood sugar level and (b) the stimulation of hemoglobin synthesis in anaemias by the hormone erythropoietin.

5. ***Product inhibition.*** *This appears to be the major mechanism for the regulation of metabolic pathways.* Very often the sufficient supply of the product acts as repressor to 'shut off' the synthesis of

usually the first of the series of enzymes of a particular pathway. This is particularly advantageous as accumulation of the later metabolites in the pathway, which may be deleterious to the cell, is prevented. This could occur if a later enzyme in the pathway were the one suppressed. Such type of inhibition is also called *feedback* (or *end product*) *inhibition*. A classic example is the inhibition of porphyrin heme biosynthesis by the end product heme. Here the biosynthesis of the first enzyme in the metabolic sequence, δ-*aminolevulinic acid synthetase is repressed*.

6. ***Endocrine control.*** The metabolic pathways are controlled by hormonal secretions in many ways. Conversion of an inactive form of enzyme to the active form (*e.g.*, conversion of phosphorylase b to active phosphorylase a by epinephrine and glucagon *via* cyclic-AMP) is one such mechanism. Other mechanisms are the regulation of transport through the cell membrane (*e.g.*, insulin), the enzyme induction and repression etc.

7. ***Neural control.*** The effects of nerve stimulation on metabolic pahtways are probably indirect by hormonal or other mechanisms. For example, the effect of psychologic factors such as fright or anger on carbohydrate metabolism, specifically in increasing the blood sugar level, apparently results from an increased secretion of epinephrine which, in turn, accelerates glucogenolysis by the mechanism described under `Product Need'. Neural effects by way of an increased secretion of neurohormones are still other examples of indirect means of affecting metabolic pathways.

8. ***Cofactor availability.*** The enzyme-catalyzed reactions, requiring a cofactor, are affected by the amount of the cofactor available. As an example, relatively large amount of nicotinamide adenine dinucleotide (NAD^+) as cofactor is required for the metabolism of ethanol. Evidently, during ethanol metabolism, lesser NAD^+ may then be available for other metabolic pathways requiring NAD^+ as cofactor. These pathways may, therefore, become partially or even completely blocked. However, metabolic sequences requiring $NADH_2$ (which is formed from NAD^+ during alcohol oxidation) as a cofactor may then be stimulated. Conversion of pyruvic acid to lactic acid is one such example.

EVOLUTION OF METABOLIC PATHWAYS

Until the 1980s, all biological catalysts, termed *enzymes*, were believed to be proteins. Then,

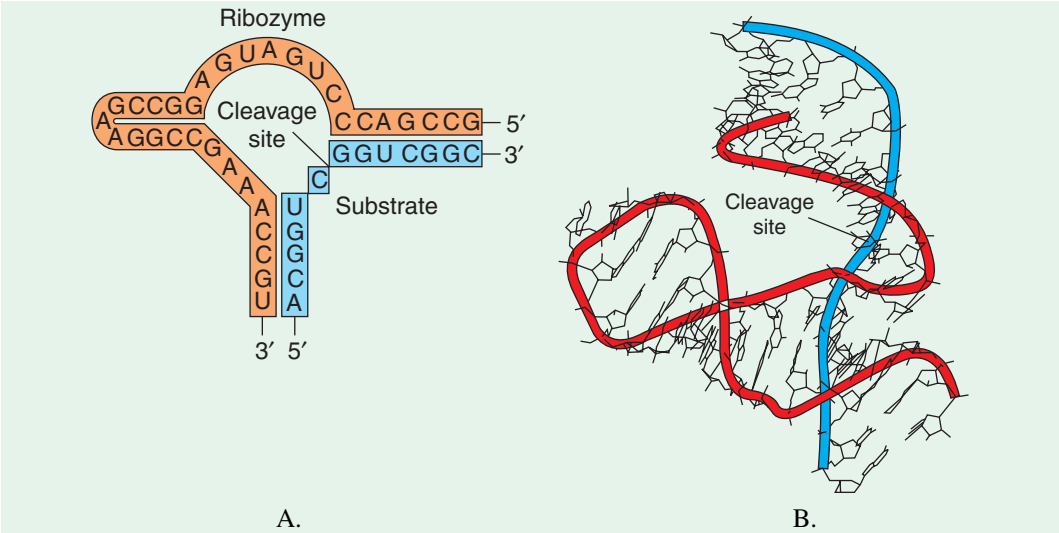


Fig. 19–20. The catalytic RNA

A. The base-pairing pattern of a 'hammerhead' ribozyme and its substrate

B. The folded conformation of the complex

The ribozyme cleaves the bond at the cleavage site. The paths of the nucleic acid backbones are highlighted in red and blue.

(*Adapted from Berg, JM Tymoczko JL and Stryer L, 2002*)

Sidney Altman and Thomas R. Cech (Nobel Laureates, 1997) independently discovered that certain RNA molecules can be effective catalysts. These RNA catalysts have come to be known as *ribozymes.* The discovery of ribozymes suggested the possibility that catalytic RNA molecules could have played fundamental roles early in the evolution of life.

The catalytic ability of RNA molecules is related to their ability to adopt specific yet complex structures. This principle is illustrated by a *'hammerhead' ribozyme,* an RNA structure first identified in plant viruses (Fig. 19–20). This RNA molecule promotes the cleavage of specific RNA molecules at specific sites (This cleavage is necessary for certain aspects of the viral life cycle). The ribozyme requires Mg^{2+} ion or other ion for the cleavage step and forms a complex with its substrate RNA molecule, that can adopt a reactive conformation. The existence of RNA molecules that possess specific binding and catalytic properties makes plausible the idea of an early **'RNA World'**, inhabited by life forms dependent on RNA molecules to play all major roles, including those important in biosynthesis and energy metabolism. Thus, RNA served as catalysts and information-storage molecules.

The activated carriers such as ATP, NADH, FAD and coenzyme A all contain adenosine diphosphate units (Fig. 19–21). What can be the reason behind it ? A possible explanation is that these molecules evolved from the early RNA catalysts. Non-RNA units such as the isoalloxazine ring may have been recruited to serve as efficient carriers of activated electrons and chemical units, a function not readily performed by RNA itself. The adenine ring of $FADH_2$ binds to a uracil unit in a niche of a ribozyme by base-pairing, whereas the isoalloxazine ring bulges out and functions as an electron carrier. When the more versatile proteins replaced RNA as the major catalysts, the

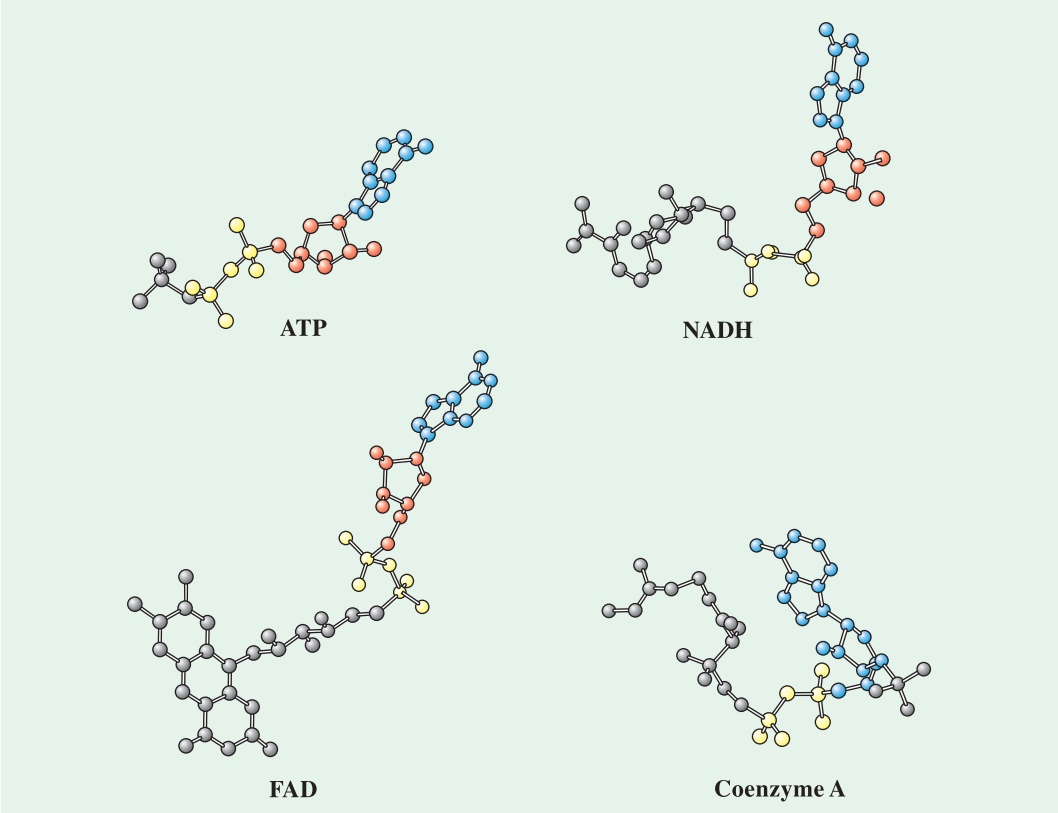


Fig. 19–21. Commonality of adenosine diphosphate (ADP) unit in ATP, NADH, FAD and CoA

The adenine unit is shown in blue, the ribose unit in red, and the diphosphate unit in yellow.

ribonucleotide coenzymes stayed essentially unchanged because they were already well adapted to their metabolic roles. For example, the nicotinamide unit of NADH can readily transfer electrons, regardless of whether the adenine unit interacts with a base in an RNA enzyme or with amino acid residues in a protein enzyme. With the advent of protein enzymes, these important cofactors evolved as free molecules, without losing the adenosine disphosphate vestige of their RNA-world ancestry. That modules and motifs of metabolism are common to all forms of life testifies to their common origin and to the retention of functioning modules through billions of years of evolution.

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PROBLEM

1. Write a balanced equation for the complete oxidation of each of the following, and calculate the respiratory quotient for each substance.
   (*a*) Ethanol
   (*b*) Acetic acid
   (*c*) Stearic acid
   (*d*) Oleic acid
   (*e*) Linoleic acid