29 Pathways of Sugar Metabolism: Pentose Phosphate Pathway, Fructose, and Galactose Metabolism

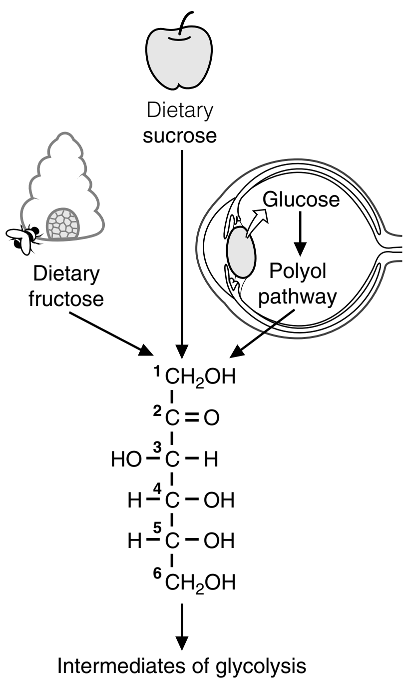

Fig. 29.1. Fructose. The sugar fructose is found in the diet as the free sugar in foods such as honey or as a component of the disaccharide sucrose in fruits and sweets. It also can be synthesized from glucose via the polyol pathway. In the lens of the eye, the polyol pathway contributes to the formation of cataracts. Fructose is metabolized by conversion to intermediates of glycolysis.

Enzymes can generally use either NADPH or NADH, but not both. Reactions requiring the input of electrons as hydride ions are usually catalyzed by enzymes specific for NADPH.

Glucose is at the center of carbohydrate metabolism and is the major dietary sugar. Other sugars in the diet are converted to intermediates of glucose metabolism, and their fates parallel that of glucose. When carbohydrates other than glucose are required for the synthesis of diverse compounds such as lactose, glycoproteins, or glycolipids, they are synthesized from glucose.

Fructose*, the second most common sugar in the adult diet, is ingested principally as the monosaccharide or as part of* ***sucrose*** *(Fig. 29.1). It is metabolized principally in the liver (and to a lesser extent in the small intestine and kidney) by phosphorylation at the 1-position to form* ***fructose 1-P****, followed by conversion to intermediates of the glycolytic pathway. The major products of its metabolism in liver are, therefore, the same as glucose (including lactate, blood glucose, and glycogen).* ***Essential fructosuria*** *(****fructokinase deficiency****) and* ***hereditary fructose intolerance*** *(a deficiency of the fructose 1-phosphate cleavage by* ***aldolase B****) are inherited disorders of fructose metabolism.*

Fructose synthesis from glucose in the ***polyol pathway*** *occurs in seminal vesicles and other tissues.* ***Aldose reductase*** *converts glucose to the sugar alcohol sorbitol (a polyol), which is then oxidized to fructose. In the lens of the eye, elevated levels of* ***sorbitol*** *in diabetes mellitus may contribute to* ***cataract*** *formation.*

Galactose is ingested principally as ***lactose,*** *which is converted to galactose and glucose in the intestine.* ***Galactose*** *is converted to glucose principally in the liver. It is phosphorylated to galactose 1-phosphate by* ***galactokinase*** *and activated to a UDP-sugar by* ***galactosyl uridylyltransferase.*** *The metabolic pathway subsequently generates glucose 1-phosphate.* ***Classical galactosemia****, a deficiency of galactosyl uridylyltransferase, results in the accumulation of galactose 1-phosphate in the liver and the inhibition of hepatic glycogen metabolism and other pathways that require UDP sugars. Cataracts can occur from accumulation of galactose in the blood, which is converted to* ***galactitol*** *(the sugar alcohol of galactose) in the lens of the eye.*

The ***pentose phosphate pathway*** *consists of both* ***oxidative*** *and* ***nonoxidative*** *components (Fig. 29.2). In the oxidative pathway, glucose 6-phosphate is oxidized to* ***ribulose 5-phosphate****, CO_2, and* ***NADPH****. Ribulose 5-phosphate, a pentose, can be converted to* ***ribose 5-phosphate*** *for nucleotide biosynthesis. The NADPH is used for reductive pathways, such as* ***fatty acid biosynthesis****,* ***detoxification*** *of drugs by monooxygenases, and the* ***glutathione defense system*** *against injury by reactive oxygen species (ROS).*

In the nonoxidative phase of the pathway, ribulose 5-phosphate is converted to ribose 5-phosphate and to intermediates of the glycolytic pathway. This portion of

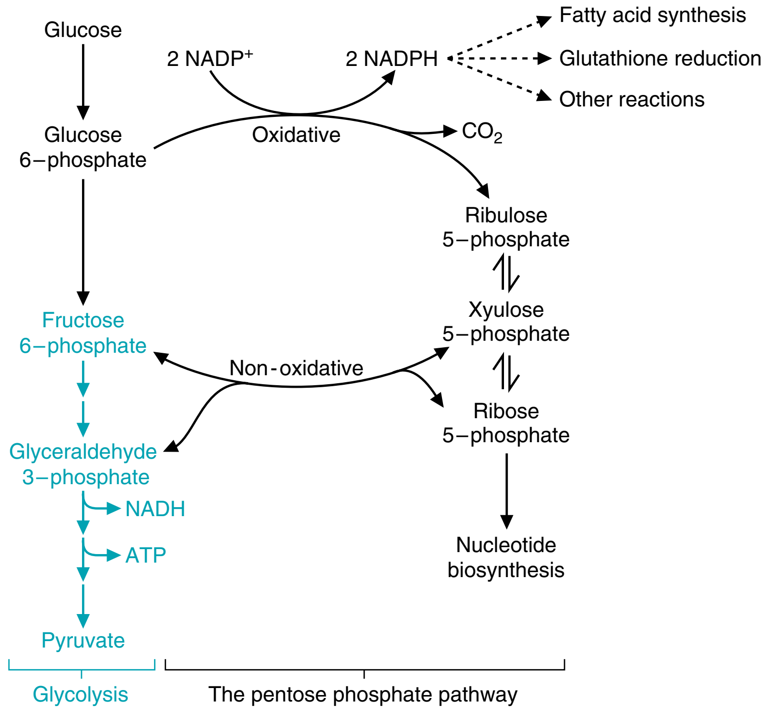

Fig. 29.2. Overview of the pentose phosphate pathway. The pentose phosphate pathway generates NADPH for reactions requiring reducing equivalents (electrons) or ribose 5-phosphate for nucleotide biosynthesis. Glucose 6-phosphate is a substrate for both the pentose phosphate pathway and glycolysis. The 5-carbon sugar intermediates of the pentose phosphate pathway are reversibly interconverted to intermediates of glycolysis. The portion of glycolysis that is not part of the pentose phosphate pathway is shown in blue.

The pentose phosphate pathway is also called the hexose monophosphate shunt (HMP shunt). It shunts hexoses from glycolysis, forming pentoses, which may be reconverted to glycolytic intermediates.

the pathway is reversible; therefore, ribose 5-phosphate can also be formed from intermediates of glycolysis. One of the enzymes involved in these sugar interconversions, ***transketolase****, uses* ***thiamine pyrophosphate*** *as a coenzyme.*

The sugars produced by the pentose phosphate pathway enter glycolysis as fructose 6-phosphate and glyceraldehyde 3-phosphate, and their further metabolism in the glycolytic pathway generates NADH, adenosine triphsphate (ATP), and pyruvate. The overall equation for the conversion of glucose 6-phosphate to fructose 6-phosphate and glyceraldehyde 3-phosphate through both the oxidative and nonoxidative reactions of the pentose phosphate pathway is:

$$3\ glucose\text{-}6\text{-}P + 6\ NADP^+ \rightarrow 3\ CO_2 + 6\ NADPH + 6\ H^+ + 2\ fructose\text{-}6\text{-}P + glyceraldehyde\text{-}3\text{-}P.$$

THE WAITING ROOM

Candice Sucher is an 18-year-old girl who presented to her physician for a precollege physical examination. While taking her medical history, the doctor learned that she carefully avoided eating all fruits and any foods that contained table sugar. She related that, from a very early age, she had learned that these foods caused severe weakness and symptoms suggestive of low blood sugar, such as tremulousness and sweating. Her medical history also indicated that her mother had described her as having been a very irritable baby who often cried incessantly, especially after meals, and vomited frequently. At these times, Candice's abdomen had become distended, and she became drowsy and apathetic. Her mother had intuitively eliminated certain foods from Candice's diet, after which the severity and frequency of these symptoms diminished.

Erin Galway is a 3-week-old female infant who began vomiting 3 days after birth, usually within 30 minutes after breastfeeding. Her abdomen became distended at these times, and she became irritable and cried frequently. When her mother noted that the whites of Erin's eyes were yellow, she took her to a pediatrician. The doctor agreed that Erin was slightly jaundiced. He also noted an enlargement of her liver and questioned the possibility of early cataract formation in the lenses of Erin's eyes. He ordered liver and kidney function tests and did two separate dipstick urine tests in his office, one designed to measure only glucose in the urine and the other capable of detecting any of the reducing sugars.

Al Martini developed a fever of 101.5°F on the second day of his hospitalization for acute alcoholism. He had a cough productive of gray sputum. A chest x-ray showed right lower lobe pneumonia. A stain of his sputum showed many small pleomorphic Gram-negative bacilli. Sputum was sent for culture and a determination of which antibiotics would be effective in treating the causative organism (sensitivity testing). Because his landlady stated that he had an allergy to penicillin, he was started on a course of the antibiotic combination of trimethoprim and sulfamethoxazole (TMP/sulfa). To his landlady's knowledge, he had never been treated with a sulfa drug previously.

On the third day of therapy with TMP/sulfa for his pneumonia, **Al Martini** was slightly jaundiced. His hemoglobin level had fallen by 3.5 g/dL from the value on admission, and his urine was red-brown because of the presence of free hemoglobin. Mr. Martini had apparently suffered acute hemolysis (lysis or destruction of some of his red blood cells) induced by his infection and exposure to the sulfa drug.

I. FRUCTOSE

Fructose is found in the diet as a component of sucrose in fruit, as a free sugar in honey, and in high-fructose corn syrup (see Fig. 29.1). Fructose enters epithelial cells and other types of cells by facilitated diffusion on the GLUT V transporter. It is metabolized to intermediates of glycolysis. Problems with fructose absorption and metabolism are relatively more common than with other sugars.

A. Fructose Metabolism

Fructose is metabolized by conversion to glyceraldehyde-3-P and dihydroxyacetone phosphate, which are intermediates of glycolysis (Fig. 29.3). The steps parallel those of glycolysis. The first step in the metabolism of fructose, as with glucose, is phosphorylation. Fructokinase, the major kinase involved, phosphorylates fructose in the 1-position. Fructokinase has a high V_{max}, and rapidly phosphorylates fructose as it enters the cell. The fructose 1-phosphate formed is not an intermediate of glycolysis but rather is cleaved by aldolase B to dihydroxyacetone phosphate (an intermediate of glycolysis) and glyceraldehyde. Glyceraldehyde is then phosphorylated to glyceraldehyde-3-P by triose kinase. Dihydroxyacetone phosphate and glyceraldehyde 3-phosphate are intermediates of the glycolytic pathway and can proceed through it to pyruvate, the TCA cycle, and fatty acid synthesis. Alternately, these intermediates can also be converted to glucose by gluconeogenesis. In other words, the fate of fructose parallels that of glucose.

When individuals with defects of aldolase B ingest fructose, the extremely high levels of fructose 1-phosphate that accumulate in the liver and kidney cause a number of adverse effects. Hypoglycemia results from inhibition of glycogenolysis and gluconeogenesis. Glycogen phosphorylase (and possibly phosphoglucomutase and other enzymes of glycogen metabolism) are inhibited by the accumulated fructose 1-phosphate. Aldolase B is required for glucose synthesis from glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, and its low activity in aldolase B–deficient individuals is further decreased by the accumulated fructose 1-phosphate. The inhibition of gluconeogenesis results in lactic acidosis.

The accumulation of fructose 1-phosphate also substantially depletes the phosphate pools. The fructokinase reaction uses ATP at a rapid rate such that the mitochondria regenerate ATP rapidly, which leads to a drop in free phosphate levels. The low levels of phosphate release inhibition of AMP deaminase, which converts AMP to inosine monophosphate (IMP). The nitrogenous base of IMP (hypoxanthine) is degraded to uric acid. The lack of phosphate and depletion of adenine nucleotides lead to a loss of ATP, further contributing to the inhibition of biosynthetic pathways, including gluconeogenesis.

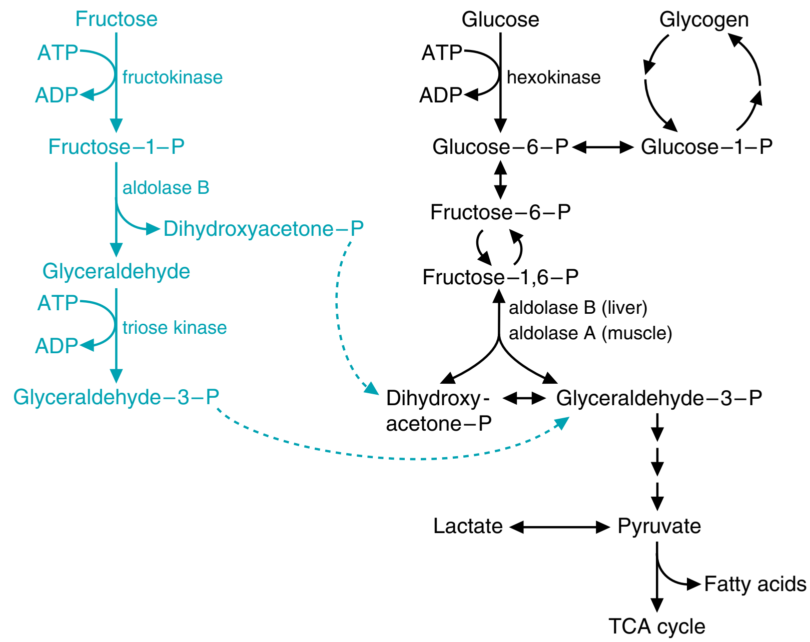

Fig. 29.3. Fructose metabolism. The pathway for the conversion of fructose to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate is shown in blue. These two compounds are intermediates of glycolysis and are converted in the liver principally to glucose, glycogen, or fatty acids. In the liver, aldolase B cleaves both fructose 1-phosphate in the pathway for fructose metabolism, and fructose 1,6-bisphosphate in the pathway for glycolysis.

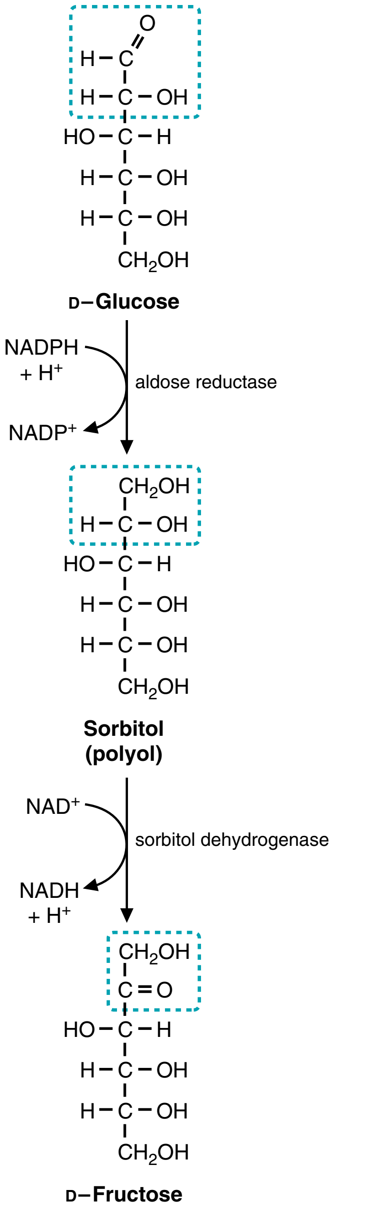

Fig. 29.4. The polyol pathway converts glucose to fructose.

Q: Essential fructosuria is a rare and benign genetic disorder caused by a deficiency of the enzyme fructokinase. Why is this disease benign, when a deficiency of aldolase B (hereditary fructose intolerance) can be fatal? Could **Candice Sucher** have essential fructosuria?

The metabolism of fructose occurs principally in the liver and to a lesser extent in the small intestinal mucosa and proximal epithelium of the renal tubule, because these tissues have both fructokinase and aldolase B. Aldolase exists as several isoforms: aldolases A, B, C, and fetal aldolase. Although all of these aldolase isoforms can cleave fructose 1,6-bisphosphate, the intermediate of glycolysis, only aldolase B can also cleave fructose 1-phosphate. Aldolase A, present in muscle and most other tissues, and aldolase C, present in brain, have almost no ability to cleave fructose 1-phosphate. Fetal aldolase, present in the liver before birth, is similar to aldolase C.

Aldolase B is the rate-limiting enzyme of fructose metabolism, although it is not a rate-limiting enzyme of glycolysis. It has a much lower affinity for fructose l-phosphate than fructose 1,6-bisphosphate (although the k_{cat} is the same) and is very slow at physiologic levels of fructose 1-phosphate. As a consequence, after ingesting a high dose of fructose, normal individuals accumulate fructose 1-phosphate in the liver while it is slowly converted to glycolytic intermediates. Individuals with hereditary fructose intolerance (a deficiency of aldolase B) accumulate much higher amounts of fructose 1-phosphate in their livers.

Other tissues also have the capacity to metabolize fructose but do so much more slowly. The hexokinase isoforms present in muscle, adipose tissue, and other tissues can convert fructose to fructose 6-phosphate, but react so much more efficiently with glucose. As a result, fructose phosphorylation is very slow in the presence of physiologic levels of intracellular glucose and glucose 6-phosphate.

B. Synthesis of Fructose in the Polyol Pathway

Fructose can be synthesized from glucose in the polyol pathway. The polyol pathway is named for the first step of the pathway in which sugars are reduced to the sugar alcohol by the enzyme aldose reductase (Fig. 29.4) Glucose is reduced to the sugar alcohol sorbitol, and sorbitol is then oxidized to fructose.

This pathway is present in seminal vesicles, which synthesize fructose for the seminal fluid. Spermatozoa use fructose as a major fuel source while in the seminal fluid and then switch to glucose once in the female reproductive tract. Utilization of fructose is thought to prevent acrosomal breakdown of the plasma membrane (and consequent activation) while the spermatozoa are still in the seminal fluid.

The polyol pathway is present in many tissues, but its function in all tissues is not understood. Aldose reductase is relatively nonspecific, and its major function may be the metabolism of an aldehyde sugar other than glucose. The activity of this enzyme can lead to major problems in the lens of the eye, where it is responsible for the production of sorbitol from glucose and galactitol from galactose. When the concentration of glucose or galactose is elevated in the blood, their respective sugar alcohols are synthesized in the lens more rapidly than they are removed, resulting in increased osmotic pressure within the lens.

II. GALACTOSE METABOLISM—METABOLISM TO GLUCOSE-1-P

Dietary galactose is metabolized principally by phosphorylation to galactose 1-phosphate, and then conversion to UDP-galactose and glucose 1-phosphate (Fig. 29.5). The phosphorylation of galactose, again an important first step in the pathway, is carried out by a specific kinase, galactokinase. The formation of UDP-galactose is accomplished by attack of the phosphate oxygen on galactose 1-phosphate on the α phosphate of UDP-glucose, releasing glucose 1-phosphate while forming UDP-galactose. The enzyme that catalyzes this reaction is galactose l-phosphate uridylyltransferase. The UDP-galactose is then converted to UDP-glucose by the reversible UDP-glucose epimerase (the configuration of the hydroxyl group on carbon four is reversed in this reaction). The net result of this sequence of reactions is that galactose is converted to glucose 1-phosphate, at the expense of 1 high-energy bond of ATP. The sum of these reactions is indicated in the equations that follow:

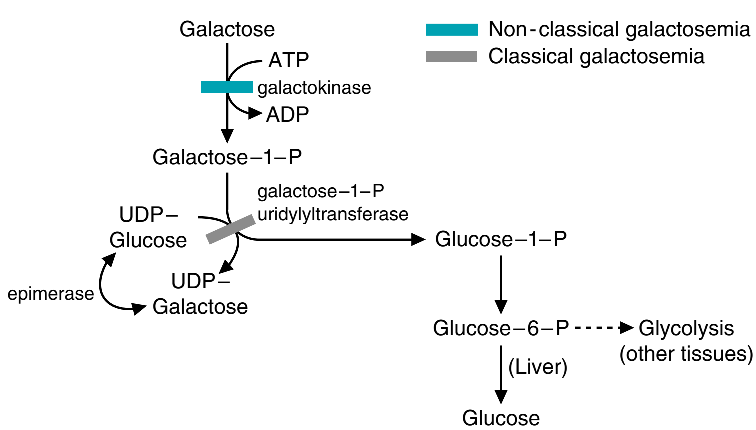

Fig. 29.5. Metabolism of galactose. Galactose is phosphorylated to galactose 1-phosphate by galactokinase. Galactose 1-phosphate reacts with UDP-glucose to release glucose 1-phosphate. Galactose thus can be converted to blood glucose, enter glycolysis, or enter any of the metabolic routes of glucose. In classical galactosemia, a deficiency of galactose 1-phosphate uridylyltransferase (shown in grey) results in the accumulation of galactose 1-phosphate in tissues and the appearance of galactose in the blood and urine. In nonclassical galactosemia, a deficiency of galactokinase results in the accumulation of galactose.

The accumulation of sorbitol in muscle and nerve tissues may contribute to the peripheral neuropathy characteristic of patients with poorly controlled diabetes mellitus. This is one of the reasons it is so important for **Di Abietes** (who has type 1 diabetes mellitus) and **Ann Sulin** (who has type 2 diabetes mellitus) to achieve good glycemic control.

The accumulation of sugars and sugar alcohols in the lens of patients with hyperglycemia (e.g., diabetes mellitus) results in the formation of cataracts. Glucose levels are elevated and increase the synthesis of sorbitol and fructose. As a consequence, a high osmotic pressure is created in the lens. The high glucose and fructose levels also result in nonenzymatic glycosylation of lens proteins. The result of the increased osmotic pressure and the glycosylation of the lens protein is an opaque cloudiness of the lens known as a cataract. **Erin Galway** seemed to have an early cataract, probably caused by the accumulation of galactose and its sugar alcohol galactitol.

A: In essential fructosuria, fructose cannot be converted to fructose 1-phosphate. This condition is benign because no toxic metabolites of fructose accumulate in the liver, and the patient remains nearly asymptomatic. Some of the ingested fructose is slowly phosphorylated by hexokinase in nonhepatic tissues and metabolized by glycolysis, and some appears in the urine. There is no renal threshold for fructose; the appearance of fructose in the urine (fructosuria) does not require a high fructose concentration in the blood.

Hereditary fructose intolerance, conversely, results in the accumulation of fructose 1-phosphate and fructose. By inhibiting glycogenolysis and gluconeogenesis, the high levels of fructose 1-phosphate caused the hypoglycemia that **Candice Sucher** experienced as an infant when she became apathetic and drowsy, and as an adult when she experienced sweating and tremulousness.

Erin Galway's urine was negative for glucose when measured with the glucose oxidase strip but was positive for the presence of a reducing sugar. The reducing sugar was identified as galactose. Her liver function tests showed an increase in serum bilirubin and in several liver enzymes. Albumin was present in her urine. These findings and the clinical history increased her physician's suspicion that Erin had classical galactosemia.

Classical galactosemia is caused by a deficiency of galactose 1-phosphate uridylyltransferase. In this disease, galactose 1-phosphate accumulates in tissues, and galactose is elevated in the blood and urine. This condition differs from the rarer deficiency of galactokinase (nonclassical galactosemia), in which galactosemia and galactosuria occur but galactose 1-phosphate is not formed. Both enzyme defects result in cataracts from galactitol formation by aldose reductase in the polyol pathway. Aldose reductase has a relatively high K_m for galactose, approximately 12 to 20 mM, so that galactitol is formed only in galactosemic patients who have eaten galactose. Galactitol is not further metabolized and diffuses out of the lens very slowly. Thus, hypergalactosemia is even more likely to cause cataracts than hyperglycemia. **Erin Galway**, although only 3 weeks old, appeared to have early cataracts forming in the lens of her eyes.

One of the most serious problems of classical galactosemia is an irreversible mental retardation. Realizing this problem, **Erin Galway's** physician wanted to begin immediate dietary therapy. A test that measures galactose 1-phosphate uridylyltransferase in erythrocytes was ordered. The enzyme activity was virtually absent, confirming the diagnosis of classical galactosemia.

$$\text{(1) Galactose + ATP} \xrightarrow{\textit{galactokinase}} \text{Galactose-1-P + ADP}$$

$$\text{(2) Galactose-1-P + UDP-glucose} \xrightarrow[\textit{uridylyltransferase}]{\textit{galactose-1-P}} \text{UDP-galactose+glucose-1-P}$$

$$\text{(3) UDP-galactose} \xrightarrow{\textit{UDP-glucose epimerase}} \text{UDP-glucose}$$

$$\textit{Net Equation: } \text{Galactose+ATP} \longrightarrow \text{Glucose-1-P + ADP}$$

The enzymes for galactose conversion to glucose 1-phosphate are present in many tissues, including the adult erythrocyte, fibroblasts, and fetal tissues. The liver has a high activity of these enzymes, and can convert dietary galactose to blood glucose and glycogen. The fate of dietary galactose, like that of fructose, therefore, parallels that of glucose. The ability to metabolize galactose is even higher in infants than in adults. Newborn infants ingest up to 1 g galactose per kg per feeding (as lactose). Yet the rate of metabolism is so high that the blood level in the systemic circulation is less than 3 mg/dL, and none of the galactose is lost in the urine.

III. THE PENTOSE PHOSPHATE PATHWAY

The pentose phosphate pathway is essentially a scenic bypass route around the first stage of glycolysis that generates NADPH and ribose-5-P (as well as other pentose sugars). Glucose 6-phosphate is the common precursor for both pathways. The oxidative first stage of the pentose phosphate pathway generates two moles of NADPH per glucose 6-phosphate oxidized. The second stage of the pentose phosphate pathway generates ribose-5-P and converts unused intermediates to fructose-6-P and glyceraldehyde-3-P in the glycolytic pathway (see Fig. 29.2). All cells require NADPH for reductive detoxification, and most cells require ribose-5-P for nucleotide synthesis. Consequently, the pathway is present in all cells. The enzymes reside in the cytosol, as do the enzymes of glycolysis.

A. Oxidative Phase of the Pentose Phosphate Pathway

1. NADPH PRODUCTION

In the oxidative first phase of the pentose phosphate pathway, glucose 6-phosphate is oxidatively decarboxylated to a pentose sugar, ribulose 5-phosphate (Fig. 29.6). The first enzyme of this pathway, glucose 6-phosphate dehydrogenase, oxidizes the aldehyde at C1 and reduces $NADP^+$ to NADPH. The gluconolactone that is formed is rapidly hydrolyzed to 6-phosphogluconate, a sugar acid with a carboxylic acid group at C1. The next oxidation step releases this carboxyl group as CO_2, with the electrons being transferred to $NADP^+$. This reaction is mechanistically very similar to the one catalyzed by isocitrate dehydrogenase in the TCA cycle. Thus, two moles of NADPH per mole of glucose 6-phosphate are formed from this portion of the pathway.

NADPH, rather than NADH, is generally used in the cell for pathways that require the input of electrons for reductive reactions because the ratio of $NADPH/NADP^+$ is much greater than the $NADH/NAD^+$ ratio. The NADH generated from fuel oxidation is rapidly oxidized back to NAD^+ by NADH dehydrogenase in the electron transport chain, so the level of NADH is very low in the cell.

NADPH can be generated from a number of reactions in the liver and other tissues, but not the red blood cell. For example, in tissues with mitochondria, an energy-requiring transhydrogenase located near the complexes of the electron transport chain can transfer reducing equivalents from NADH to $NADP^+$ to generate NADPH.

NADPH, however, cannot be directly oxidized by the electron transport chain, and the ratio of NADPH to $NADP^+$ in cells is greater than one. The reduction potential of NADPH therefore can contribute to the energy needed for biosynthetic processes and provide a constant source of reducing power for detoxification reactions.

2. RIBOSE 5-PHOSPHATE FROM THE OXIDATIVE ARM OF THE PATHWAY

To generate ribose 5-phosphate from the oxidative pathway, the ribulose 5-phosphate formed from the action of the two oxidative steps is isomerized to produce ribose 5-phosphate (a ketose-to-aldose conversion, similar to fructose 6-phosphate being isomerized to glucose 6-phosphate; see section III.B.1 below). The ribose 5-phosphate can then enter the pathway for nucleotide synthesis, if needed, or can be converted to glycolytic intermediates, as described below for the nonoxidative phase of the pentose phosphate pathway. The pathway through which the ribose 5-phosphate travels is determined by the needs of the cell at the time of its synthesis.

B. The Nonoxidative Phase of the Pentose Phosphate Pathway

The nonoxidative reactions of this pathway are reversible reactions that allow intermediates of glycolysis (specifically glyceraldehyde-3-P and fructose-6-P) to be converted to five-carbon sugars (such as ribose-5-P), and vice versa. The needs of the cell will determine in which direction this pathway proceeds. If the cell has produced ribose-5-P, but does not need to synthesize nucleotides, then the ribose-5-P will be converted to glycolytic intermediates. If the cell still requires NADPH, the ribose-5-P will be converted back into glucose-6-P using nonoxidative reactions (see below). And finally, if the cell already has a high level of NADPH, but needs to produce nucleotides, the oxidative reactions of the pentose phosphate pathway will be inhibited, and the glycolytic intermediates fructose-6-P and glyceraldehyde-3-P will be used to produce the five carbon sugars using exclusively the nonoxidative phase of the pentose phosphate pathway.

1. THE CONVERSION OF RIBOSE 5-PHOSPHATE TO GLYCOLYTIC INTERMEDIATES

The nonoxidative portion of the pentose phosphate pathway consists of a series of rearrangement and transfer reactions that first convert ribulose 5-phosphate to ribose 5-phosphate and xylulose 5-phosphate, and then the ribose 5-phosphate and xyulose 5-phosphate are converted to intermediates of the glycolytic pathway. The enzymes involved are an epimerase, an isomerase, transketolase, and transaldolase.

The epimerase and isomerase convert ribulose 5-phosphate to two other 5-carbon sugars (Fig. 29.7). The isomerase converts ribulose 5-phosphate to ribose 5-phosphate. The epimerase changes the stereochemical position of one hydroxyl group (at carbon 3), converting ribulose 5-phosphate to xylulose 5-phosphate.

Transketolase transfers 2-carbon fragments of keto sugars (sugars with a keto group at C2) to other sugars. Transketolase picks up a 2-carbon fragment from xylulose 5-phosphate by cleaving the carbon–carbon bond between the keto group and the adjacent carbon, thereby releasing glyceraldehyde 3-phosphate (Fig. 29.8). The 2-carbon fragment is covalently bound to thiamine pyrophosphate, which transfers

Xylulose 5-phosphate has recently been identified as an activator of protein phosphatase 2A (PP2A). PP2A removes phosphates from PFK-2 and from a transcription factor that binds to carbohydrate response elements in promoters of genes such as pyruvate kinase. The hydrolysis of the phosphates activates both proteins, such that xylulose 5-phosphate can regulate pathways relating to both carbohydrate and fat metabolism.

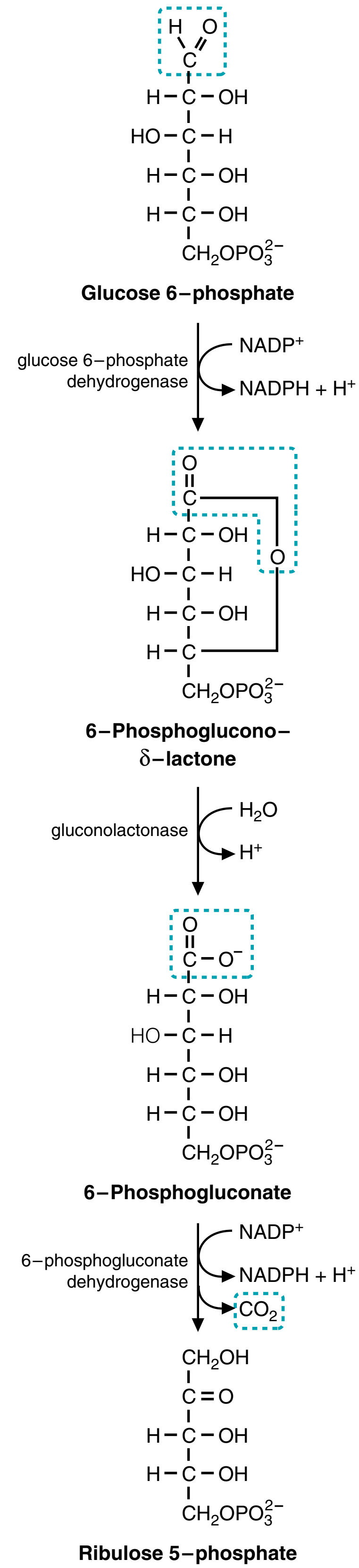

Fig. 29.6. Oxidative portion of the pentose phosphate pathway. Carbon 1 of glucose 6-phosphate is oxidized to an acid and then released as CO_2 in an oxidative decarboxylation reaction. Each oxidation step generates an NADPH.

Doctors suspected that the underlying factor in the destruction of **Al Martini's** red blood cells was an X-linked defect in the gene that codes for glucose 6-phosphate dehydrogenase. The red blood cell is dependent on this enzyme for a source of NADPH to maintain reduced levels of glutathione, one of its major defenses against oxidative stress (see Chapter 24). Glucose 6-phosphate dehydrogenase deficiency is the most common known enzymopathy, and affects approximately 7% of the world's population and about 2% of the U.S. population. Most glucose 6-phosphate dehydrogenase–deficient individuals are asymptomatic but can undergo an episode of hemolytic anemia if exposed to certain drugs, to certain types of infections, or if they ingest fava beans. When questioned, Al Martini replied that he did not know what a fava bean was and had no idea whether he was sensitive to them.

it to the aldehyde carbon of another sugar, forming a new ketose. The role of thiamine-pyrophosphate here is thus very similar to its role in the oxidative decarboxylation of pyruvate and α-ketoglutarate (see Chapter 20, section I.B). Two reactions in the pentose phosphate pathway use transketolase; in the first, the 2-carbon keto fragment from xylulose 5-phosphate is transferred to ribose 5-phosphate to form sedoheptulose 7-phosphate, and in the other, a 2-carbon keto fragment (usually derived from xylulose 5-phosphate) is transferred to erythrose 4-phosphate to form fructose 6-phosphate.

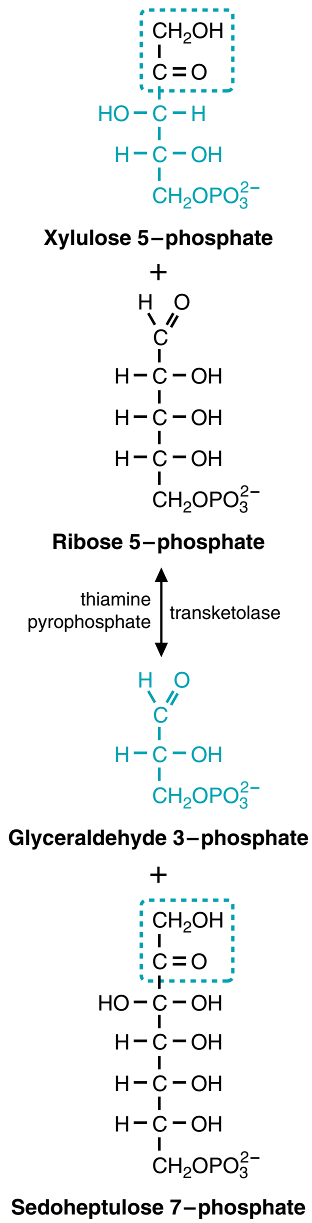

Fig. 29.8. Two-carbon unit transferred by transketolase. Transketolase cleaves the bond next to the keto group and transfers the 2-carbon keto fragment to an aldehyde. Thiamine pyrophosphate carries the 2-carbon fragment, forming a covalent bond with the carbon of the keto group.

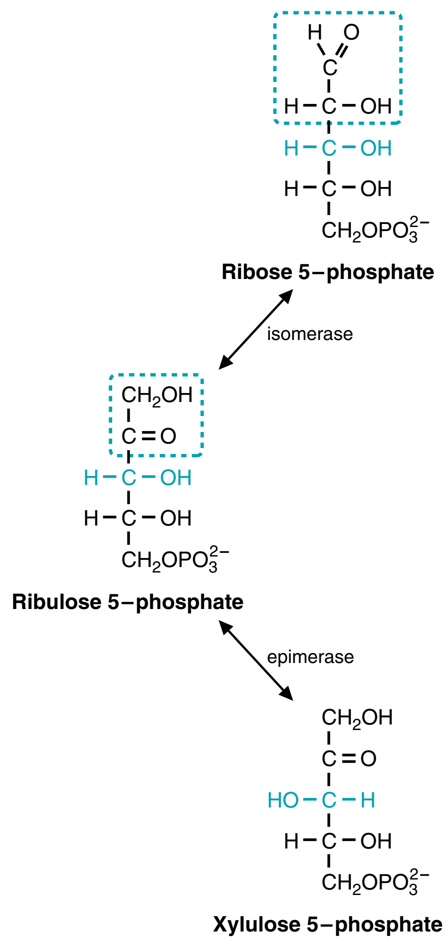

Fig. 29.7. Ribulose 5-phosphate is epimerized (to xylulose 5-phosphate) and isomerized (to ribose 5-phosphate).

Transaldolase transfers a 3-carbon keto fragment from sedoheptulose 7-phosphate to glyceraldehyde 3-phosphate to form erythrose 4-phosphate and fructose 6-phosphate (Fig. 29.9). The aldol cleavage occurs between the two hydroxyl carbons adjacent to the keto group (on carbons 3 and 4 of the sugar). This reaction is similar to the aldolase reaction in glycolysis, and the enzyme uses an active amino group, from the side chain of lysine, to catalyze the reaction.

The net result of the metabolism of 3 moles of ribulose 5-phosphate in the pentose phosphate pathway is the formation of 2 moles of fructose 6-phosphate and 1 mole of glyceraldehyde 3-phosphate, which then continue through the glycolytic pathway with the production of NADH, ATP, and pyruvate. Because the pentose phosphate pathway begins with glucose 6-phosphate, and feeds back into the

The transketolase activity of red blood cells is used to measure thiamine nutritional status and diagnose the presence of thiamine deficiency. The activity of transketolase is measured in the presence and absence of added thiamine pyrophosphate. If the thiamine intake of a patient is adequate, the addition of thiamine pyrophosphate does not increase the activity of transketolase because it already contains bound thiamine pyrophosphate. If the patient is thiamine deficient, transketolase activity will be low, and adding thiamine pyrophosphate will greatly stimulate the reaction. **Al Martini** was diagnosed in Chapter 19 as having beriberi heart disease resulting from thiamine deficiency. The diagnosis was based on laboratory tests confirming the thiamine deficiency.

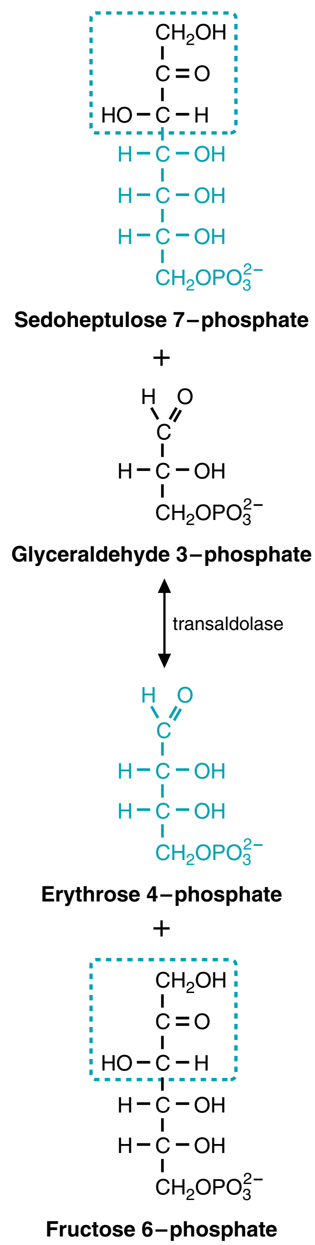

Fig. 29.9. Transaldolase transfers a 3-carbon fragment that contains an alcohol group next to a keto group.

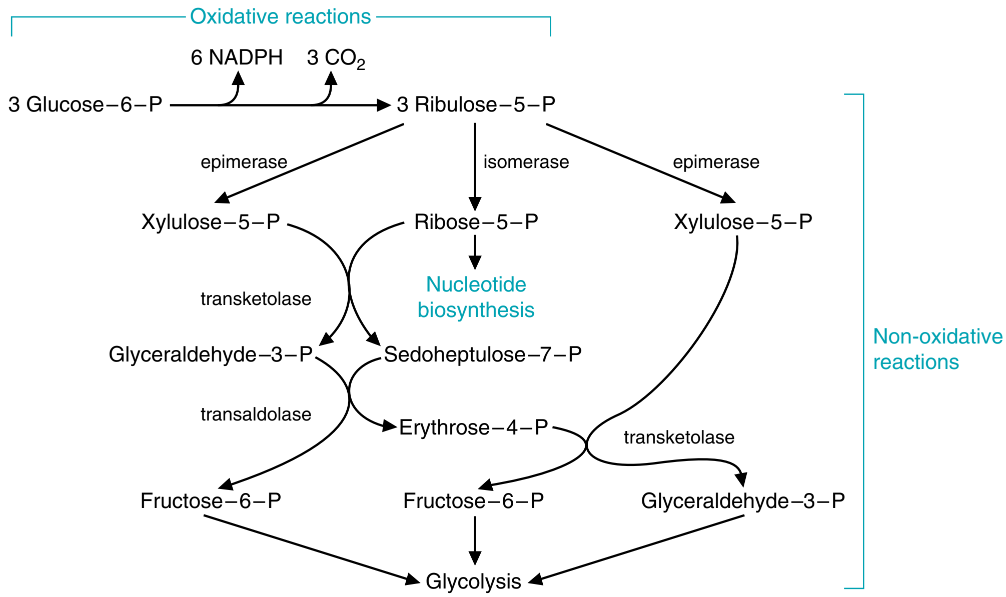

Fig. 29.10. A balanced sequence of reactions in the pentose phosphate pathway. The interconversion of sugars in the pentose phosphate pathway results in conversion of 3 glucose 6-phosphate to 6 NADPH, 3 CO_2, 2 fructose 6-phosphate, and one glyceraldehyde 3-phosphate.

glycolytic pathway, it is sometimes called the hexose monophosphate shunt (a shunt or a pathway for glucose 6-phosphate). The reaction sequence starting from glucose-6-P, and involving both the oxidative and nonoxidative phases of the pathway, is shown in Figure 29.10.

2. GENERATION OF RIBOSE 5-PHOSPHATE FROM INTERMEDIATES OF GLYCOLYSIS

The reactions catalyzed by the epimerase, isomerase, transketolase, and transaldolase are all reversible reactions under physiologic conditions. Thus, ribose 5-phosphate required for purine and pyrimidine synthesis can be generated from intermediates of the glycolytic pathway, as well as from the oxidative phase of the pentose phosphate pathway. The sequence of reactions that generate ribose 5-phosphate from intermediates of glycolysis is indicated below.

(1) Fructose-6-P + glyceraldehyde-3-P $\xleftrightarrow{\textit{Transketolase}}$ Erythrose-4-P + Xylulose-5-P

(2) Erythrose-4-P + Fructose-6-P $\xleftrightarrow{\textit{Transaldolase}}$ Sedoheptulose-7-P + Glyceraldehyde-3-P

(3) Sedoheptulose-7-P+ Glyceraldehyde-3-P $\xleftrightarrow{\textit{Transketolase}}$ Ribose-5-P + Xylulose-5-P

(4) 2 Xylulose-5-P $\xleftrightarrow{\textit{Epimerase}}$ 2 Ribulose-5-P

(5) 2 Ribulose-5-P $\xleftrightarrow{\textit{Isomerase}}$ 2 Ribose-5-P

Net Equation : 2 Fructose-6-P + Glyceraldehyde-3-P $\longleftrightarrow$ *3 Ribose-5-P*

C. Role of the Pentose Phosphate Pathway in the Generation of NADPH

In general, the oxidative phase of the pentose phosphate pathway is the major source of NADPH in cells. NADPH provides the reducing equivalents for biosynthetic reactions and for oxidation–reduction reactions involved in protection against the toxicity of ROS (see Chapter 24). The glutathione-mediated defense against oxidative stress is common to all cell types (including the red blood cell), and the requirement for NADPH to maintain levels of reduced glutathione probably accounts for the universal distribution of the pentose phosphate pathway among different types of cells. Fig. 29.11 illustrates the importance of this pathway in maintaining the membrane integrity of the red blood cell. NADPH is also used for anabolic pathways, such as fatty acid synthesis, cholesterol synthesis, and fatty acid chain elongation (Table 29.1). It is the source of reducing equivalents for cytochrome P450 hydroxylation of aromatic compounds, steroids, alcohols, and drugs. The highest concentrations of glucose 6-phosphate dehydrogenase are found in phagocytic cells, where NADPH oxidase uses NADPH to form superoxide from molecular oxygen. The superoxide then generates hydrogen peroxide, which kills the microorganisms taken up by the phagocytic cells (see Chapter 24).

The entry of glucose 6-phosphate into the pentose phosphate pathway is controlled by the cellular concentration of NADPH. NADPH is a strong product inhibitor of glucose 6-phosphate dehydrogenase, the first enzyme of the pathway. As NADPH is oxidized in other pathways, the product inhibition of glucose 6-phosphate dehydrogenase is relieved, and the rate of the enzyme is accelerated to produce more NADPH.

Table 29.1. Pathways That Require NADPH

Detoxification
• Reduction of oxidized glutathione
• Cytochrome P450 monooxygenases
Reductive synthesis
• Fatty acid synthesis
• Fatty acid chain elongation
• Cholesterol synthesis
• Neurotransmitter synthesis
• Nucleotide synthesis
• Superoxide synthesis

Q: How does the net energy yield from the metabolism of 3 moles of glucose 6-phosphate through the pentose phosphate pathway to pyruvate compare with the yield of 3 moles of glucose 6-phosphate through glycolysis?

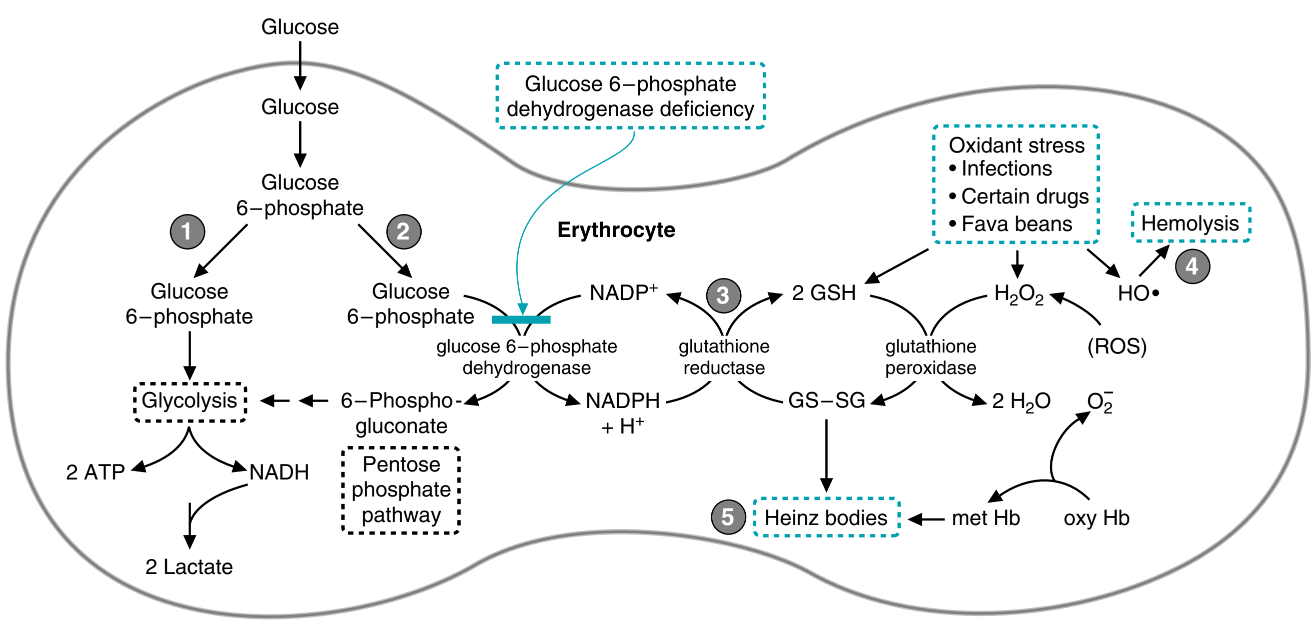

Fig. 29.11. Hemolysis caused by reactive oxygen species. **1.** Maintenance of the integrity of the erythrocyte membrane depends on its ability to generate ATP and NADH from glycolysis. **2.** NADPH is generated by the pentose phosphate pathway. **3.** NADPH is used for the reduction of oxidized glutathione to reduced glutathione. Glutathione is necessary for the removal of H_2O_2 and lipid peroxides generated by reactive oxygen species (ROS). **4.** In the erythrocytes of healthy individuals, the continuous generation of superoxide ion from the nonenzymatic oxidation of hemoglobin provides a source of reactive oxygen species. The glutathione defense system is compromised by glucose 6-phosphate dehydrogenase deficiency, infections, certain drugs, and the purine glycosides of fava beans. **5.** As a consequence, Heinz bodies, aggregates of cross-linked hemoglobin, form on the cell membranes and subject the cell to mechanical stress as it tries to go through small capillaries. The action of the ROS on the cell membrane as well as mechanical stress from the lack of deformability result in hemolysis.

A: The net energy yield from 3 moles of glucose 6-phosphate metabolized through the pentose phosphate pathway and then the last portion of the glycolytic pathway is 6 moles of NADPH, 3 moles of CO_2, 5 moles of NADH, 8 moles of ATP, and 5 moles of pyruvate. In contrast, the metabolism of 3 moles of glucose 6-phosphate through glycolysis is 6 moles of NADH, 9 moles of ATP, and 6 moles of pyruvate.

Table 29.2. Cellular Needs Dictate the Direction of the Pentose Phosphate Pathway Reactions

Cellular Need	Direction of Pathway
NADPH only	Oxidative reactions produce NADPH; nonoxidative reactions convert ribulose-5-P to glucose-6-P to produce more NADPH
NADPH + ribose-5-P	Oxidative reactions produce NADPH and ribulose-5-P; the isomerase converts ribulose-5-P to ribose-5-P.
Ribose-5-P only	Only the nonoxidative reactions. High NADPH inhibits glucose- 6-P dehydrogenase, so transketolase and transaldolase will be used to convert fructose-6-P and glyceraldehyde-3-P to ribose-5-P.
NADPH and pyruvate	Both the oxidative and nonoxidative reactions are used. The oxidative reactions generate NADPH and ribulose-5-P. The nonoxidative reactions convert the ribulose-5-P to fructose-6-P and glyceraldehyde-3-P, and glycolysis will convert these intermediates to pyruvate.

In the liver, the synthesis of fatty acids from glucose is a major route of NADPH reoxidation. The synthesis of liver glucose 6-phosphate dehydrogenase, like the key enzymes of glycolysis and fatty acid synthesis, is induced by the increased insulin/glucagon ratio after a high-carbohydrate meal. A summary of the possible routes glucose-6-P may follow using the pentose phosphate pathway is presented in Table 29.2.

CLINICAL COMMENTS

Hereditary fructose intolerance (HFI) is caused by a low level of fructose 1-phosphate aldolase activity in aldolase B, an isozyme of fructose 1,6-bisphosphate aldolase that is also capable of cleaving fructose 1-phosphate. In patients of European descent, the most common defect is a single missense mutation in exon 5 (G $\rightarrow$ C), resulting in an amino acid substitution (Ala $\rightarrow$ Pro). As a result of this substitution, a catalytically impaired aldolase B is synthesized in abundance. The exact prevalence of HFI in the United States is not established but is approximately 1 per 15,000 to 25,000 population. The disease is transmitted by an autosomal recessive inheritance pattern.

When affected patients such as **Candice Sucher** ingest fructose, fructose is converted to fructose 1-phosphate. Because of the deficiency of aldolase B, fructose 1-phosphate cannot be further metabolized to dihydroxyacetone phosphate and glyceraldehyde and accumulates in those tissues that have fructokinase (liver, kidney, and small intestine). Fructose is detected in the urine with the reducing sugar test (see Chapter 5). A DNA screening test (based on the generation of a new restriction site by the mutation) now provides a safe method to confirm a diagnosis of hereditary fructose intolerance.

In the infant and small child, the major symptoms include poor feeding, vomiting, intestinal discomfort, and failure to thrive. The greater the ingestion of dietary fructose, the more severe the clinical reaction. The result of prolonged ingestion of fructose is ultrastructural changes in the liver and kidney resulting in hepatic and renal failure. Hereditary fructose intolerance is usually a disease of infancy, because adults with fructose intolerance who have survived avoid the ingestion of fruits, table sugar, and other sweets.

Erin Galway has galactosemia, which is caused by a deficiency of galactose 1-phosphate uridylyltransferase; it is one of the most common genetic diseases. Galactosemia is an autosomal recessive disorder of galactose metabolism that occurs in about 1 in 60,000 newborns. Approximately two thirds of the states in the United States screen newborns for this disease because failure to begin immediate treatment results in mental retardation. Failure to thrive is the most common initial clinical symptom. Vomiting or diarrhea is found in most patients, usually starting within a few days of milk ingestion. Signs of deranged liver function, either jaundice or hepatomegaly, are present almost as frequently after the first week of life. The jaundice of intrinsic liver disease may be accentuated by the severe hemolysis in some patients. Cataracts have been observed within a few days of birth.

Management of patients requires elimination of galactose from the diet. Failure to eliminate this sugar results in progressive liver failure and death. In infants, artificial milk made from casein or soybean hydrolysate is used.

Al Martini's sputum culture sent on the second day of his admission for acute alcoholism and pneumonia grew out *Haemophilus influenzae*. This organism is sensitive to a variety of antibiotics, including TMP/sulfa. Unfortunately, it appeared that Mr. Martini had suffered an acute hemolysis (lysis or destruction of some of his red blood cells), probably induced by exposure to the sulfa drug and his infection with *H. influenzae*. The hemoglobin that escaped from the lysed red blood cells was filtered by his kidneys and appeared in his urine.

By mechanisms that are not fully delineated, certain drugs (such as sulfa drugs and antimalarials), a variety of infectious agents, and exposure to fava beans can cause red blood cell destruction in individuals with a genetic deficiency of glucose 6-phosphate dehydrogenase. Presumably, these patients cannot generate enough reduced NADPH to defend against the ROS. Although erythrocytes lack most of the other enzymatic sources of NADPH for the glutathione antioxidant system, they do have the defense mechanisms provided by the antioxidant vitamins E and C and catalase. Thus, individuals who are not totally deficient in glucose 6-phosphate dehydrogenase remain asymptomatic unless an additional oxidative stress, such as an infection, generates additional oxygen radicals.

Some drugs, such as the antimalarial primaquine and the sulfonamide which Al Martini is taking, affect the ability of red blood cells to defend against oxidative stress. Fava beans, which look like fat string beans and are sometimes called broad beans, contain the purine glycosides vicine and isouramil. These compounds react with glutathione. It has been suggested that cellular levels of reduced glutathione (GSH) decrease to such an extent that critical sulfhydryl groups in some key proteins cannot be maintained in reduced form.

The highest prevalence rates for glucose 6-phosphate dehydrogenase deficiency are found in tropical Africa and Asia, in some areas of the Middle East and the Mediterranean, and in Papua New Guinea. The geographic distribution of this deficiency is similar to that of sickle cell trait, and is probably also related to the relative resistance it confers against the malaria parasite.

Because the individuals with this deficiency are asymptomatic unless exposed to an "oxidant challenge," the clinical course of the hemolytic anemia is usually self-limited if the causative agent is removed. However, genetic polymorphism accounts for a substantial variability in the severity of the disease. Severely affected patients may have a chronic hemolytic anemia and other sequelae even without known exposure to drugs, infection, and other causative factors. In such patients, neonatal jaundice is also common and can be severe enough to cause death.

BIOCHEMICAL COMMENTS

Before the metabolic toxicity of fructose was appreciated, substitution of fructose for glucose in intravenous solutions, and of fructose for sucrose in enteral tube feeding or diabetic diets, was frequently recommended.

(Enteral tube feeding refers to tubes placed into the gut; parenteral tube feeding refers to tubes placed into the vein, feeding intravenously.) Administration of intravenous fructose to patients with diabetes mellitus or other forms of insulin resistance avoided the hyperglycemia found with intravenous glucose, possibly because fructose metabolism in the liver bypasses the insulin-regulated step at phosphofructokinase-1. However, because of the unregulated flow of fructose through glycolysis, intravenous fructose feeding frequently resulted in lactic acidosis (see Fig. 29.3). In addition, the fructokinase reaction is very rapid, and tissues would become depleted of ATP and phosphate when large quantities of fructose were metabolized over a short period. This would lead to cell death. Fructose is less toxic in the diet or in enteral feeding because of the relatively slow rate of fructose absorption.

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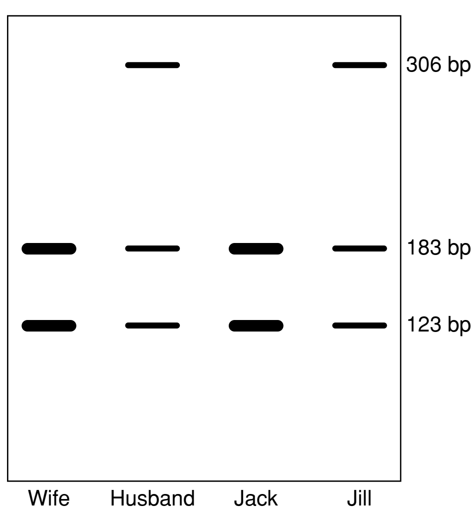
REVIEW QUESTIONS—CHAPTER 29

1. Hereditary fructose intolerance is a rare recessive genetic disease that is most commonly caused by a mutation in exon 5 of the aldolase B gene. The mutation fortuitously creates a new AhaII recognition sequence. To test for the mutation, DNA was extracted from a wife, husband, and their two children, Jack and Jill. The DNA for exon 5 of the aldolase B gene was amplified by polymerase chain reaction (PCR), treated with AhaII, subjected to electrophoresis on an agarose gel, and stained with a dye that binds to DNA.



Which of the following conclusions can be made from the data presented?

(A) Both of the children have the disease.
(B) Neither of the children has the disease.
(C) Jill has the disease, Jack does not.
(D) Jack has the disease, Jill does not.
(E) There is not enough information to make a determination

2. On examining the gel himself, the husband became concerned that he might not be the biologic father of one or both of the children. From the pattern on the gel, you can reasonably conclude which of the following?

(A) He is probably not Jill's father.
(B) He is probably not Jack's father.
(C) He could be the father of both children.
(D) He is probably not the father of either child.
(E) There is not enough information to make a determination

3. An alcoholic is brought to the Emergency Room for a hypoglycemic coma. Because alcoholics are frequently malnourished, which of the following enzymes can be used to test for a thiamine deficiency?

(A) Aldolase
(B) Transaldolase
(C) Transketolase
(D) Glucose 6-phosphate dehydrogenase
(E) UDP-galactose epimerase

4. Intravenous fructose feeding can lead to lactic acidosis caused by which of the following?

(A) Bypassing the regulated pyruvate kinase step
(B) Bypassing the regulated PFK-1 step
(C) Allosterically activating aldolase B
(D) Allosterically activating lactate dehydrogenase
(E) Increasing the [ATP]/[ADP] ratio in liver

5. The polyol pathway of sorbitol production and the HMP shunt pathway are linked by which of the following?

(A) The HMP shunt produces 6-phosphogluconate, an intermediate in the polyol pathway.
(B) The HMP shunt produces NADPH, which is required for the polyol pathway.
(C) The HMP shunt produces ribitol, an intermediate of the polyol pathway.
(D) Both pathways use glucose as the starting material.
(E) Both pathways use fructose as the starting material.