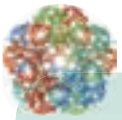
CHAPTER 8

Carbohydrate Metabolism

Wine: A Product of Fermentation
Humans use microorganisms, in this case yeast, to metabolize sugar in the absence of oxygen. The aging of wine in oak barrels improves its taste and aroma.

OUTLINE



Metabolism and Jet Engines

Can systems biology improve our understanding of biochemical pathways such as glycolysis? Modern species are the result of billions of years of rigorous natural selection, which has adapted organisms to their various environments. This selection process also governs the metabolic pathways that manage the biochemical transformations that sustain life. As systems biologists analyzed metabolic processes, it became apparent that evolution, operating under thermodynamic and kinetic constraints, has converged again and again into a relatively small set of designs. Catabolic pathways, those that degrade organic molecules and release energy, provide an important example. These pathways typically have two characteristics: optimal ATP production and kinetic efficiency (i.e., minimal response time to changes in cellular metabolic requirements). Living organisms have optimized catabolic pathways, in part, through highly exergonic reactions at the beginning of a pathway. The early "activation" of nutrient molecules thus makes subsequent ATP-producing reactions (usually near the end of the pathway) to run thermodynamically downhill. As a result, the pathway can produce ATP under varying substrate and product concentrations. The term "turbo design," inspired by the turbo engines in jet aircraft, describes this phenomenon. A good example is glycolysis, the energy-capturing reaction pathway that converts the hexose glucose into pyruvate (Figure 8.1).

A jet engine creates propulsion by mixing air with fuel to create hot, expanding, fast-moving exhaust gases, which blast out the back. Some of the air drawn in at the front of the engine is diverted into compressors, where its pressure increases substantially before flowing into combustion chambers and mixing with fuel molecules. As the burning fuel molecules expand, they flow through turbines fitted with fan blades that drive the compressors. An important feature of this process is that hot exhaust gases are also fed back into the engine to accelerate the fuel input step. This chapter reveals how carbohydrate fuel is burned by living cells with the same remarkable efficiency.

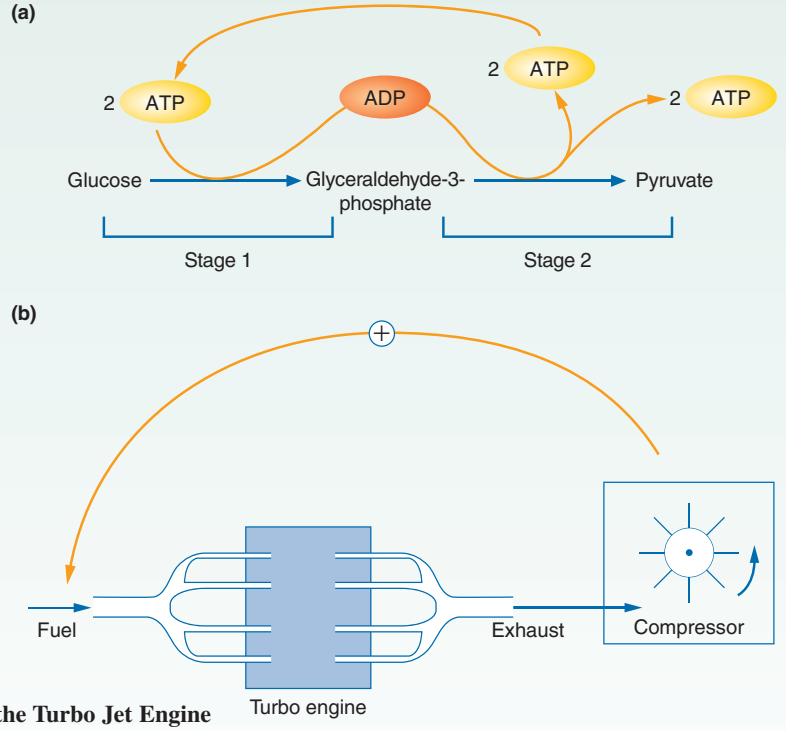


FIGURE 8.1

Comparison of Glycolysis and the Turbo Jet Engine

(a) Glycolysis is a two-stage catabolic pathway. Two of the four ATPs produced in stage 2 are used to activate an incoming glucose molecule (stage 1). The ADPs used in stage 2 are generated from the two ATPs used in stage 1 and in ATP-requiring reactions throughout the cell. (b) The schematic representation of a turbo jet engine illustrates how energy generated by an engine can be used to improve efficiency. Before exiting the engine, hot exhaust gases are diverted around the compressor turbines, raising the temperature and increasing the efficiency of fuel combustion.

Overview

CARBOHYDRATES PLAY SEVERAL CRUCIAL ROLES IN THE METABOLIC PROCESSES OF LIVING ORGANISMS. THEY SERVE AS ENERGY SOURCES AND as structural elements in living cells. This chapter looks at the role of carbohydrates in energy production. Because the monosaccharide glucose is a prominent energy source in almost all living cells, major emphasis is placed on its synthesis, degradation, and storage.

Living cells are in a state of ceaseless activity. To maintain its "life," each cell depends on highly coordinated biochemical reactions. Carbohydrates are an important source of the energy that drives these reactions. This chapter discusses the energy-generating pathways of carbohydrate metabolism are discussed. During **glycolysis**, an ancient pathway found in almost all organisms, a small amount of energy is captured as a glucose molecule is converted to two molecules of pyruvate. Glycogen, a storage form of glucose in vertebrates, is synthesized by **glycogenesis** when glucose levels are high and degraded by **glycogenolysis** when glucose is in short supply. Glucose can also be synthesized from noncarbohydrate precursors by reactions referred to as **gluconeogenesis**. The **pentose phosphate pathway** enables cells to convert glucose-6-phosphate, a derivative of glucose, to ribose-5-phosphate (the sugar used to synthesize nucleotides and nucleic acids) and other types of monosaccharides. NADPH, an important cellular reducing agent, is also produced by this pathway. In Chapter 9, the *glyoxylate cycle*, used by some organisms (primarily plants) to manufacture carbohydrate from fatty acids, is considered. *Photosynthesis*, a process in which light energy is captured to drive carbohydrate synthesis, is described in Chapter 13.

Any discussion of carbohydrate metabolism focuses on the synthesis and usage of glucose, a major fuel for most organisms. In vertebrates, glucose is transported throughout the body in the blood. If cellular energy reserves are low, glucose is degraded by the glycolytic pathway. Glucose molecules not required for immediate energy production are stored as glycogen in liver and muscle. The energy requirements of many tissues (e.g., brain, red blood cells, and exercising skeletal muscle cells) depend on an uninterrupted flow of glucose. Depending on a cell's metabolic requirements, glucose can also be used to synthesize, for example, other monosaccharides, fatty acids, and certain amino acids. Figure 8.2 summarizes the major pathways of carbohydrate metabolism in animals.

8.1 GLYCOLYSIS

Glycolysis, occurs, at least in part, in almost every living cell. This series of reactions is believed to be among the oldest of all the biochemical pathways. Both the enzymes and the number and mechanisms of the steps in the pathway are highly conserved in prokaryotes and eukaryotes. Also, glycolysis is an anaerobic process, which would have been necessary in the oxygen-poor atmosphere of pre-eukaryotic Earth.

In glycolysis, also referred to as the *Embden-Meyerhof-Parnas pathway*, each glucose molecule is split and converted to two three-carbon units (pyruvate). During this process several carbon atoms are oxidized. The small amount of energy captured during glycolytic reactions (about 5% of the total available) is stored temporarily in two molecules each of ATP and NADH (the reduced form of the coenzyme NAD^+). The subsequent metabolic fate of pyruvate depends on the organism being considered and its metabolic circumstances. In **anaerobic organisms**

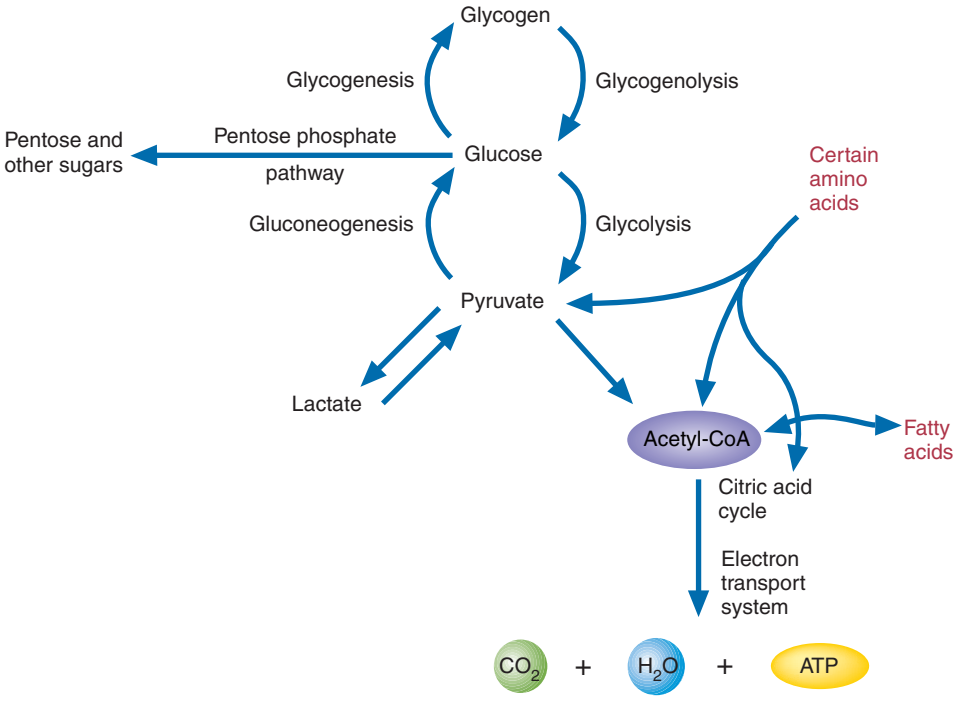


FIGURE 8.2

Major Pathways in Carbohydrate Metabolism

In animals, excess glucose is converted to its storage form, glycogen, by glycogenesis. When glucose is needed as a source of energy or as a precursor molecule in biosynthetic processes, glycogen is degraded by glycogenolysis. Glucose can be converted to ribose-5-phosphate (a component of nucleotides) and NADPH (a powerful reducing agent) by means of the pentose phosphate pathway. Glucose is oxidized by glycolysis, an energy-generating pathway that converts it to pyruvate. In the absence of oxygen, pyruvate is converted to lactate. When oxygen is present, pyruvate is further degraded to form acetyl-CoA. Significant amounts of energy in the form of ATP can be extracted from acetyl-CoA by the citric acid cycle and the electron transport system. Note that carbohydrate metabolism is inextricably linked to the metabolism of other nutrients. For example, acetyl-CoA is also generated from the breakdown of fatty acids and certain amino acids. When acetyl-CoA is present in excess, a different pathway converts it into fatty acids.

(those that do not use oxygen to generate energy), pyruvate may be converted to waste products such as ethanol, lactic acid, acetic acid, and similar molecules. Using oxygen as a terminal electron acceptor, aerobic organisms such as animals and plants completely oxidize pyruvate to form CO_2 and H_2O in an elaborate stepwise mechanism known as **aerobic respiration** (Chapters 9 and 10).

Glycolysis (Figure 8.3), which consists of 10 reactions, occurs in two stages:

1. Glucose is phosphorylated twice and cleaved to form two molecules of glyceraldehyde-3-phosphate (G-3-P). The two ATP molecules consumed during this stage are like an investment, because this stage creates the actual substrates for oxidation in a form that is trapped inside the cell.
2. Glyceraldehyde-3-phosphate is converted to pyruvate. Four ATP and two NADH molecules are produced. Because two ATP were consumed in stage 1, the net production of ATP per glucose molecule is 2.

The glycolytic pathway can be summed up in the following equation:

$$\text{D-Glucose} + 2\ \text{ADP} + 2\ P_i + 2\ \text{NAD}^+ \rightarrow 2\ \text{pyruvate} + 2\ \text{ATP} + 2\ \text{NADH} + 2\text{H}^+ + 2\text{H}_2\text{O}$$

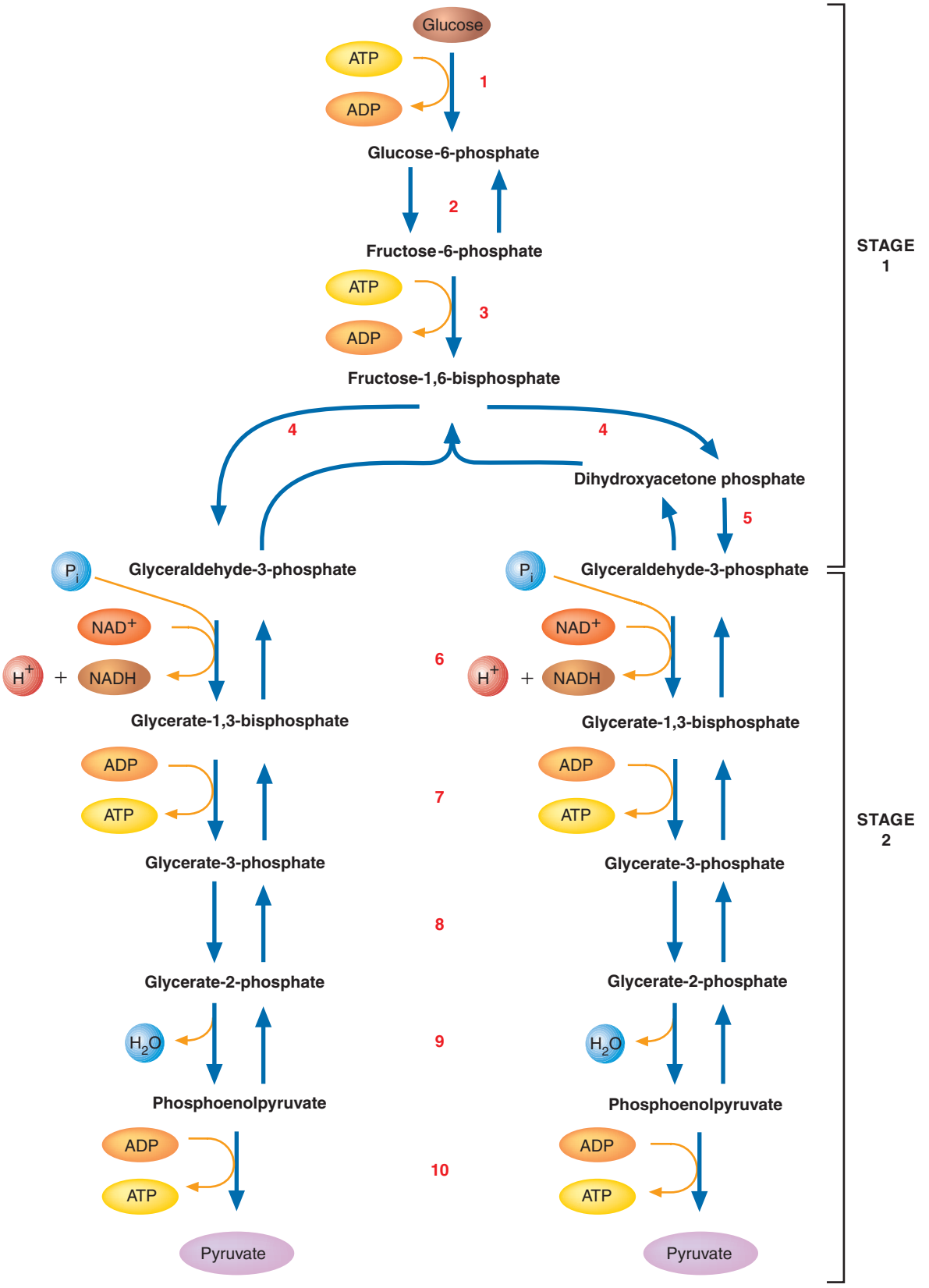


FIGURE 8.3

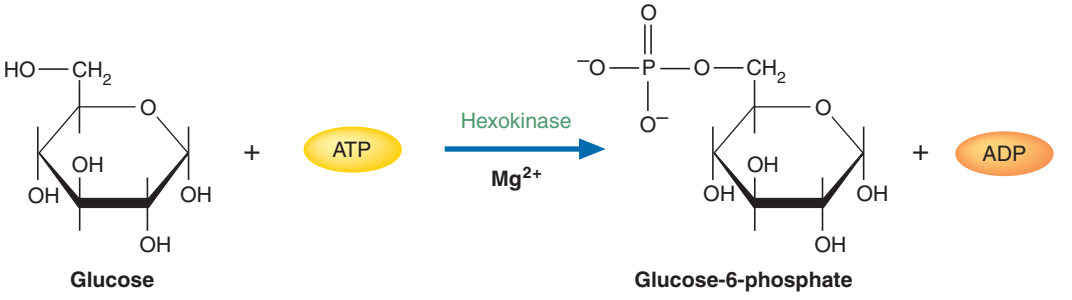
The Glycolytic Pathway

In glycolysis, a pathway with 10 reactions, each glucose molecule is converted into two pyruvate molecules. In addition, two molecules each of ATP and NADH are produced. Reactions with double arrows are reversible reactions, and those with single arrows are irreversible reactions that serve as control points in the pathway.

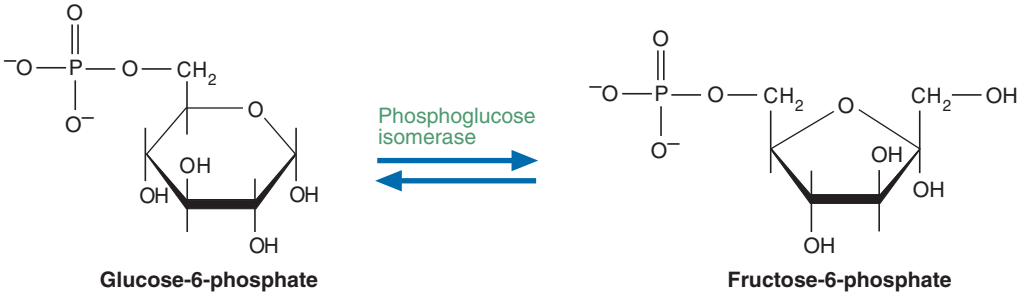
The Reactions of the Glycolytic Pathway

Glycolysis is summarized in Figures 8.3. The 10 reactions of the glycolytic pathway are as follows.

1. **Synthesis of glucose-6-phosphate**. Immediately after entering a cell, glucose and other sugar molecules are phosphorylated. Phosphorylation prevents transport of glucose out of the cell and increases the reactivity of the oxygen in the resulting phosphate ester. Several enzymes, called the hexokinases, catalyze the phosphorylation of hexoses in all cells in the body. ATP, a cosubstrate in the reaction, is complexed with Mg^{2+}. (ATP-Mg^{2+} complexes are common in kinase-catalyzed reactions.) Under intracellular conditions the reaction is irreversible; that is, the enzyme has no ability to retain or accommodate the product of the reaction in its active site, regardless of the concentration of G-6-P.

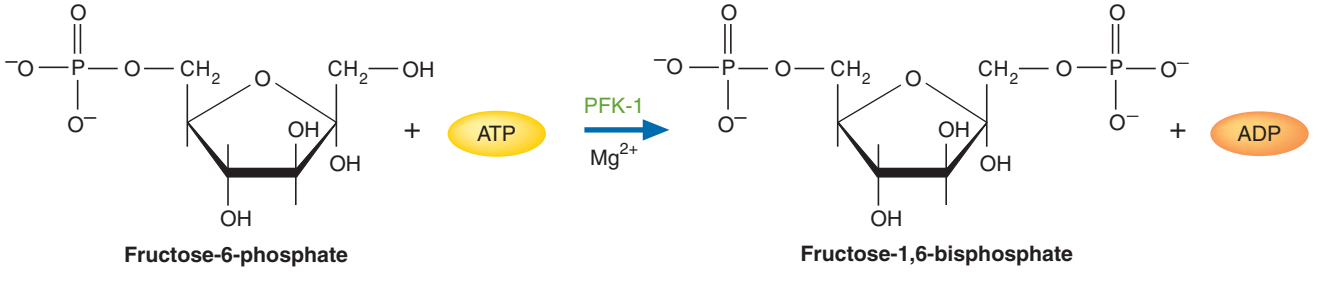


2. **Conversion of glucose-6-phosphate to fructose-6-phosphate**. During reaction 2 of glycolysis, the open chain form of the aldose glucose-6-phosphate is converted to the open chain form of the ketose fructose-6-phosphate by phosphoglucose isomerase (PGI) in a readily reversible reaction:



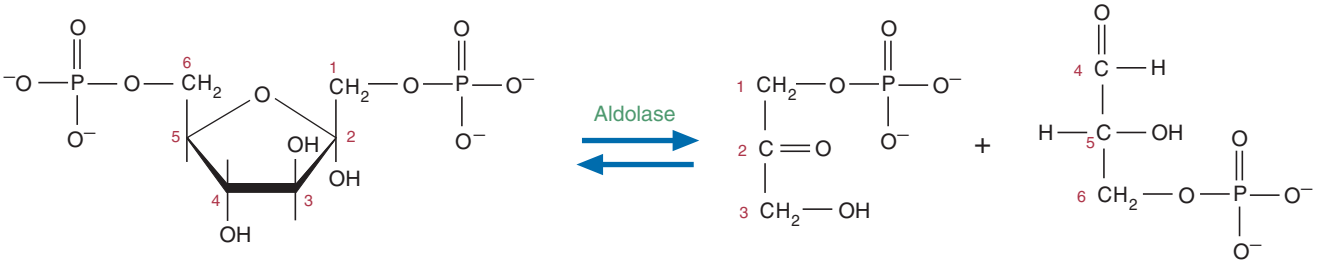
Recall that the isomerization reaction of glucose and fructose involves an enediol intermediate (Figure 7.16). This transformation makes C-1 of the fructose product available for phosphorylation. The hemiacetal hydroxy group of glucose-6-phosphate is more difficult to phosphorylate.

3. **The phosphorylation of fructose-6-phosphate**. Phosphofructokinase-1 (PFK-1) irreversibly catalyzes the phosphorylation of fructose-6-phosphate to form fructose-1,6-bisphosphate:



The PFK-1-catalyzed reaction is irreversible under cellular conditions. It is, therefore, the first committed step in glycolysis. Unlike glucose-6-phosphate and fructose-6-phosphate, the substrate and product, respectively, of the previous reaction, fructose-1,6-bisphosphate cannot be diverted into other pathways. Investing a second molecule of ATP serves several purposes. First of all, because ATP is used as the phosphorylating agent, the reaction proceeds with a large decrease in free energy. After fructose-1,6-bisphosphate has been synthesized, the cell is committed to glycolysis. Because fructose-1,6-bisphosphate eventually splits into two trioses, another purpose for phosphorylation is to prevent any later product from diffusing out of the cell because charged molecules cannot easily cross membranes.

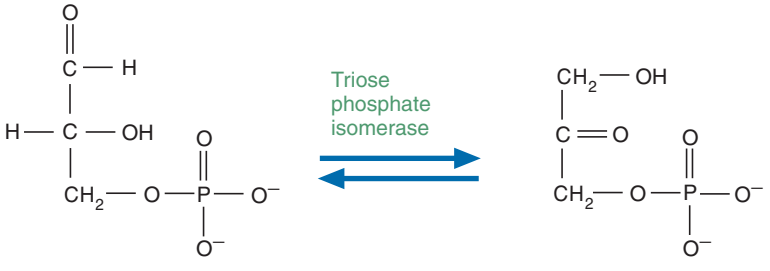
4. **Cleavage of fructose-1,6-bisphosphate**. Stage 1 of glycolysis ends with the cleavage of fructose-1,6-bisphosphate into two three-carbon molecules: glyceraldehyde-3-phosphate (G-3-P) and dihydroxyacetone phosphate (DHAP). This reaction is an **aldol cleavage**, hence the name of the enzyme: aldolase. Aldol cleavages are the reverse of aldol condensations, described on p. xxx. In aldol cleavages an aldehyde and a ketone are products.



Fructose-1,6-bisphosphate | **Dihydroxyacetone phosphate** | **Glyceraldehyde-3-phosphate**

Although the cleavage of fructose-1,6-bisphosphate is thermodynamically unfavorable ($\Delta G^{\circ\prime} = +23.8$ kJ/mol), the reaction proceeds because the products are rapidly removed.

5. **The interconversion of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate**. Of the two products of the aldolase reaction, only G-3-P serves as a substrate for the next reaction in glycolysis. To prevent the loss of the other three-carbon unit from the glycolytic pathway, triose phosphate isomerase catalyzes the reversible conversion of DHAP to G-3-P:

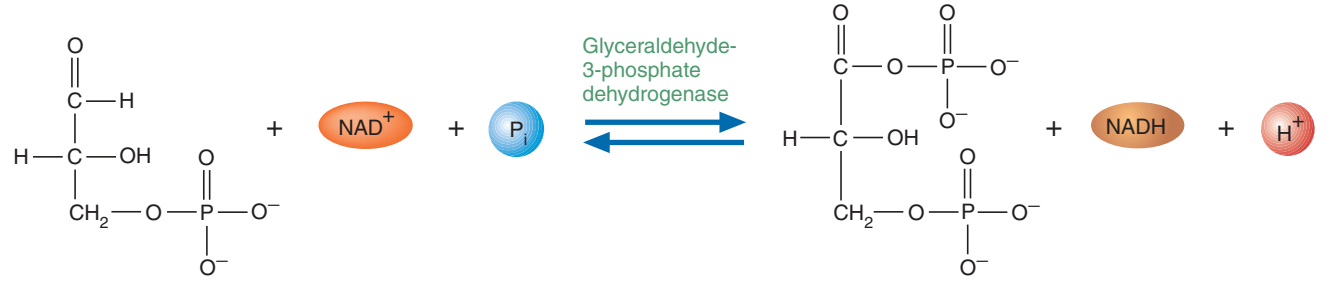


Glyceraldehyde-3-phosphate | **Dihydroxyacetone phosphate**

After this reaction, the original molecule of glucose has been converted to two molecules of G-3-P.

6. **Oxidation of glyceraldehyde-3-phosphate**. During reaction 6 of glycolysis, G-3-P undergoes oxidation and phosphorylation. The product,

glycerate-1,3-bisphosphate, contains a high-energy phosphoanhydride bond, which may be used in the next reaction to generate ATP:

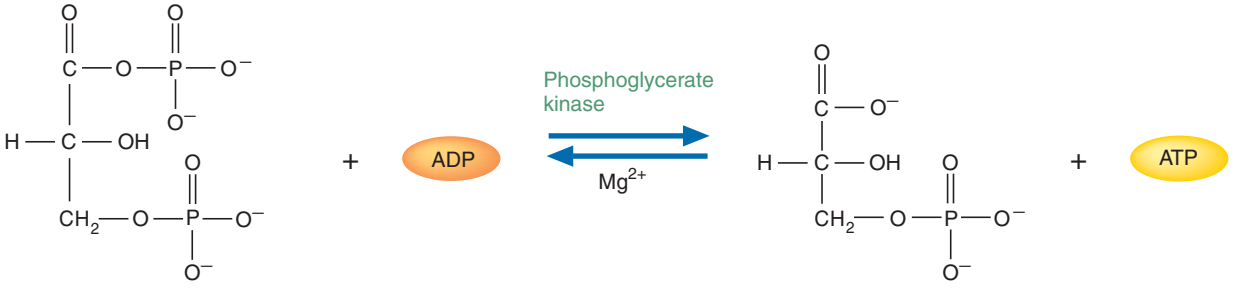


Glyceraldehyde-3-phosphate

Glycerate-1,3-bisphosphate

This complex process is catalyzed by glyceraldehyde-3-phosphate dehydrogenase, a tetramer composed of four identical subunits. Each subunit contains one binding site for G-3-P and another for NAD^+, an oxidizing ogent. As the enzyme forms a covalent thioester bond with the substrate (Figure 8.4), a hydride ion ($H:^-$) is transferred to NAD^+ in the active site. NADH, the reduced form of NAD^+, then leaves the active site and is replaced by an incoming NAD^+. The acyl enzyme adduct is attacked by inorganic phosphate and the product leaves the active site.

7. **Phosphoryl group transfer**. In this reaction ATP is synthesized as phosphoglycerate kinase catalyzes the transfer of the high-energy phosphoryl group of glycerate-1,3-bisphosphate to ADP:



Glycerate-1,3-bisphosphate

Glycerate-3-phosphate

Reaction 7 is an example of a substrate-level phosphorylation. Because the synthesis of ATP is endergonic, it requires an energy source. In **substrate-level phosphorylations**, ATP is produced by the transfer of a phosphoryl group from a substrate with a high phosphoryl transfer potential (glycerate-1,3-bisphosphate) (refer to Table 4.1) to produce a compound with a lower transfer potential (ATP) and therefore $\Delta G < 0$. Because two molecules of glycerate-1,3-bisphosphate are formed for every glucose molecule, this reaction produces two ATP molecules, and the investment of phosphate bond energy is recovered. ATP synthesis later in the pathway represents a net gain.

8. **The interconversion of 3-phosphoglycerate and 2-phosphoglycerate**. Glycerate-3-phosphate has a low phosphoryl group transfer potential. As such, it is a poor candidate for further ATP synthesis ($\Delta G^{\circ\prime}$ for ATP synthesis is –30.5 kJ/mol). Cells convert glycerate-3-phosphate with its energy-poor phosphate ester to phosphoenolpyruvate (PEP), which has an exceptionally high phosphoryl group transfer potential. (The standard free energies of hydrolysis of glycerate-3-phosphate and PEP are −12.6 and −61.9 kJ/mol, respectively.) In the first step in this conversion (reaction 8), phosphoglycerate mutase catalyzes the conversion of a C-3 phosphorylated compound to a C-2 phosphorylated compound through a two-step addition/elimination cycle.

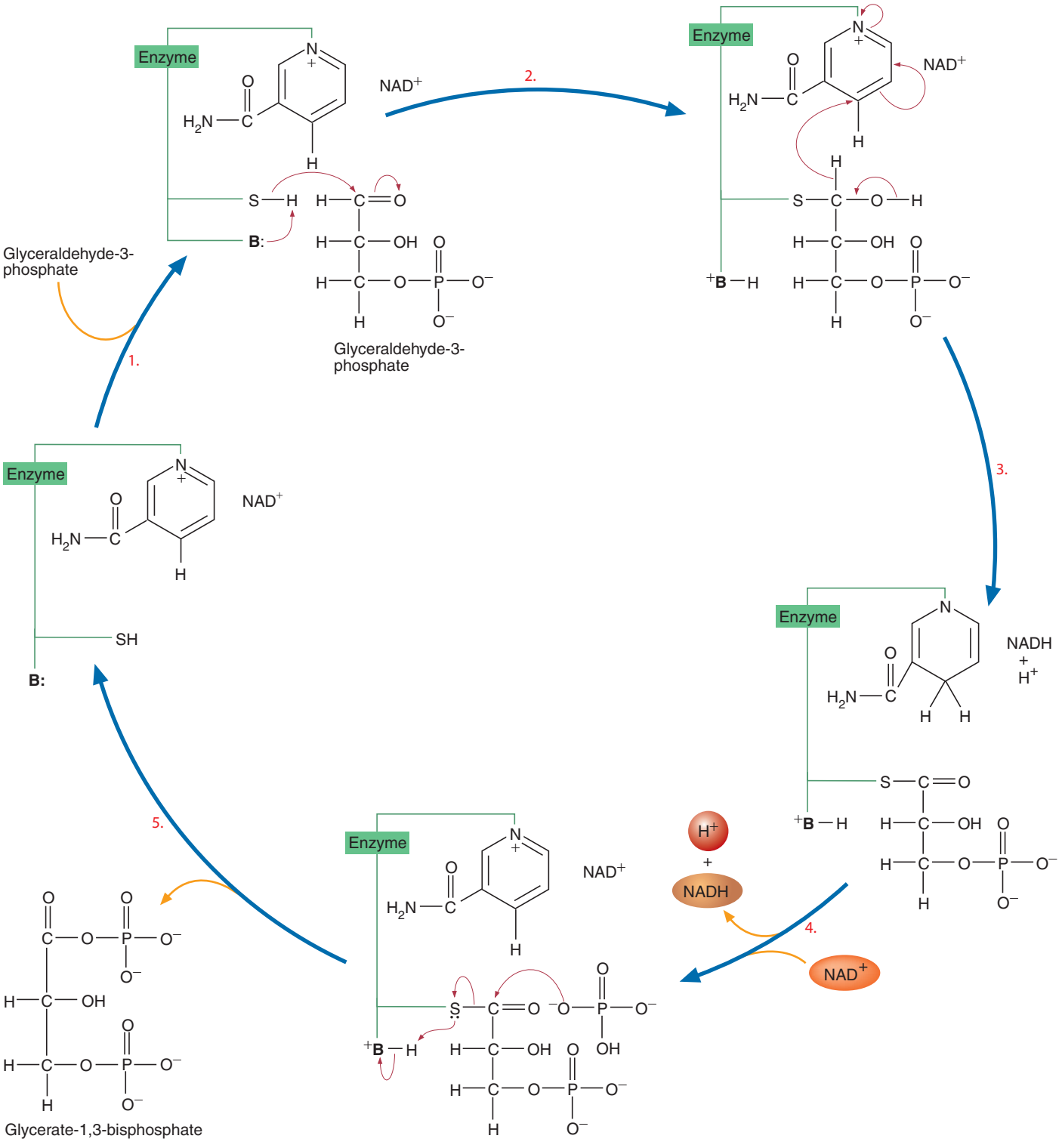
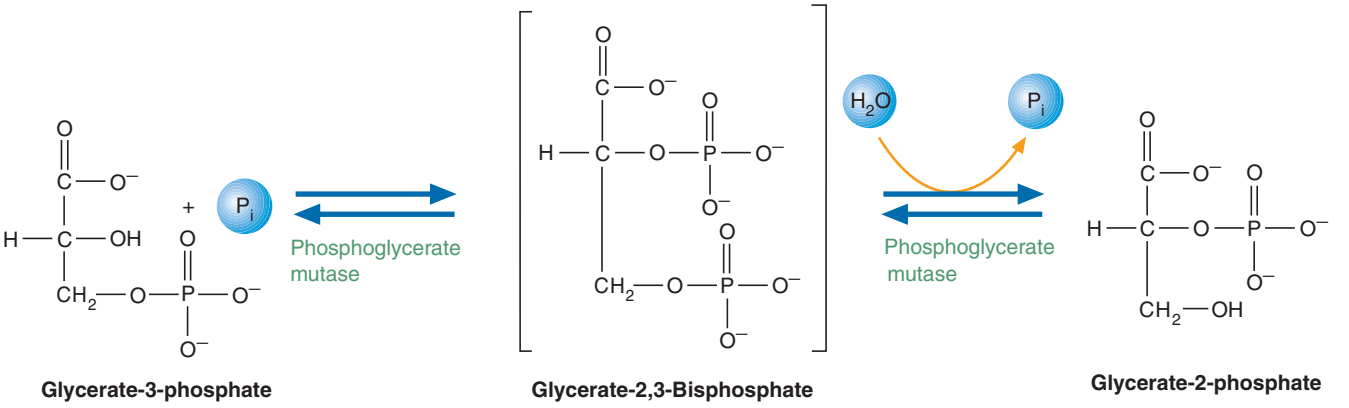


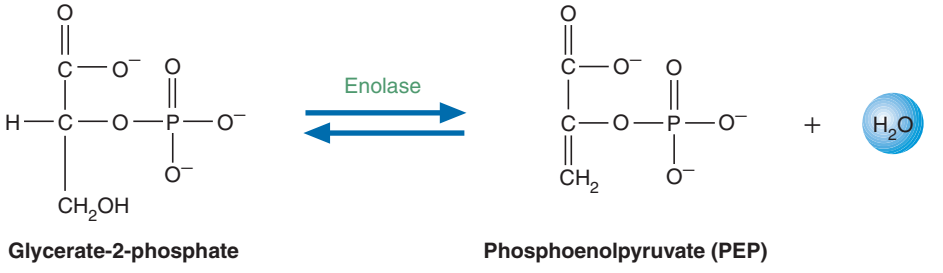
FIGURE 8.4

Glyceraldehyde-3-Phosphate Dehydrogenase Reaction

In the first step the substrate, glyceraldehyde-3-phosphate, enters the active site. As the enzyme catalyzes the reaction of the substrate with a sulfhydryl group within the active site (step 2), the substrate is oxidized (step 3). The noncovalently bound NADH is exchanged for a cytoplasmic NAD^+ (step 4). Displacement of the enzyme by inorganic phosphate (step 5) liberates the product, glycerate-1, 3-bisphosphate, thus returning the enzyme to its original form.



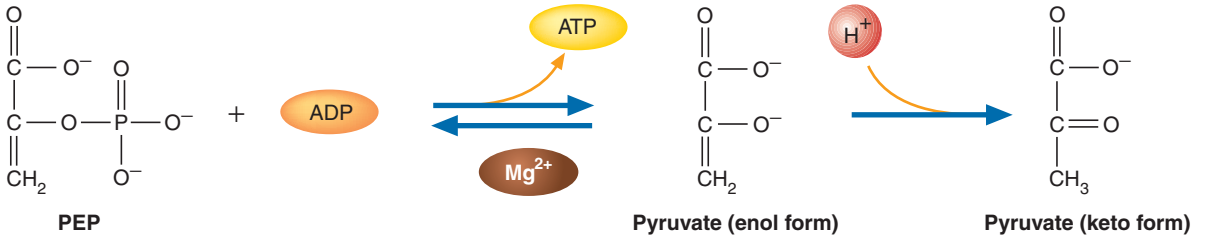
9. **Dehydration of 2-phosphoglycerate.** Enolase catalyzes the dehydration of glycerate-2-phosphate to form PEP:



PEP has a higher phosphoryl group transfer potential than does glycerate-2-phosphate because it contains an enol-phosphate group instead of a simple phosphate ester. The reason for this difference is made apparent in the next reaction. Aldehydes and ketones have two isomeric forms. The *enol* form contains a carbon-carbon double bond and a hydroxyl group. Enols exist in equilibrium with the more stable carbonyl-containing *keto* form. The interconversion of keto and enol forms, also called **tautomers**, is referred to as **tautomerization**:

This tautomerization is restricted by the presence of the phosphate group, as is the resonance stabilization of the free phosphate ion. As a result, phosphoryl transfer to ADP in reaction 10 is highly favored.

10. **Synthesis of pyruvate**. In the final reaction of glycolysis, pyruvate kinase catalyzes the transfer of a phosphoryl group from PEP to ADP. Two molecules of ATP are formed for each molecule of glucose.



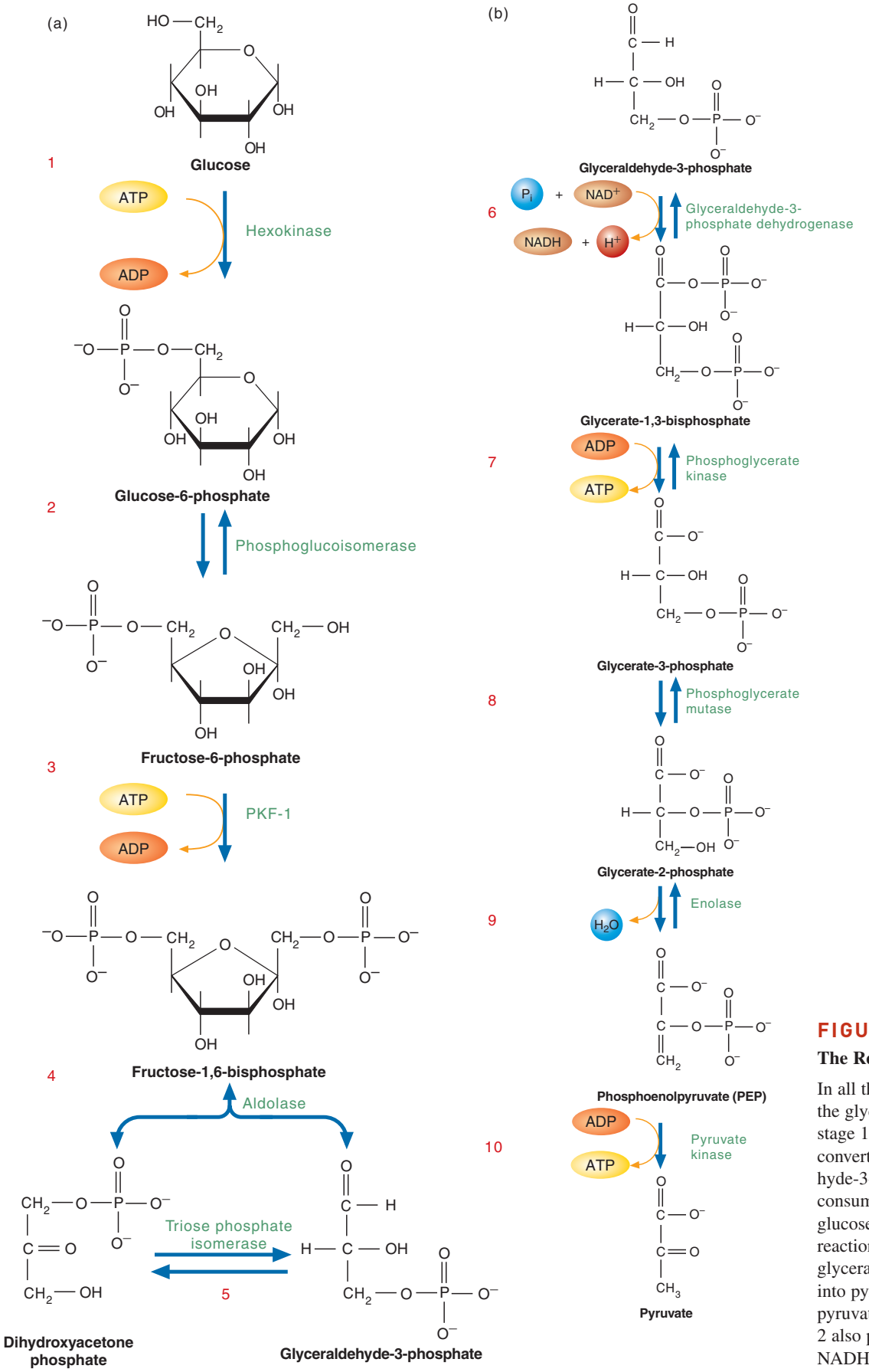


FIGURE 8.5

The Reactions of Glycolysis

In all there are 10 reactions in the glycolytic pathway. (a) In stage 1, reactions 1 through 5 convert glucose into glyceraldehyde-3-phosphate. Two ATP are consumed in state 1 for each glucose molecule. (b) In stage 2 reactions 6 though 10 convert glyceraldehyde-3-phosphate into pyruvate. In addition to pyruvate, the reactions of stage 2 also produce 4 ATP and 2 NADH per glucose molecule.

PEP is irreversibly converted to pyruvate because in this reaction—the transfer of a phosphoryl group from a molecule with a high transfer potential to one with a lower transfer potential—there is an exceptionally large free energy loss (refer to Table 4.1). This energy loss is associated with the spontaneous conversion (tautomerization) of the enol form of pyruvate to the more stable keto form. The 10 reactions of glycolysis are illustrated in Figure 8.5.

The Fates of Pyruvate

In terms of energy, the result of glycolysis is the production of two ATPs and two NADHs per molecule of glucose. Pyruvate, the other product of glycolysis, is still an energy-rich molecule, which can yield a substantial amount of ATP. Whether or not further energy can be produced, however, depends on the cell type and the availability of oxygen. Under aerobic conditions, most cells in the body convert pyruvate into acetyl-CoA, the entry-level substrate for the **citric acid cycle**, an amphibolic pathway that completely oxidizes the two acetyl carbons to form CO_2 and and the reduced molecules NADH and $FADH_2$. (An **amphibolic pathway** functions in both anabolic and catabolic processes.) The **electron transport system**, a series of oxidation-reduction reactions, transfers electrons from NADH and $FADH_2$ to O_2 to form water. The energy that is released during electron transport is coupled to a mechanism that synthesizes ATP. Under anaerobic conditions, further oxidation of pyruvate is impeded. A number of cells and organisms compensate by converting this molecule to a more reduced organic compound and regenerating the NAD^+ required for glycolysis to continue (Figure 8.6). (Recall

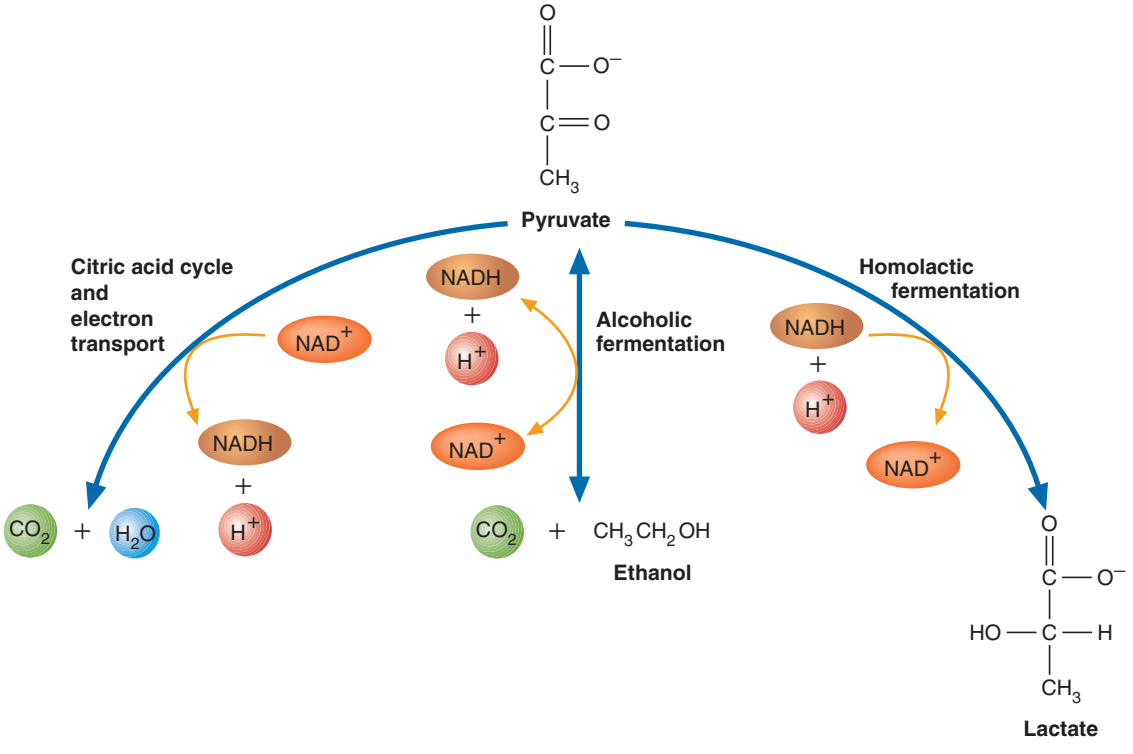
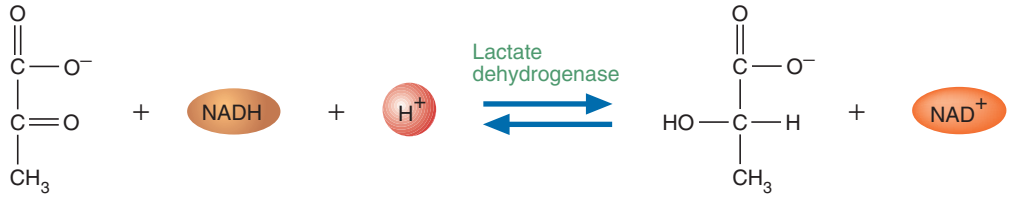


FIGURE 8.6

The Fates of Pyruvate

When oxygen is available (*left*), aerobic organisms completely oxidize pyruvate to CO_2 and H_2O. In the absence of oxygen, pyruvate can be converted to several types of reduced molecules. In some cells (e.g., yeast), ethanol and CO_2 are produced (*middle*). In others (e.g., muscle cells), homolactic fermentation occurs in which lactate is the only organic product (*right*). Some microorganisms use heterolactic fermentation reactions (not shown) that produce other acids or alcohols in addition to lactate. In all fermentation processes, the principal purpose is to regenerate NAD^+ so that glycolysis can continue.

that the hydride ion acceptor molecule NAD^+ is a cosubstrate in the reactoin catalyzed by glyceraldehyde-3-phosphate dehydrogenase.) This process of NAD^+ regeneration is referred to as **fermentation**. Muscle cells, red blood cells, and certain bacterial species (e.g., *Lactobacillus*) produce NAD^+ by transforming pyruvate into lactate:



In rapidly contracting muscle cells, the demand for energy is high. After the O_2 supply is depleted, *lactic acid fermentation* provides sufficient NAD^+ to allow glycolysis (with its low level of ATP production) to continue for a short time (Figure 8.7).

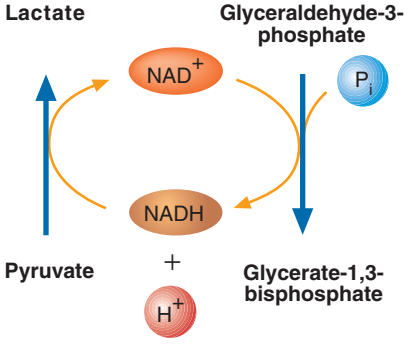


FIGURE 8.7

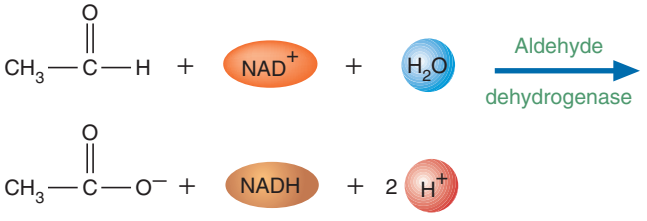
Recycling of NADH During Anaerobic Glycolysis

The NADH produced during the conversion of glyceraldehyde-3-phosphate to glycerate-1,3-bisphosphate is oxidized when pyruvate is converted to lactate. This process allows the cell to continue producing ATP under anaerobic conditions as long as glucose is available.

QUESTION 8.1

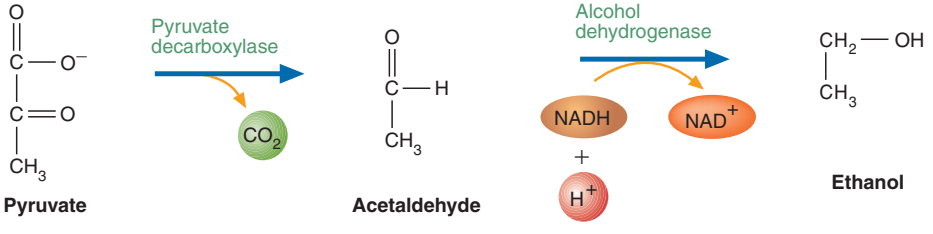
Most molecules of ethanol are detoxified in the liver by two reactions. In the first, ethanol is oxidized to form acetaldehyde. This reaction, catalyzed by alcohol dehydrogenase, produces large amounts of NADH:

Soon after its production, acetaldehyde is converted to acetate by aldehyde dehydrogenase, which catalyzes a reaction that also produces NADH:



One common effect of alcohol intoxication is the accumulation of lactate in the blood. Can you explain why this effect occurs?

In yeast and certain bacterial species, pyruvate is decarboxylated to form acetaldehyde, which is then reduced by NADH to form ethanol. (In a **decarboxylation** reaction, an organic acid loses a carboxyl group as CO_2.)



This process, called alcoholic fermentation, is used commercially to produce wine, beer, and bread. Certain bacterial species produce organic molecules other than ethanol. For example, *Clostridium acetobutylicum*, an organism related to the causative agents of botulism and tetanus, produces butanol. Until recently, this organism was used commercially to synthesize butanol, an alcohol used to produce detergents and synthetic fibers. A petroleum-based synthetic process has now replaced microbial fermentation.

COMPANION WEBSITE Visit the companion website at www.oup.com/us/mckee to read the Biochemistry in Perspective essay on fermentation.

KEY CONCEPTS

- During glycolysis, glucose is converted to two molecules of pyruvate. A small amount of energy is captured in two molecules each of ATP and NADH.
- In anaerobic organisms, pyruvate is converted to waste products in a process called fermentation.
- In the presence of oxygen the cells of aerobic organisms convert pyruvate into CO_2 and H_2O.

The Energetics of Glycolysis

During glycolysis, the energy released as glucose is broken down to pyruvate is coupled to the phosphorylation of ADP with a net yield of 2 ATP. However, evaluation of the standard free energy changes of the individual reactions (Figure 8.8) does not explain the efficiency of this pathway. A more useful method for evaluating free energy changes takes into account the conditions (e.g., pH and metabolite concentrations) under which cells actually operate. As illustrated in Figure 8.8, free energy changes measured in red blood cells indicate that only three reactions (1, 3, and 10, see pp. 269–270) have significantly negative ΔG values. These reactions, catalyzed by hexokinase, PFK-1, and pyruvate kinase, respectively, are for all practical purposes irreversible; that is, each goes to completion as written.

FIGURE 8.8

Free Energy Changes During Glycolysis in Red Blood Cells

Note that the standard free energy changes ($\Delta G^{\circ\prime}$) for the reactions in glycolysis show no consistent pattern (upper plot). In contrast, actual free energy values (ΔG) based on metabolite concentrations measured in red blood cells (lower plot) clearly illustrate why reactions 1, 3, and 10 (the conversions of glucose to glucose-6-phosphate, fructose-6-phosphate to fructose-1,6-bisphosphate, and phosphoenolpyruvate to pyruvate, respectively) are irreversible. The ready reversibility of the remaining reactions is indicated by their near-zero ΔG values. (GLU = glucose, G6P = glucose-6-phosphate, F6P = fructose-6-phosphate, FBP = fructose-1,6-bisphosphate, GAP = glyceraldehyde phosphate, PG3 = glycerate-3-phosphate, PG2 = glycerate-2-phosphate, PEP = phosphoenolpyruvate, PYR = pyruvate, LAC = lactate) Note that the conversion of DHAP to GAP is not counted in this list, since FBP is broken into GAP and DHAP, which is reconverted into GAP.

Biochemestry IN PERSPECTIVE

Saccharomyces cerevisiae and the Crabtree Effect

What unique properties of *Saccharomyces cerevisiae* make it so useful in the production of wine, beer, and bread?

The yeast *Saccharomyces cerevisiae,* a eukaryotic microorganism, has had a profound effect on humans. Beginning in the Neolithic age and continuing to the present day, this organism is used to convert carbohydrate-containing food into the wine, beer, and bread that many humans consider indispensible to life. What properties of *S. cerevisiae* make it uniquely suited for the oldest of biotechnologies? Although many yeast species can ferment carbohydrates to form ethanol and CO_2, only *S. cerevisiae* efficiently produces these molecules in large quantities. A simple experiment helps explain why. If fleshy fruits such as grapes are crushed and placed in a vat, they will begin to ferment. At the beginning of fermentation, a survey of the microorganisms present reveals many different types of microorganisms, but relatively few *S. cerevisiae*. As the fermentation process proceeds (and the ethanol content increases), however, *S. cerevisiae* cells become a larger proportion of the microbes until eventually they virtually become the only microbes present. How *S. cerevisiae* performs this feat has been the subject of considerable research—and not only because of the economic importance of traditional fermentation biotechnologies. The goal of producing ethanol biofuel from cellulose (not from a food staple such as corn) in an efficient, cost-effective manner still remains elusive. The principal physiological reason that allows *S. cerevisiae* to ferment carbohydrates efficiently and dominate its environment is explained by the Crabtree effect, described next.

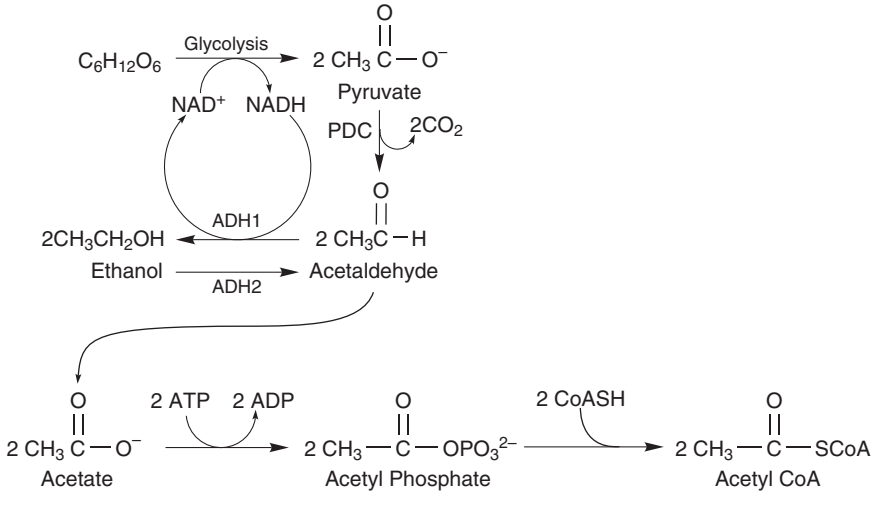
The Crabtree Effect

S. cerevisiae is a *facultative anaerobe*: it is capable of generating energy in both the presence and the absence of O_2 using aerobic metabolism (the citric acid cycle, the electron transport system, and oxidative phosphorylation) and fermentation, respectively. Unlike most fermenting organisms, *S. cerevisiae* can also ferment sugar in the presence of O_2. As glucose or fructose levels rise, pyruvate is diverted away from the citric acid cycle (the first phase of aerobic energy generation) into ethanol synthesis by conversion to acetaldehyde and CO_2 by pyruvate decarboxylase. This phenomenon, in which glucose represses aerobic metabolism, is the **Crabtree effect**. (In most organisms that use oxygen the **Pasteur effect** is observed: glycolysis is depressed when the gas is available.) In *S. cerevisiae* cells high glucose levels result in changes in gene expression. These changes promote the insertion of hexose transporters into the plasma membrane (resulting in rapid transport of glucose into the cell), the synthesis of glycolytic enzymes, and the inhibition

FIGURE 8A

Ethanol Metabolism in *S. cerevisiae*

When glucose levels are high, yeast cells shift into the "make, accumulate, consume" ethanol pathway. At first, glucose is converted into ethanol molecules to regenerate NAD^+. Ethanol is then released into the environment where it kills competing microbes. Once glucose is depleted, glucose repression ends. Among the results of derepression is that ADH2, the enzyme that converts ethanol back into acetaldehyde, is synthesized. Acetaldehyde is subsequently converted into acetyl-CoA, the substrate for the citric acid cycle. Note that although the "make, accumulate, consume" strategy is expensive (i.e., the energy expended to synthesize the enzymes needed to convert ethanol into acetyl-CoA and the ATPs used to synthesize acetyl phosphate), yeast cells manage to kill off the competition and retrieve a waste product that they then use as an energy source.



Biochemistry IN PERSPECTIVE cont

of aerobic respiration enzyme synthesis. The diversion of pyruvate into ethanol production is also believed to result from an overflow phenomenon. There are too few pyruvate dehydrogenase molecules to convert pyruvate entirely into acetyl-CoA. Consequently, pyruvate decarboxylase converts excess pyruvate molecules into acetaldehyde.

At first glance the glucose-induced repression of aerobic metabolism appears inefficient because, the production of ATP in fermentation (2 ATP per glucose) is minor compared with that of oxidative phosphorylation (approximately 30 ATP per glucose). However, rapid synthesis of ethanol along with its release into the environment by ethanol-tolerant *S. cerevisiae* has the effect of eliminating microbial competitors and predators. Once glucose levels are depleted and O_2 is available, there is a significant change in gene expression, called the *diauxic shift*, which alters yeast energy metabolism. Glucose repression ends, and the yeast cells proceed to reabsorb ethanol and reconvert it to acetaldehyde using ADH2 (p. xxx). Acetaldehyde is then converted to acetyl-CoA, the citric acid cycle substrate. In effect, as a result of its unique energy metabolism, referred to as "make, accumulate, consume," *S. cerevisiae* cells can use their waste product as an energy source (Figure 8A). Humans take advantage of the first phase of yeast energy metabolism in the commercial production of wine and beer. Early in the process, aerobic fermentation is accompanied by O_2-requiring reactions that facilitate cell division, thereby increasing the number of yeast. As the oxygen in the fermentation vessel is depleted, ethanol production accelerates. Eventually the sugar level drops and fermentation slows. By excluding O_2 from the fermentation at this stage of the process, the ethanol content of the product is maximized because the shift to aerobic degradation of ethanol is prevented.

SUMMARY: A metabolic adaptation in the ancestors of *S. cerevisiae* allowed them to produce large quantities of ethanol, a toxic molecule that eliminated microbial competitors. Humans take advantage of this adaptation when they use *S. cerevisiae* in the production of alcoholic beverages and bread.

The values for the remaining reactions (2, 4–9) are so close to zero that they operate near equilibrium. Consequently, these latter reactions are easily reversible; small changes in substrate or product concentrations can alter the direction of each reaction. Not surprisingly, in gluconeogenesis (Section 8.2), the pathway by which glucose can be generated from pyruvate and certain other substrates, all of the glycolytic enzymes are involved except for those that catalyze reactions 1, 3, and 10. Gluconeogenesis uses different enzymes to bypass the irreversible steps of glycolysis.

Regulation of Glycolysis

The rate at which the glycolytic pathway operates in a cell is directly controlled primarily by the kinetic properties of its hexokinase isoenzymes and the allosteric regulation of the enzymes that catalyze the three irreversible reactions: hexokinase, PFK-1, and pyruvate kinase.

THE HEXOKINASES The animal liver has four hexokinases. Three of these enzymes (hexokinases I, II, and III) are found in varying concentrations in other body tissues, where they bind reversibly to an anion channel (called a *porin*) in the outer membrane of mitochondria. As a result, ATP is readily available. These isozymes have high affinities for glucose relative to its concentration in blood; that is, they are half-saturated at concentrations of less than 0.1 mM, although blood glucose levels are approximately 4 to 5 mM. In addition, hexokinases I, II, and III are inhibited from phosphorylating glucose molecules by glucose-6-phosphate, the product of the reaction. When blood glucose levels are low, these properties allow cells such as those in brain and muscle to obtain sufficient glucose. When

blood glucose levels are high, cells do not phosphorylate more glucose molecules than required to meet their immediate needs. The fourth enzyme, called hexokinase IV (or glucokinase), catalyzes the same reaction but has significantly different kinetic properties. Glucokinase (GK), found in liver as well as in certain cells in pancreas, intestine, and brain, requires much higher glucose concentrations for optimal activity (about 10 mM), and it is not inhibited by glucose-6-phosphate. In liver, GK diverts glucose into storage as glycogen. This capacity provides the resources used to maintain blood glucose levels, a major role of the liver. Consequently, after a carbohydrate meal the liver does not remove large quantities of glucose from the blood for glycogen synthesis until other tissues have satisfied their requirements for this molecule. In cell types where it occurs, GK is believed to be a *glucose sensor.* Because glucokinase does not usually work at maximum velocity, it is highly sensitive to small changes in blood glucose levels. Its activity is linked to a signal transduction pathway. For example, the release of insulin (the hormone that promotes the uptake of glucose into muscle and adipose tissue cells) by pancreatic β-cells in response to rising blood levels of glucose is initiated by GK. GK regulation involves its binding to GK regulator protein (GKRP), a process that is triggered by high fructose-6-phosphate levels. GKRP/GK then moves into the nucleus. When blood glucose levels rise after a meal, GKRP releases GK (caused by exchange with fructose-1-phosphate), and GK moves back through the nuclear pores and can again phosphorylate glucose.

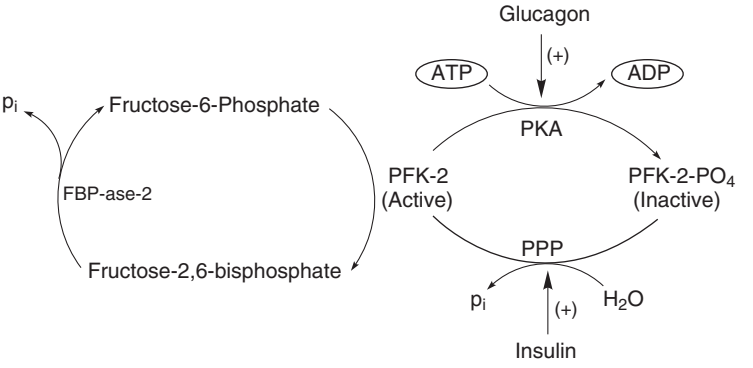
ALLOSTERIC REGULATION OF GLYCOLYSIS The reactions catalyzed by hexokinases I, II, and III, PFK-1, and pyruvate kinase can be switched on and off by allosteric effectors. In general, allosteric effectors are molecules whose cellular concentrations are sensitive indicators of a cell's metabolic state. Some allosteric effectors are product molecules. For example, hexokinases I, II, and III are inhibited by excess glucose-6-phosphate. Several energy-related molecules also act as allosteric effectors. For example, a high AMP concentration (an indicator of low energy production) activates pyruvate kinase. In contrast, pyruvate kinase is inhibited by a high ATP concentration (an indicator that the cell's energy requirements are being met). Acetyl-CoA, which accumulates when ATP is in rich supply, inhibits pyruvate kinase.

Of the three key enzymes in glycolysis, PFK-1 is the most carefully regulated. Its activity is allosteically inhibited by high levels of ATP and citrate, which are indicators that the cell's energy charge is high and that the citric acid cycle, a major component of the cell's energy-generating capacity, has slowed down. AMP is an allosteric activator of PFK-1. AMP levels, which increase when the energy charge of the cell is low, are a better predictor of energy deficit than ADP levels. Fructose-2,6-bisphosphate, allosteric activator of PFK-1 activity in the liver, is synthesized by phosphofructokinase-2 (PFK-2) in response to hormonal signals correlated to blood glucose levels (Figure 8.9). When serum glucose levels are high, hormone-stimulated increase in fructose-2,6-bisphosphate coordinately increases the activity of PFK-1 (activates glycolysis) and decreases the activity of the enzyme that catalyzes the reverse reaction, fructose-1,6-bisphosphatase (inhibits gluconeogenesis, Section 8.2). AMP is an allosteric inhibitor of fructose-1,6-bisphosphatase. PFK-2 is a bifunctional enzyme that behaves as a phosphatase when phosphorylated in response to the hormone glucagon (released into blood in reponse to low blood sugar, see later). It functions as a kinase when dephosphorylated in response to the hormone insulin (high blood sugar). Fructose-2,6-bisphosphate, produced via hormone-induced covalent modification of PFK-2, is an indicator of high levels of available glucose and allosterically activates PFK-1. Accumulated fructose-1,6-bisphosphate activates pyruvate kinase, providing a feed-forward mechanism of control (i.e., fructose-1,6-bisphosphate is an allosteric activator). The allosteric regulation of glycolysis is summarized in Table 8.1.

FIGURE 8.9

Fructose-2,6-Bisphosphate Level Regulation

Glycolysis is stimulated when fructose-2,6-bisphosphate, the activator of PFK-1, is synthesized by PFK-2. PFK-2 is, in turn, activated by a dephosphorylation reaction catalyzed by phosphoprotein phosphatase (PPP), an enzyme activated by insulin. PFK-2 is a bifunctional protein with two enzymatic activities: PFK-2 and fructose-2-6-bisphosphatase-2 (FBPase-2). As a result, the dephosphorylation reaction also inhibits FBP-ase-2, the enzymatic activity th at converts fructose-2,6-bisphosphate to fructose-6-phosphate. Glycolysis is inhibited when fructose-2-6-bisphosphate levels are low, which is the result of a glucagon-stimulated and protein kinase A (PKA)-catalyzed phosphorylation reaction that inactivates PFK-2.



HORMONAL REGULATION Glycolysis is also regulated by the peptide hormones glucagon and insulin. **Glucagon**, released by pancreatic α-cells when blood glucose is low, activates the phosphatase function of PFK-2, thereby reducing the level of fructose-2,6-bisphosphate in the cell. As a result, PFK-1 activity and flux through glycolysis are decreased. In liver, glucagon also inactivates pyruvate kinase. Glucagon's effects, triggered by binding to its receptor on target cell surfaces, are mediated by cyclic AMP. Cyclic AMP (cAMP) (p. xxx) is synthesized from ATP in a reaction catalyzed by adenylate cyclase, a plasma membrane protein. Once synthesized, cAMP binds to and activates protein kinase A (PKA). PKA then initiates a signal cascade of phosphorylation/dephosphorylation reactions that alter the activities of a diverse set of enzymes and transcription factors. **Transcription factors** are proteins that regulate or initiate RNA synthesis by binding to specific DNA sequences called **response elements.**

Insulin is a peptide hormone released from pancreatic β-cells when blood glucose levels are high. The effects of insulin on glycolysis include activation of the kinase function of PFK-2, which increases the level of fructose-2,6-bisphosphate in the cell, in turn increasing glycolytic flux. In cells containing insulin-sensitive glucose transporters (muscle and adipose tissue but not liver or brain) insulin promotes the translocation of glucose transporters to the cell surface. When insulin binds to its cell-surface receptor, the receptor protein undergoes several autophosphorylation reactions, which trigger numerous intracellular signal cascades that involve phosphorylation and dephosphorylation of target enzymes and transcription factors. Many of insulin's effects on gene expression are mediated by the transcription factor SREBP1c, a sterol regulatory element binding protein (p. xxx). As a result of SREBP1c activation, there is increased synthesis of glucokinase and pyruvate kinase.

AMPK: A METABOLIC MASTER SWITCH AMP-activated protein kinase (AMPK) is an enzyme that plays a central role in energy metabolism. First discovered as a regulator of lipid metabolism, AMPK is now known to affect glucose metabolism as well. Once AMPK is activated, as a result of an increase in a

TABLE 8.1 Allosteric Regulation of Glycolysis

Enzyme	Activator	Inhibitor
Hexokinase		Glucose-6-phosphate, ATP
PFK-1	Fructose-2,6-bisphosphate, AMP	Citrate, ATP
Pyruvate kinase	Fructose-1,6-bisphosphate, AMP	Acetyl-CoA, ATP

cell's AMP:ATP ratio, it phosphorylates target proteins (enzymes and transcription factors). AMPK switches off anabolic pathways (e.g., protein and lipid synthesis) and switches on catabolic pathways (e.g., glycolysis and fatty acid oxidation). AMPK's regulatory effects on glycolysis include the following. In cardiac and skeletal muscle, AMPK promotes glycolysis by facilitating the stress- or exercise-induced recruitment of glucose transporters to the plasma membrane. In cardiac cells, AMPK stimulates glycolysis by activating PFK-2. AMPK structure and its functional properties are described in Chapter 12.

QUESTION 8.2

Glucose Tolerance

Insulin is a hormone secreted by the pancreas when blood sugar increases. Its most easily observable function is to reduce the blood sugar level to normal. The binding of insulin to its target cells promotes the transport of glucose across the plasma membrane. The capacity of an individual to respond to a carbohydrate meal by reducing blood glucose concentration quickly is referred to as *glucose tolerance.* Chromium-deficient animals show a decreased glucose tolerance; that is, they cannot remove glucose from blood quickly enough. The metal is believed to facilitate the binding of insulin to cells. Do you think the chromium is acting as an allosteric activator or cofactor?

QUESTION 8.3

Louis Pasteur, the great nineteenth-century French chemist and microbiologist, was the first scientist to observe that cells that can oxidize glucose completely to CO_2 and H_2O use glucose more rapidly in the absence of O_2 than in its presence. The oxygen molecule seems to inhibit glucose consumption. Explain in general terms the significance of this finding, now referred to as the Pasteur effect.

8.2 GLUCONEOGENESIS

Gluconeogenesis, the formation of new glucose molecules from noncarbohydrate precursors, occurs primarily in the liver. Precursor molecules include lactate, pyruvate, glycerol, and certain α-keto acids (molecules derived from amino acids). Under certain conditions (i.e., metabolic acidosis or starvation) the kidney can make small amounts of new glucose. Between meals adequate blood glucose levels are maintained by the hydrolysis of liver glycogen. When liver glycogen is depleted (e.g., owing to prolonged fasting or vigorous exercise), the gluconeogenesis pathway provides the body with adequate glucose. Brain and red blood cells rely exclusively on glucose as their energy source.

Gluconeogenesis Reactions

The reaction sequence in gluconeogenesis is largely the reverse of glycolysis. Recall, however, that three glycolytic reactions (the reactions catalyzed by hexokinase, PFK-1, and pyruvate kinase) are irreversible. In gluconeogenesis, alternate reactions catalyzed by different enzymes are used to bypass these obstacles. The reactions unique to gluconeogenesis are listed next. The entire gluconeogenic pathway and its relationship to glycolysis are illustrated in Figure 8.10. The bypass reactions of gluconeogenesis are as follows:

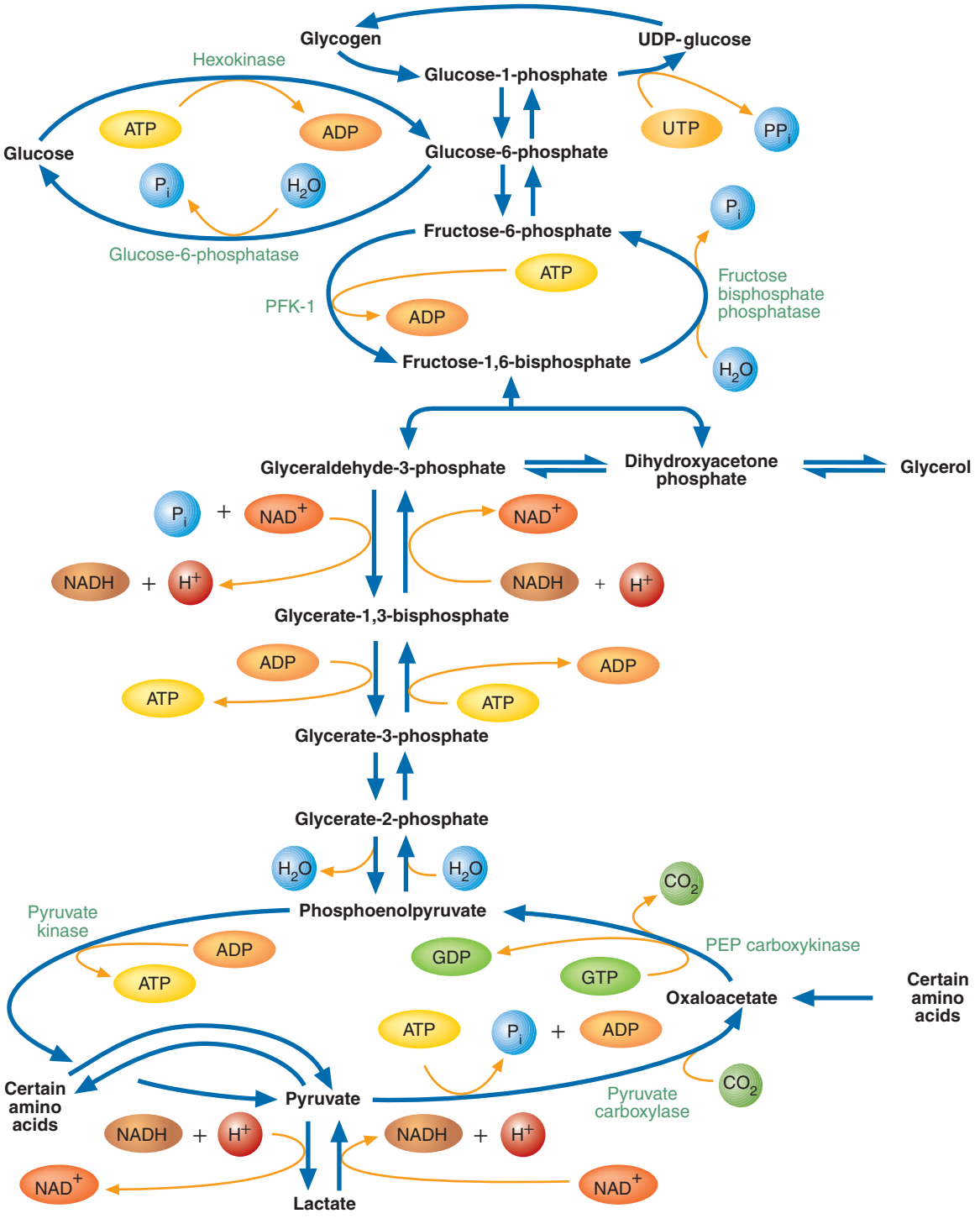


FIGURE 8.10

Carbohydrate Metabolism: Gluconeogenesis and Glycolysis

In gluconeogenesis, which occurs when blood sugar levels are low and liver glycogen is depleted, 7 of the 10 reactions of glycolysis are reversed. Three irreversible glycolytic reactions are bypassed by alternative reactions. The major substrates for gluconeogenesis are certain amino acids (derived from muscle), lactate (formed in muscle and red blood cells), and glycerol (produced from the degradation of triacylglycerols). In contrast to the reactions of glycolysis, which occur only in cytoplasm, the gluconeogenesis reactions catalyzed by pyruvate carboxylase and, in some species, PEP carboxykinase occur within the mitochondria. The reaction catalyzed by glucose-6-phosphatase takes place in the endoplasmic reticulum. Note that gluconeogenesis and glycolysis do not occur simultaneously. In glycolysis, pyruvate is converted either to acetyl-CoA (not shown) or to lactate.

Biochemistry IN PERSPECTIVE

Turbo Design Can Be Dangerous

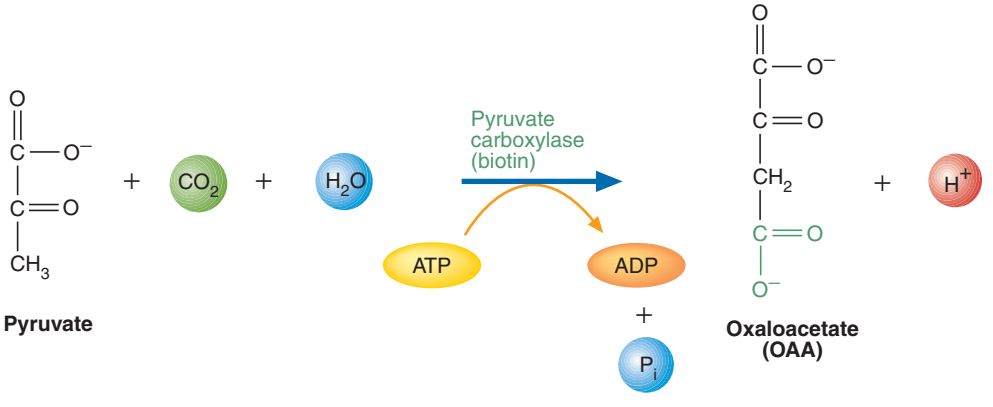
Why must turbo design pathways be rigorously controlled?

Catabolic pathways with a turbo design, such as glycolysis, are optimized and efficient. However, the early phases of such pathways must be negatively regulated to prevent buildup of intermediates and overuse of fuel. Two of the four ATPs produced from each glucose molecule are fed back to the fuel input stage of the pathway to drive the pathway forward. As indicated by its use by most modern living organisms, glycolysis has been a tremendously successful energy-generating strategy. It is not perfect, however. Under certain circumstances, the turbo design of glycolysis makes some cells vulnerable to a phenomenon called "substrate-accelerated death".

Certain types of mutant yeast cells, for example, are unable to grow anaerobically on glucose despite having a completely functional glycolytic pathway. These mutants die when exposed to large concentrations of glucose. Amazingly, research efforts have revealed that defects in *TPS1*, the gene that codes for the catalytic subunit of trehalose-6-phosphate synthase, are responsible. Trehalose-6-phosphate (Tre-6-P), an α-(1,1)-disaccharide of glucose, is a compatible solute (see the Biochemistry in Perspective box in Chapter 3 entitled Water, Abiotic Stress, and Compatible Solutes) used by yeast and various other organisms to resist several forms of abiotic stress. Apparently, Tre-6-P is a normal inhibitor of HK (and possibly a glucose transporter). In the absence of a functional TPS1 protein and when glucose becomes available, glycolytic flux in the mutant cells rapidly accelerates. In a relatively short time, as a result of the turbo design of the pathway, most available phosphate has been incorporated into glycolytic intermediates, and the cell's ATP level is too low to sustain cellular processes. This and other similar examples of substrate-accelerated cell death in other species provide insight into the importance of the intricate regulatory mechanisms observed in living organisms.

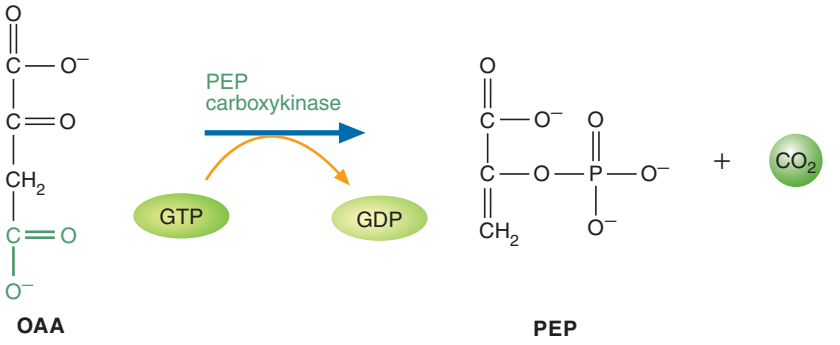
SUMMARY: Defects in the intricate regulatory mechanism that controls a turbo design pathway can make an organism vulnerable to substrate-accelerated death through uncontrolled pathway flux.

1. **Synthesis of PEP**. PEP synthesis from pyruvate requires two enzymes: pyruvate carboxylase and PEP carboxykinase. Pyruvate carboxylase, found within mitochondria, converts pyruvate to oxaloacetate (OAA):

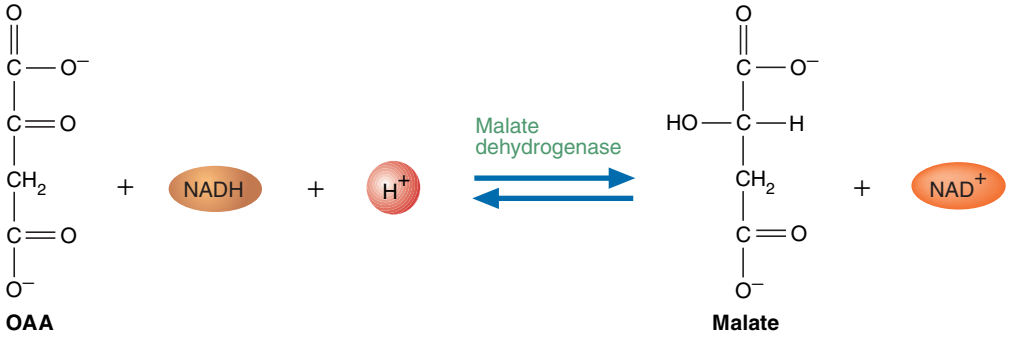


The transfer of CO_2 to form the product OAA is mediated by the coenzyme *biotin* (p. xxx), which is covalently bound within the enzyme's active site.

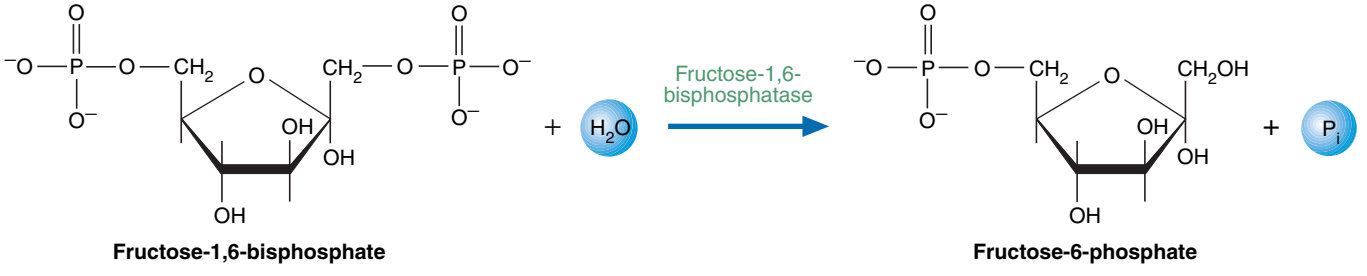
OAA is then decarboxylated and phosphorylated by PEP carboxykinase in a reaction driven by the hydrolysis of guanosine triphosphate (GTP):



PEP carboxykinase is found within the mitochondria of some species and in the cytoplasm of others. In humans this enzymatic activity is found in both compartments. Because the inner mitochondrial membrane is impermeable to OAA, cells that lack mitochondrial PEP carboxykinase transfer OAA into the cytoplasm by using the **malate shuttle**. In this process, OAA is converted into malate by mitochondrial malate dehydrogenase. After the transport of malate across mitochondrial membrane, the reverse reaction (to form OAA and NADH) is catalyzed by cytoplasmic malate dehydrogenase. The malate shuttle allows gluconeogenesis to continue because it provides the NADH required for the reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase.



2. **Conversion of fructose-1,6-bisphosphate to fructose-6-phosphate.** The irreversible PFK-1–catalyzed reaction in glycolysis is bypassed by fructose-1,6-bisphosphatase:



This exergonic reaction ($\Delta G^{\circ\prime} = -16.7$ kJ/mol) is also irreversible under cellular conditions. ATP is not regenerated, and inorganic phosphate (P_i) is also produced. Fructose-1,6-bisphosphatase is an allosteric enzyme. Its activity is stimulated by citrate and inhibited by AMP and fructose-2,6-bisphosphate.

3. **Formation of glucose from glucose-6-phosphate**. Glucose-6-phosphatase, found only in liver and kidney, catalyzes the irreversible hydrolysis of glucose-6-phosphate to form glucose and P_i. Glucose is subsequently released into the blood.

Each of the foregoing reactions is matched by an opposing irreversible reaction in glycolysis. Each set of such paired reactions is referred to as a *substrate cycle*. Because they are coordinately regulated (an activator of the enzyme catalyzing the forward reaction serves as an inhibitor of the enzyme catalyzing the reverse reaction), very little energy is wasted, even though both enzymes may be operating at some level at the same time. *Flux control* (regulation of the flow of substrate and removal of product) is more effective if transient accumulation of product is funneled back through the cycle. The catalytic velocity of the forward enzyme will remain high if the concentration of the substrate is maximized. The gain in catalytic efficiency more than makes up for the small energy loss in recycling the product.

Gluconeogenesis is an energy-consuming process. Instead of generating ATP (as in glycolysis), gluconeogenesis requires the hydrolysis of six high-energy phosphate bonds.

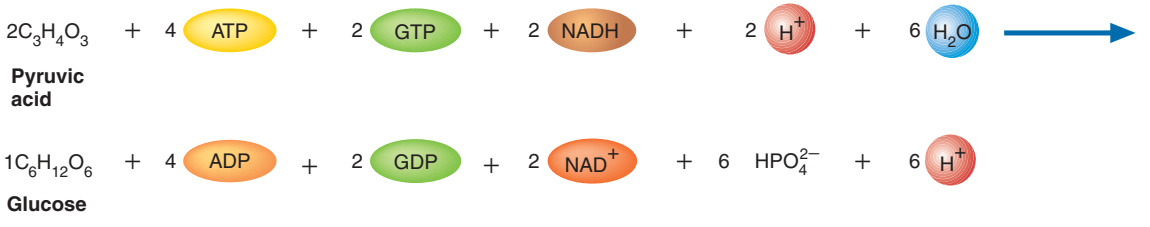
QUESTION 8.4

Malignant hyperthermia is a rare, inherited disorder triggered during surgery by certain anesthetics. A dramatic (and dangerous) rise in body temperature (as high as 112°F) is accompanied by muscle rigidity and acidosis. The excessive muscle contraction is initiated by a large release of calcium from the sarcoplasmic reticulum, a calcium-storing organelle in muscle cells. Acidosis results from excessive lactic acid production. Prompt treatment to reduce body temperature and to counteract the acidosis is essential to save the patient's life. A probable contributing factor to this disorder is wasteful cycling between glycolysis and gluconeogenesis. Explain why this is a reasonable explanation.

Malignant Hyperthermia

QUESTION 8.5

After examining the gluconeogenic pathway reaction summary illustrated here, account for each component in the equation. [*Hint:* The hydrolysis of each nucleotide releases a proton.]



QUESTION 8.6

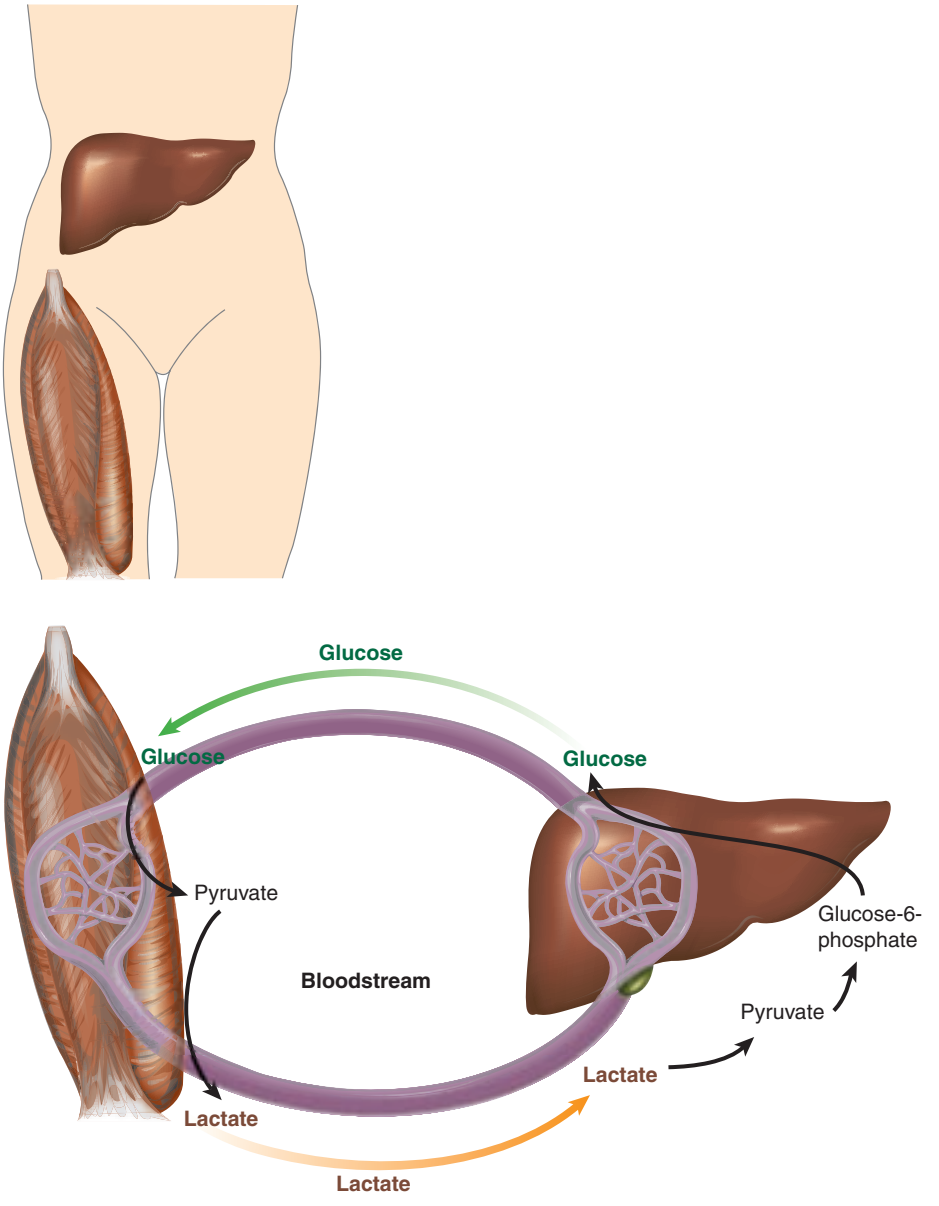
Patients with *von Gierke's disease* (a glycogen storage disease) lack glucose-6-phosphatase activity. Two prominent symptoms of this disorder are fasting hypoglycemia and lactic acidosis. Can you explain why these symptoms occur?

von Gierke's Disease

FIGURE 8.11

The Cori Cycle

During strenuous exercise, lactate is produced anaerobically in muscle cells. After passing through blood to the liver, lactate is converted to glucose by gluconeogenesis.

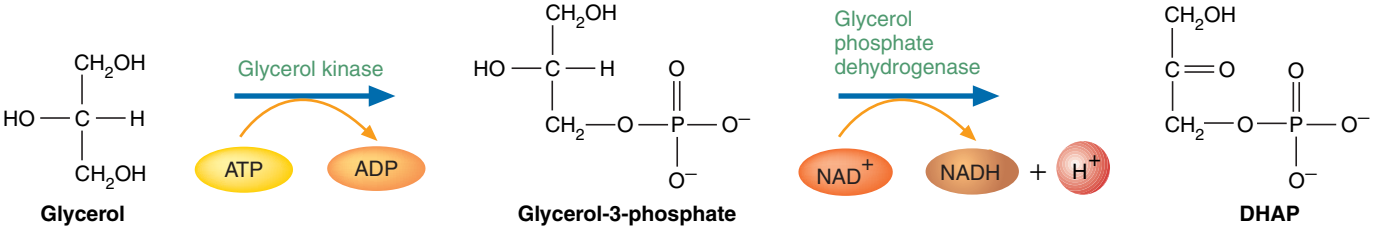


Gluconeogenesis Substrates

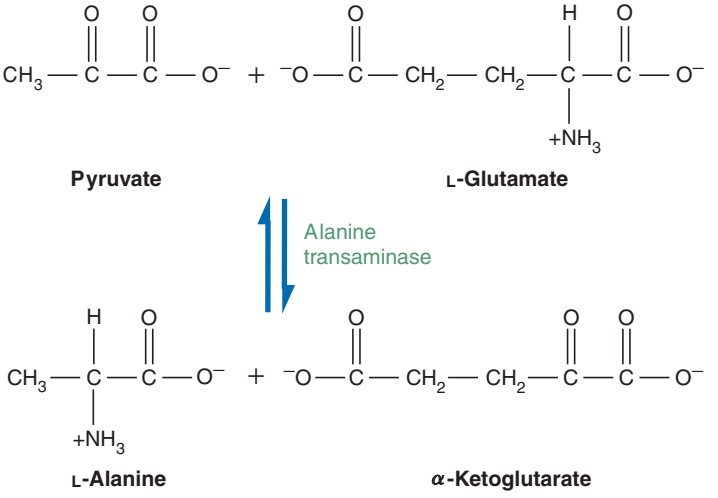
As previously mentioned, several metabolites are gluconeogenic precursors. Three of the most important substrates are described briefly.

Lactate is released by red blood cells and other cells that lack mitochondria or have low oxygen concentrations. In the **Cori cycle**, lactate is released by skeletal muscle during exercise (Figure 8.11). After lactate is transferred to the liver, it is reconverted to pyruvate by lactate dehydrogenase and then to glucose by gluconeogenesis.

Glycerol, a product of fat metabolism in adipose tissue, is transported to the liver in the blood and then converted to glycerol-3-phosphate by glycerol kinase. Oxidation of glycerol-3-phosphate to form DHAP occurs when cytoplasm NAD^+ concentration is relatively high.



Of all the amino acids that can be converted to glycolytic intermediates (molecules referred to as *glucogenic*), alanine is perhaps the most important. When exercising muscle produces large quantities of pyruvate, some of these molecules are converted to alanine by a transamination reaction involving glutamate:



After it has been transported to the liver, alanine is reconverted to pyruvate and then to glucose. The **glucose-alanine cycle** (Figure 8.12) serves several purposes. In addition to its role in recycling α-keto acids between muscle and liver, the glucose-alanine cycle is a mechanism for transporting amino nitrogen to the liver. In α-keto acids, sometimes referred to as carbon skeletons, a carbonyl group is directly attached to the carboxyl group. Once alanine reaches the liver, it is reconverted to pyruvate. The amino nitrogen is then incorparated into urea or transfered to other α-keto acids to restore the amino acid balance in the liver (Chapter 15).

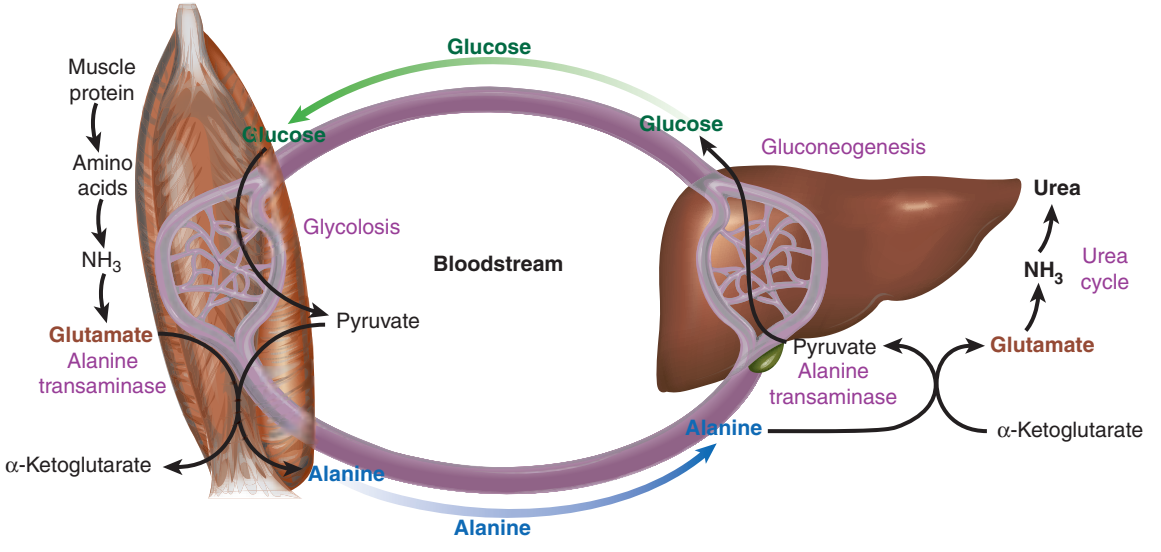


FIGURE 8.12

The Glucose-Alanine Cycle

Alanine is formed from pyruvate in muscle. After it has been transported to the liver, alanine is reconverted to pyruvate by alanine transaminase. Eventually pyruvate is used in the synthesis of new glucose. Because muscle cannot synthesize urea from amino nitrogen, the glucose-alanine cycle is used to transfer amino nitrogen to the liver.

Gluconeogenesis Regulation

As with other metabolic pathways, the rate of gluconeogenesis is affected primarily by substrate availability, allosteric effectors, and hormones. Not surprisingly, gluconeogenesis is stimulated by high concentrations of lactate, glycerol, and amino acids. A high-fat diet, starvation, and prolonged fasting make large quantities of these molecules available.

The four key enzymes in gluconeogenesis (pyruvate carboxylase, PEP carboxykinase, fructose-1,6-bisphosphatase, and glucose-6-phosphatase) are affected to varying degrees by allosteric modulators. For example, fructose-1,6-bisphosphatase is activated by citrate and inhibited by AMP and fructose-2,6-bisphosphate. Acetyl-CoA activates pyruvate carboxylase. (The concentration of acetyl-CoA, a product of fatty acid degradation, is especially high during starvation.) Figure 8.13 provide an overview of the allosteric regulation of glycolysis and gluconeogenesis.

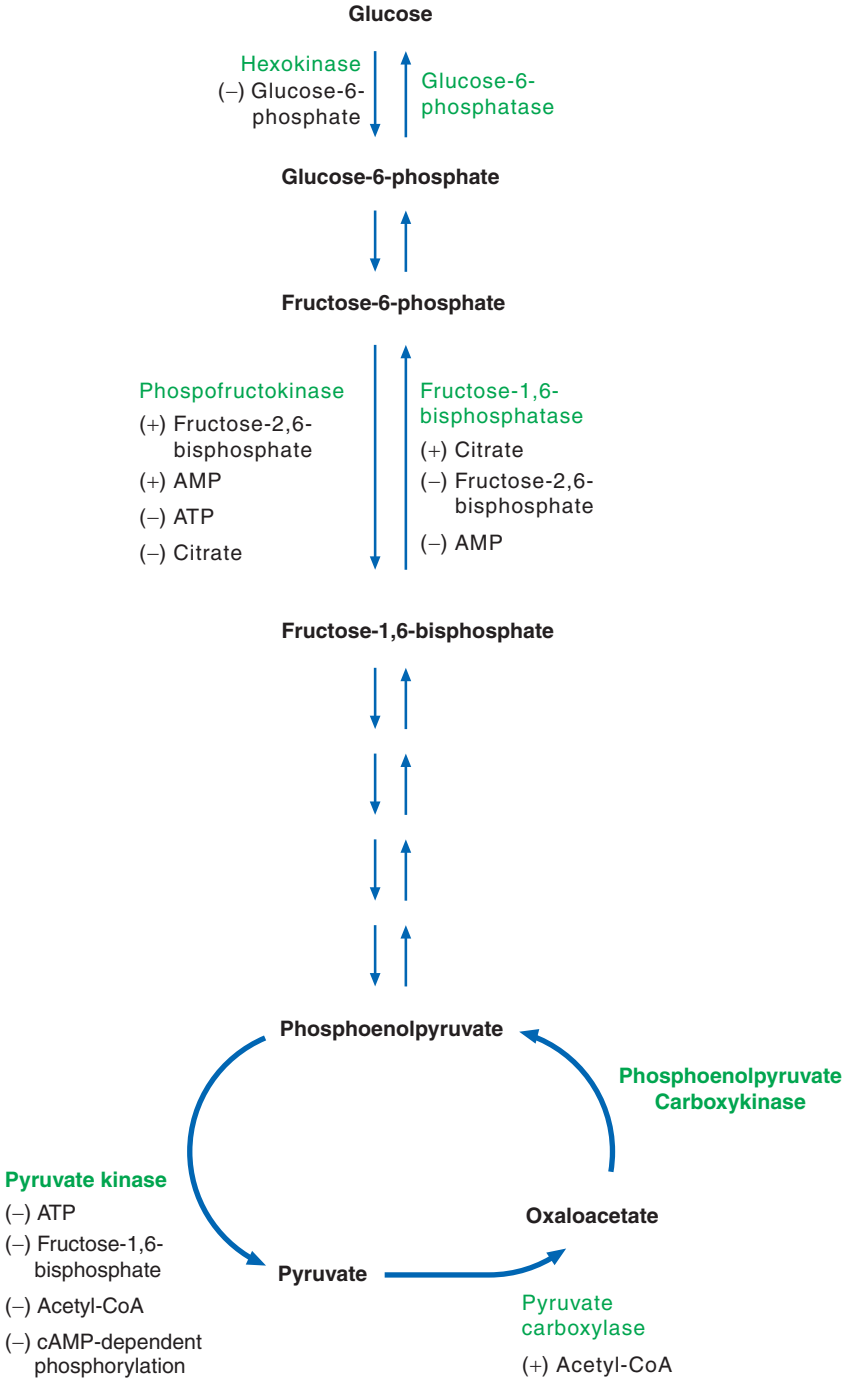


FIGURE 8.13

Allosteric Regulation of Glycolysis and Gluconeogenesis

The key enzymes in glycolysis and gluconeogenesis are regulated by allosteric effectors. Activator, +; inhibitor, −.

As with other biochemical pathways, hormones affect gluconeogenesis by altering the concentrations of allosteric effectors and the key rate-determining enzymes. As mentioned previously, glucagon depresses the synthesis of fructose-2,6-bisphosphate, which releases the inhibition of fructose-1,6-bisphosphatase, and inactivates the glycolytic enzyme pyruvate kinase. Hormones also influence gluconeogenesis by altering enzyme synthesis. For example, the synthesis of gluconeogenic enzymes is stimulated by cortisol, a steroid hormone produced in the cortex of the adrenal gland that facilitates the body's adaptation to stressful situations. Finally, insulin action leads to the synthesis of new molecules of glucokinase, PFK-1 (SREBP1c-induced), and PFK-2 (glycolysis favored). Insulin also depresses the synthesis (also via SREBP1c) of PEP carboxykinase, fructose-1,6-bisphosphatase, and glucose-6-phosphatase. Glucagon action leads to the synthesis of additional molecules of PEP carboxykinase, fructose-1,6-bisphosphatase, and glucose-6-phosphatase (gluconeogenesis favored).

The hormones that regulate glycolyses and gluconeogenesis alter the phosphorylation state of certain target proteins in the liver cell, which in turn modifies gene expression. The key point to remember is that insulin and glucagon have opposing effects on carbohydrate metabolism. The direction of metabolite flux, (i.e., whether either glycolysis or gluconeogenesis is active) is largely determined by the ratio of insulin to glucagon. After a carbohydrate meal, the insulin/glucagon ratio is high and glycolysis in the liver predominates over gluconeogenesis. After a period of fasting or following a high-fat, low-carbohydrate meal, the insulin/glucagon ratio is low and gluconeogenesis in the liver predominates over glycolysis. The availability of ATP is the second important regulator in the reciprocal control of glycolysis and gluconeogenesis in that high levels of AMP, the low-energy hydrolysis product of ATP, increase the flux through glycolysis at the expense of gluconeogenesis, and low levels of AMP increase the flux through gluconeogenesis at the expense of glycolysis. Although control at the PFK-1/fructose-1,6-bisphosphatase cycle would appear to be sufficient for this pathway, control at the pyruvate kinase step is key because it permits the maximal retention of PEP, a molecule with a very high phosphate transfer potential.

KEY CONCEPTS

- Gluconeogenesis, the synthesis of new glucose molecules from noncarbohydrate precursors, occurs primarily in the liver.
- The reaction sequence is the reverse of glycolysis except for three reactions that bypass irreversible steps in glycolysis.

8.3 THE PENTOSE PHOSPHATE PATHWAY

The pentose phosphate pathway is an alternative metabolic pathway for glucose oxidation in which no ATP is generated. Its principal products are NADPH, a reducing agent required in several anabolic processes, and ribose-5-phosphate, a structural component of nucleotides and nucleic acids. The pentose phosphate pathway occurs in the cytoplasm in two phases: oxidative and nonoxidative. In the oxidative phase of the pathway, the conversion of glucose-6-phosphate to ribulose-5-phosphate is accompanied by the production of two molecules of NADPH. The nonoxidative phase involves the isomerization and condensation of a number of different sugar molecules. Three intermediates in this process that are useful in other pathways are ribose-5-phosphate, fructose-6-phosphate, and glyceraldehyde-3-phosphate.

The oxidative phase of the pentose phosphate pathway consists of three reactions (Figure 8.14a). In the first reaction, glucose-6-phosphate dehydrogenase (G-6-PD) catalyzes the oxidation of glucose-6-phosphate. 6-Phosphogluconolactone and NADPH are products in this reaction. 6-Phospho-D-glucono-δ-lactone is then hydrolyzed to produce 6-phospho-D-gluconate. A second molecule of NADPH is produced during the oxidative decarboxylation of 6-phosphogluconate, a reaction that yields ribulose-5-phosphate.

A substantial amount of the NADPH required for reductive processes (i.e., lipid biosynthesis) is supplied by these reactions. For this reason this pathway is most active in cells in which relatively large amounts of lipids are synthesized, (e.g., adipose tissue, adrenal cortex, mammary glands, and the liver). NADPH is

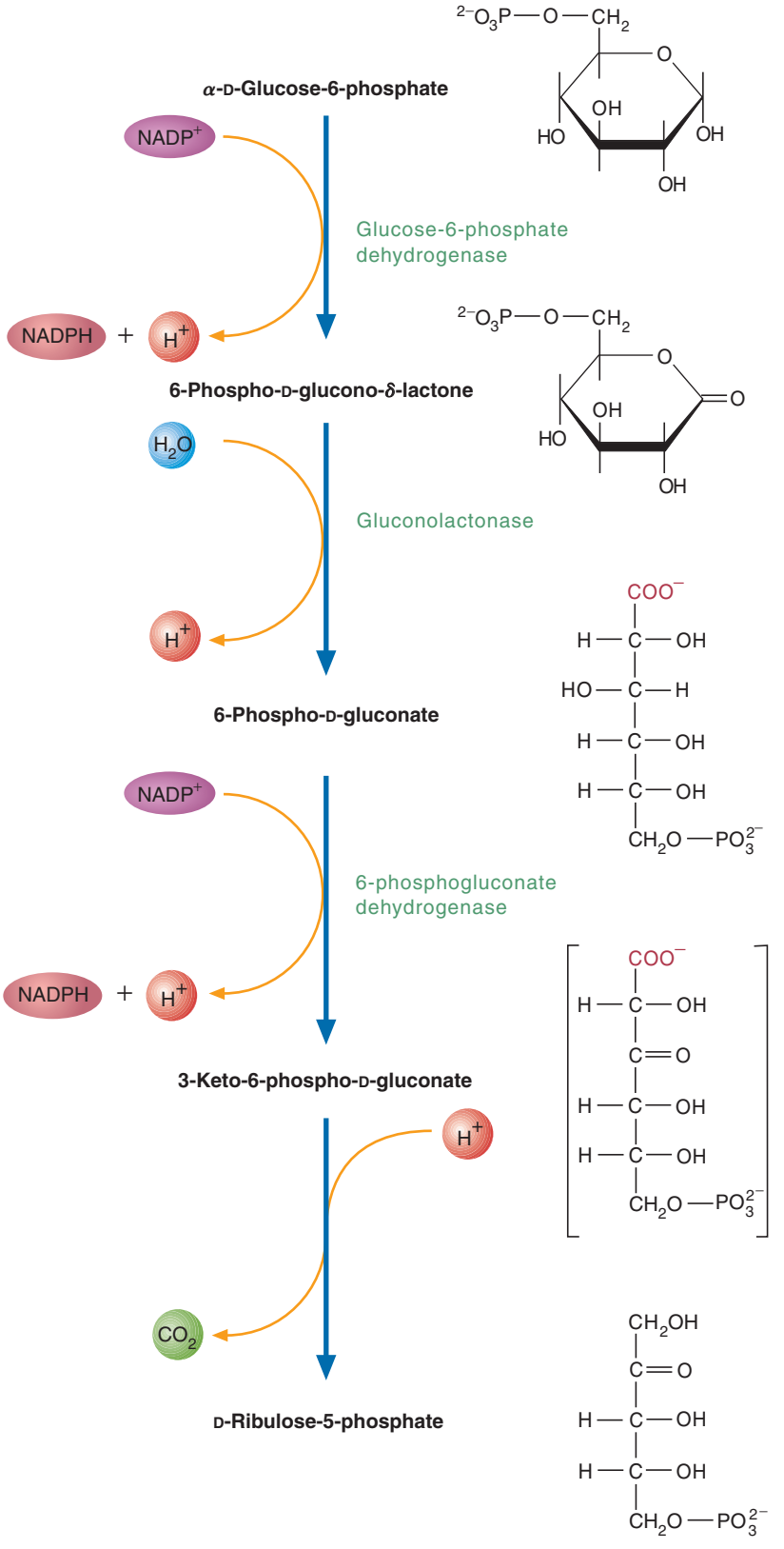


FIGURE 8.14a

The Pentose Phosphate Pathway

(a) The oxidative phase. NADPH is an important product of these reactions.

also a powerful antioxidant. (**Antioxidants** are substances that prevent the oxidation of other molecules. Their roles in living processes are described in Chapter 10.) Consequently, the oxidative phase of the pentose phosphate pathway is also quite active in cells that are at high risk for oxidative damage, such as red blood cells.

The nonoxidative phase of the pathway begins with the conversion of ribulose-5-phosphate to ribose-5-phosphate by ribulose-5-phosphate isomerase or to xylulose-5-phosphate by ribulose-5-phosphate epimerase. During the remaining reactions of the pathway (Figure 8.14b), transketolase and transaldolase catalyze the interconversions of trioses, pentoses, and hexoses. *Transketolase* is a TPP-requiring enzyme that transfers two-carbon units from a ketose to an aldose. (TPP, thiamine pyrophosphate, is the coenzyme form of thiamine, also known as vitamin

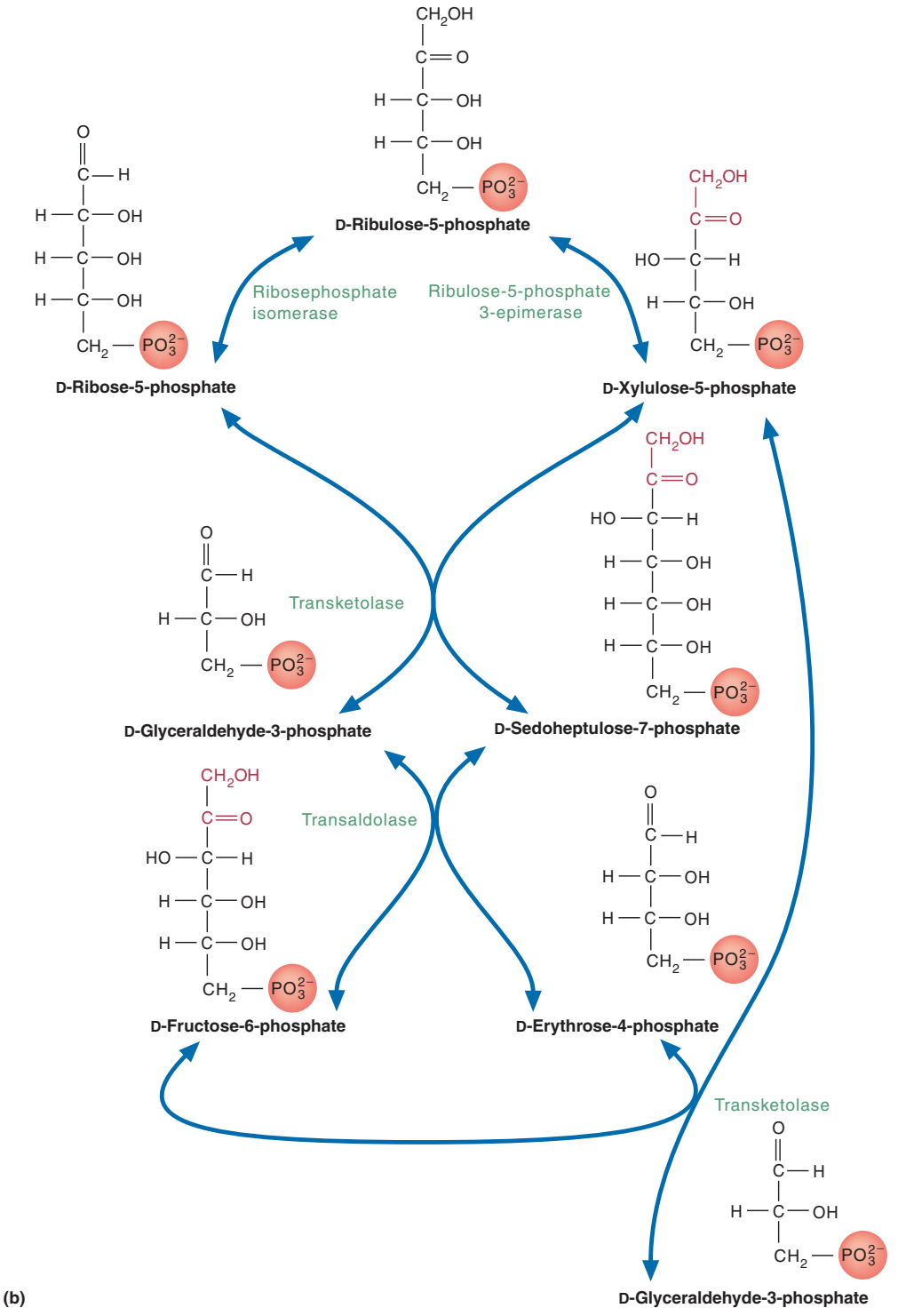


FIGURE 8.14b

The Pentose Phosphate Pathway

(b) The nonoxidative phase. When cells require more NADPH than pentose phosphates, the enzymes in the nonoxidative phase convert ribose-5-phosphate into the glycolytic intermediates fructose-6-phosphate and glyceraldehyde-3-phosphate.

B_1.) Transketolase catalyzes two reactions. In the first reaction, the enzyme transfers a two-carbon unit from xylulose-5-phosphate to ribose-5-phosphate, yielding glyceraldehyde-3-phosphate and sedoheptulose-7-phosphate. In the second transketolase-catalyzed reaction, a two-carbon unit from another xylulose-5-phosphate molecule is transferred to erythrose-4-phosphate to form a second molecule of glyceraldehyde-3-phosphate and fructose-6-phosphate. *Transaldolase* transfers three-carbon units from a ketose to an aldose. In the reaction catalyzed by transaldolase, a three-carbon unit is transferred from sedoheptulose-7-phosphate to glyceraldehyde-3-phosphate. The products formed are fructose-6-phosphate and erythrose-4-phosphate. The result of the nonoxidative phase of the pathway is the synthesis of ribose-5-phosphate and the glycolytic intermediates glyceraldehyde-3-phosphate and fructose-6-phosphate.

When pentose sugars are not required for biosynthetic reactions, the metabolites in the nonoxidative portion of the pathway are converted into glycolytic intermediates that can then be further degraded to generate energy or converted into precursor molecules for biosynthetic processes (Figure 8.15). For this

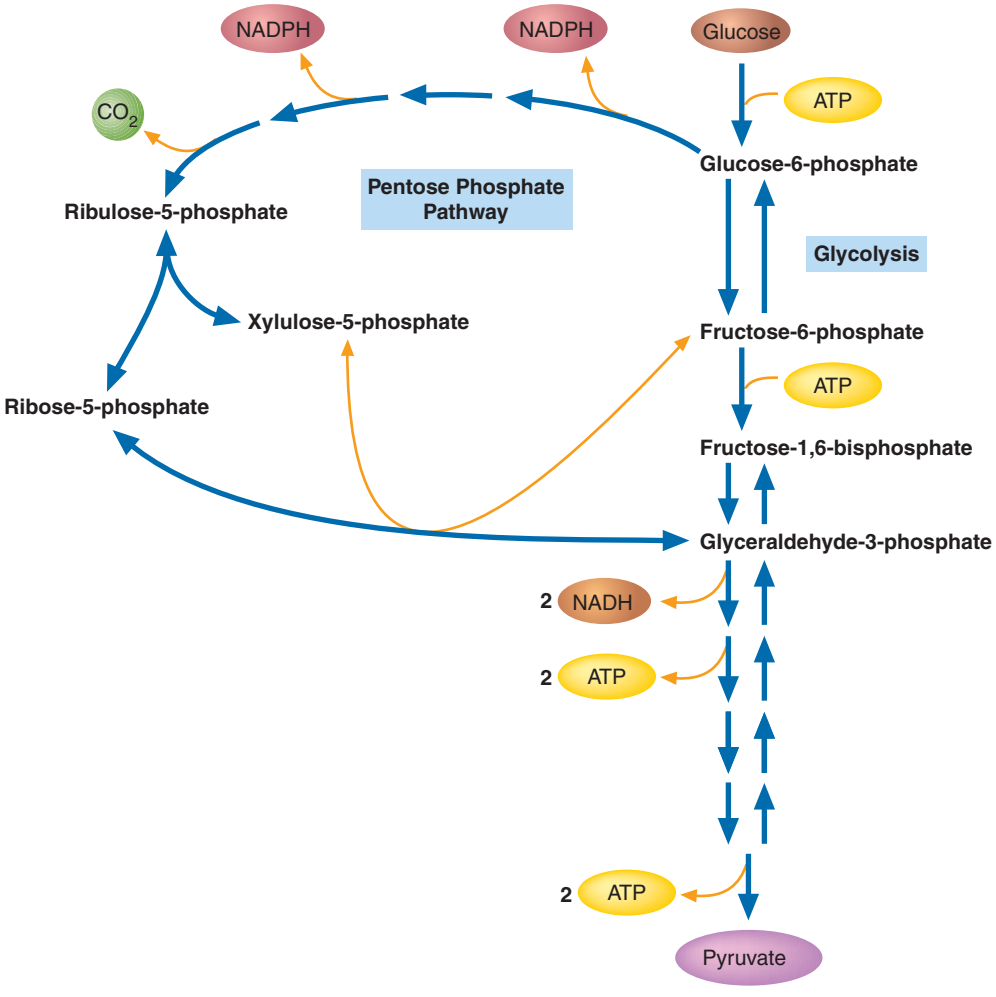


FIGURE 8.15

Carbohydrate Metabolism: Glycolysis and the Pentose Phosphate Pathway

If the cell requires more NADPH than ribose molecules, it can channel the products of the nonoxidative phase of the pentose phosphate pathway into glycolysis. As this overview of the two pathways illustrates, excess ribulose-5-phosphate can be converted into the glycolytic intermediates fructose-6-phosphate and glyceraldehyde-3-phosphate.

reason the pentose phosphate pathway is also referred to as the *hexose monophosphate shunt.* In plants, the pentose phosphate pathway is involved in the synthesis of glucose during the dark reactions of photosynthesis (Chapter 13).

The pentose phosphate pathway is regulated to meet the cell's moment-by-moment requirements for NADPH and ribose-5-phosphate. The oxidative phase is very active in cells such as red blood cells or hepatocytes in which demand for NADPH is high. In contrast, the oxidative phase is virtually absent in cells (e.g., muscle cells) that synthesize little or no lipid. (Lipid synthesis is a major consumer of NADPH.) G-6-PD catalyzes a key regulatory step in the pentose phosphate pathway. Its activity is inhibited by NADPH and stimulated by GSSG, the oxidized form of glutathione, an important cellular antioxidant (Chapter 10) and glucose-6-phosphate. In addition, diets high in carbohydrate increase the synthesis of both G-6-PD and phosphogluconate dehydrogenase.

KEY CONCEPT

The pentose phosphate pathway produces NADPH, ribose-5-phosphate, and the glycolytic intermediates fructose-6-phosphate and glyceraldehyde-3-phosphate.

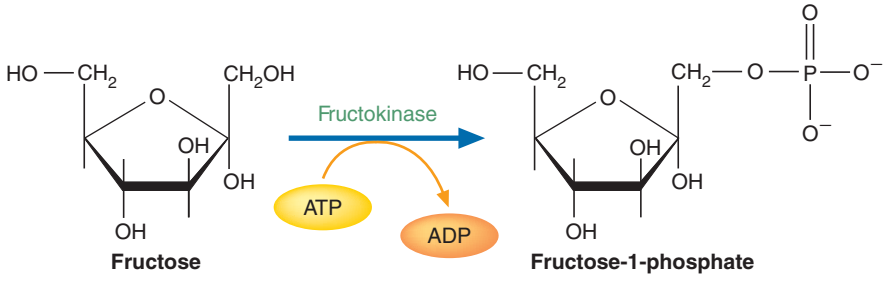
8.4 METABOLISM OF OTHER IMPORTANT SUGARS

Several sugars other than glucose are important in vertebrates. The most notable of these are fructose, galactose, and mannose. Besides glucose, these molecules are the most common sugars found in oligosaccharides and polysaccharides. They are also energy sources. The reactions by which these sugars are converted into glycolytic intermediates are illustrated in Figure 8.16. The metabolism of fructose, an important component of the human diet, is discussed.

Fructose Metabolism

Dietary sources of fructose include fruit, honey, sucrose, and high-fructose corn syrup, an inexpensive sweetener used in a wide variety of processed foods and beverages.

Fructose, second only to glucose as a source of carbohydrate in the modern human diet, can enter the glycolytic pathway by two routes. In the liver, fructose is converted to fructose-1-phosphate by fructokinase:
When fructose-1-phosphate enters the glycolytic pathway, it is first split into dihydroxyacetone phosphate (DHAP) and glyceraldehyde by fructose-1-phosphate



aldolase. DHAP is then converted to glyceraldehyde-3-phosphate by triose phosphate isomerase. Glyceraldehyde-3-phosphate is generated from glyceraldehyde and ATP by glyceraldehyde kinase.

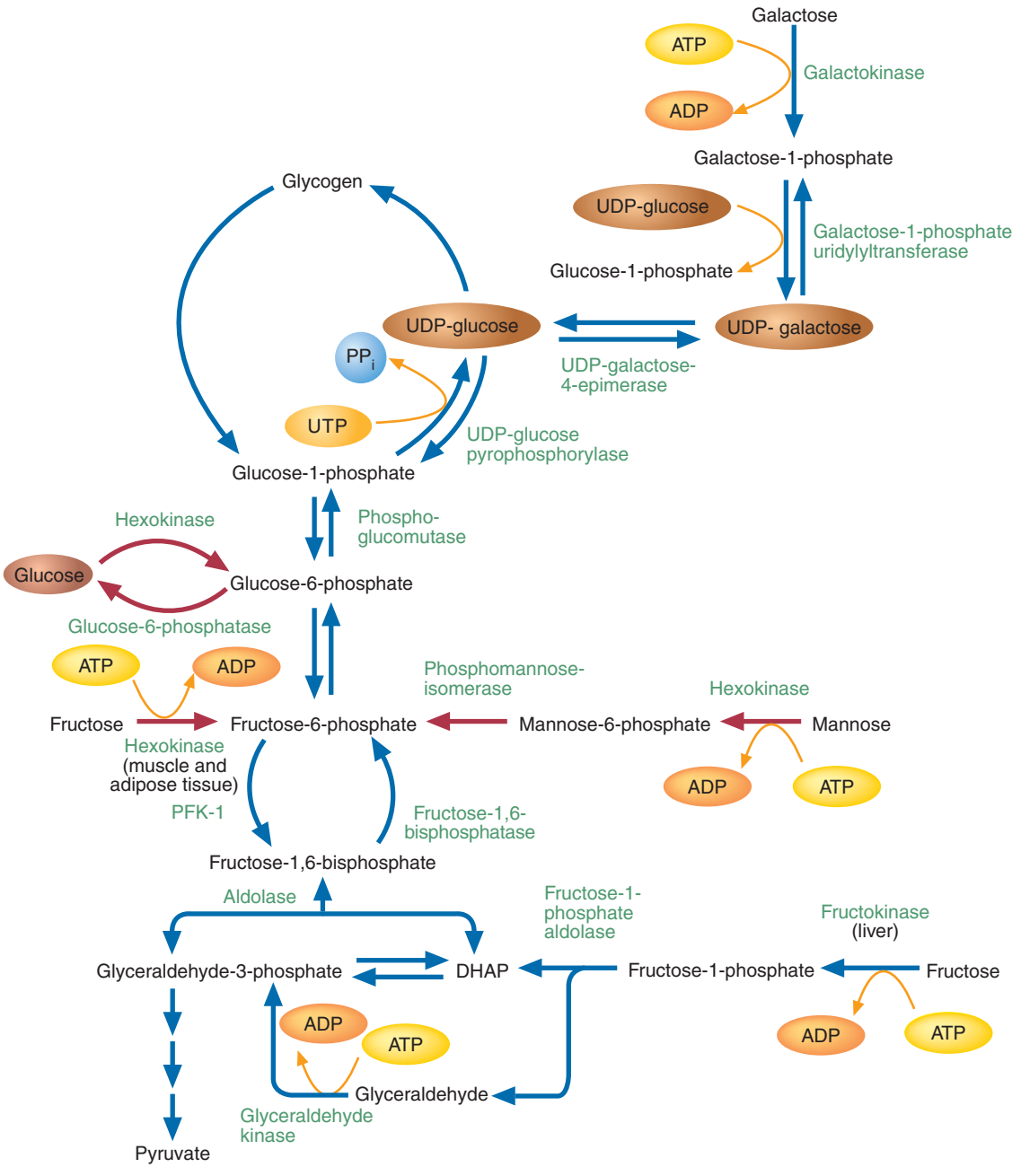
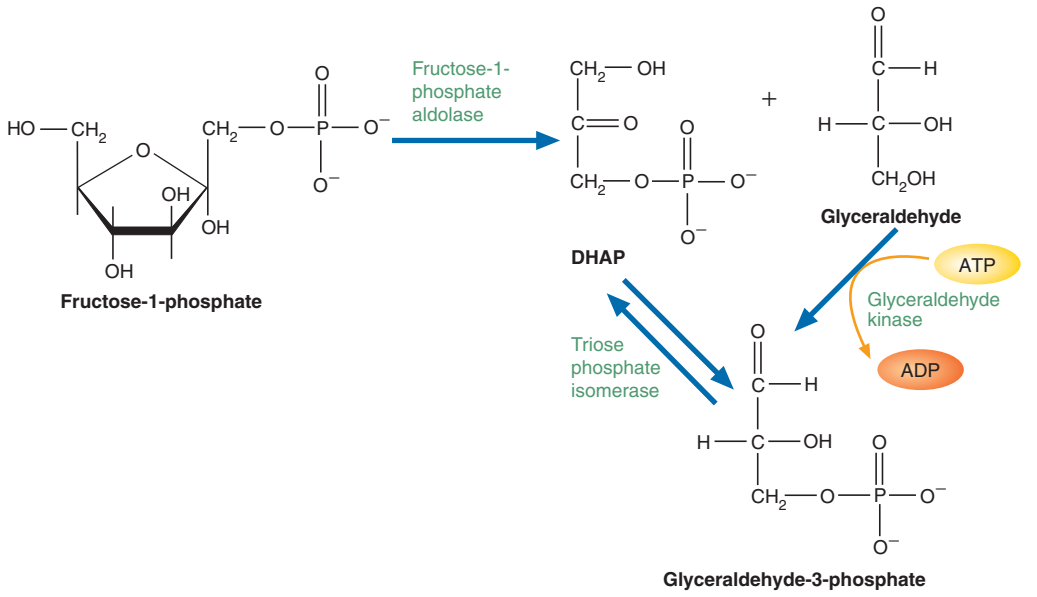


FIGURE 8.16

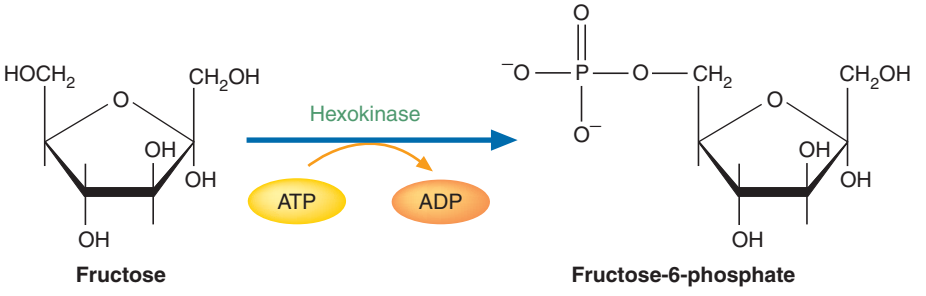
Carbohydrate Metabolism: Other Important Sugars

Fructose enters the glycolytic pathway by two routes. Fructokinase in liver cells converts fructose to fructose-1-phosphate that is then split into DHAP and glyceraldehyde. In muscle and adipose tissue, fructose is phosphorylated by hexokinase to form the glycolytic intermediate fructose-6-phosphate. Galactose is converted into galactose-1-phosphate, which then reacts with UDP-glucose to form UDP-galactose. UDP-galactose is converted to its epimer, UDP-glucose, the substrate for glycogen synthesis. Mannose is phosphorylated by hexokinase to form mannose-6-phosphate, which is then isomerized to fructose-6-phosphate.



The conversion of fructose-1-phosphate into glycolytic intermediates bypasses two regulatory steps (the reactions catalyzed by hexokinase and PFK-1); thus in comparison to glucose, the entrance of fructose into the glycolytic pathway is essentially unregulated.

In muscle and adipose tissue, fructose is converted to the glycolytic intermediate fructose-6-phosphate by hexokinase. Because the hexokinases have a low affinity for fructose, this reaction is of minor importance unless fructose consumption is exceptionally high.



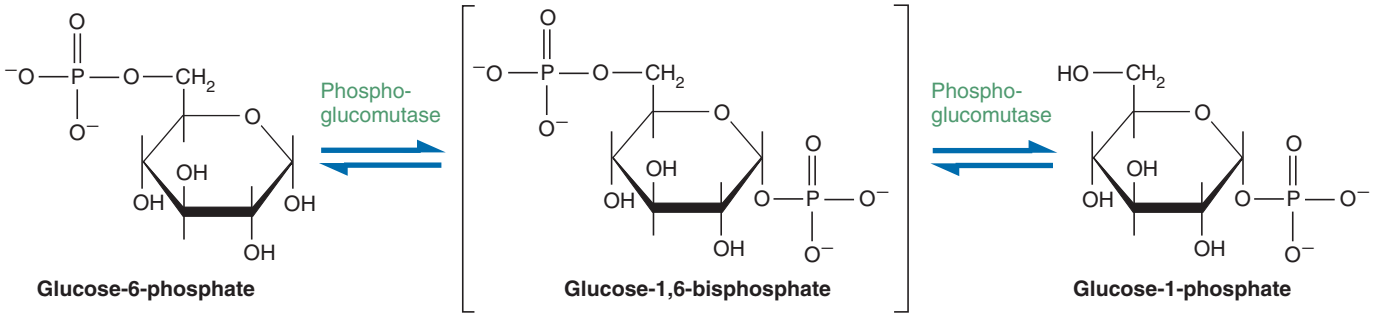
8.5 GLYCOGEN METABOLISM

Glycogen is the storage form of glucose. The synthesis and degradation of glycogen are carefully regulated so that sufficient glucose is available for the body's energy needs. Both glycogenesis and glycogenolysis are controlled primarily by three hormones: insulin, glucagon, and epinephrine.

Glycogenesis

Glycogen synthesis occurs after a meal, when blood glucose levels are high. It has long been recognized that the consumption of a carbohydrate meal is followed promptly by liver glycogenesis. The synthesis of glycogen from glucose-6-phosphate involves the following set of reactions.

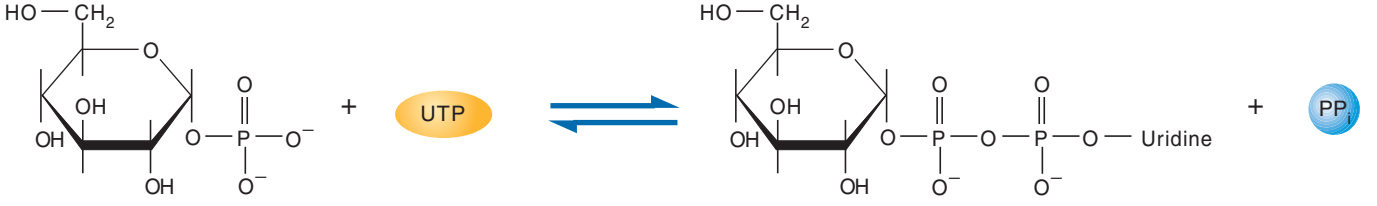
1. **Synthesis of glucose-1-phosphate**. Glucose-6-phosphate is reversibly converted to glucose-1-phosphate by phosphoglucomutase, an enzyme that contains a phosphoryl group attached to a reactive serine residue:



Glucose-6-phosphate **Glucose-1,6-bisphosphate** **Glucose-1-phosphate**

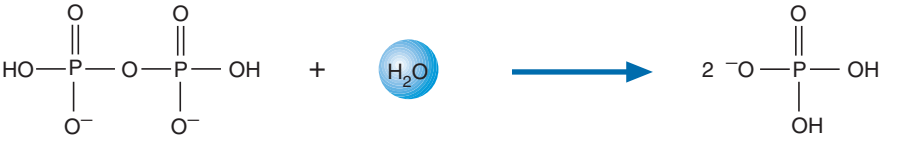
The enzyme's phosphoryl group is transferred to glucose-6-phosphate, forming glucose- 1,6-bisphosphate. As glucose-1-phosphate forms, the phosphoryl group attached to C-6 is transferred to the enzyme's serine residue.

2. **Synthesis of UDP-glucose**. Glycosidic bond formation is an endergonic process. Derivatizing the sugar with a good leaving group provides the driving force for most sugar transfer reactions. For this reason, sugar-nucleotide synthesis is a common reaction preceding sugar transfer and polymerization processes. Uridine diphosphate glucose (UDP-glucose) is more reactive than glucose and is held more securely in the active site of the enzymes catalyzing transfer reactions (referred to as a group as glycosyl transferases). Because UDP-glucose contains two phosphoryl bonds, it is a highly reactive molecule. Formation of UDP-glucose, whose $\Delta G^{\circ\prime}$ value is near zero, is a reversible reaction catalyzed by UDP-glucose pyrophosphorylase:



Glucose-1-phosphate **UDP-glucose**

However, the reaction is driven to completion because pyrophosphate (PP_i) is immediately and irreversibly hydrolyzed by pyrophosphatase with a large loss of free energy ($\Delta G^{\circ\prime} = -33.5$ kJ/mol):



PP_i **P_i**

(Recall that removing product shifts the reaction equilibrium to the right. This cellular strategy is common.)

3. **Synthesis of glycogen from UDP-glucose**. The formation of glycogen from UDP-glucose requires two enzymes: (a) glycogen synthase, which catalyzes the transfer of the glucosyl group of UDP-glucose to the nonreducing ends of glycogen (Figure 8.17a), and (b) amylo-α(1,4 → 1,6)-glucosyl transferase (branching enzyme), which creates the α(1,6) linkages for branches in the molecule (Figure 8.17b).

   Glycogen synthesis requires a preexisting tetrasaccharide composed of four α(1,4)-linked glucosyl residues. The first of these residues is linked to a specific tyrosine residue in a "primer" protein called *glycogenin.* The glycogen chain is then extended by glycogen synthase and branching enzyme. Large glycogen granules, each consisting of a single highly

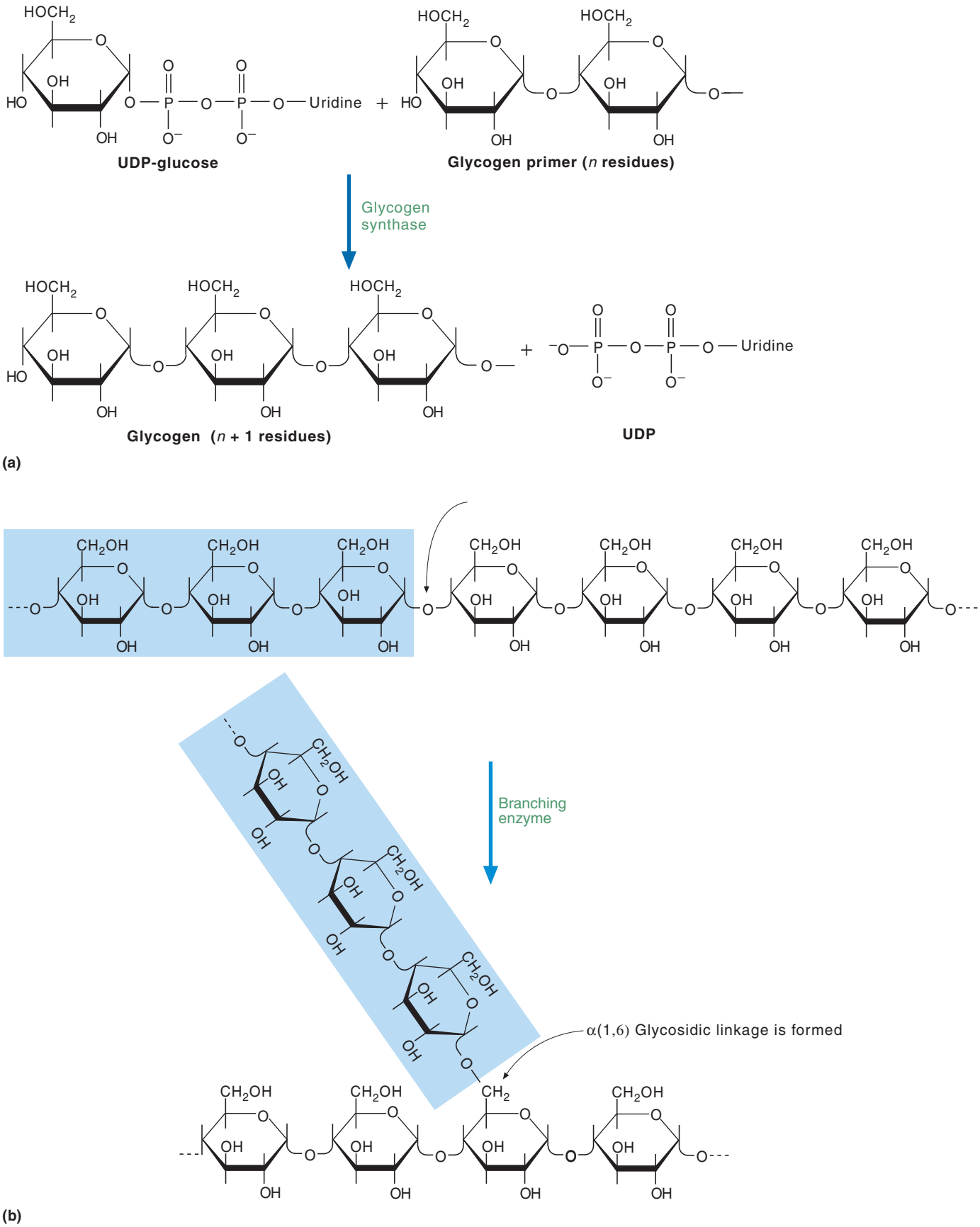


FIGURE 8.17

Glycogen Synthesis

(a) The enzyme glycogen synthase breaks the ester linkage of UDP-glucose and forms an $\alpha(1,4)$ glycosidic bond between glucose and the growing glycogen chain. (b) Branching enzyme is responsible for the synthesis of $\alpha(1,6)$ linkages in glycogen.

branched glycogen molecule, can be observed in the cytoplasm of liver and muscle cells of well-fed animals. The enzymes responsible for glycogen synthesis and degradation coat each granule's surface.

Glycogenolysis

Glycogen degradation requires the following two reactions.

1. **Removal of glucose from the nonreducing ends of glycogen**. Glycogen phosphorylase uses inorganic phosphate (P_i) to cleave the $\alpha(1,4)$ linkages on the outer branches of glycogen to yield glucose-1-phosphate. Glycogen phosphorylase stops when it comes within four glucose residues of a branch point (Figure 8.18). (A glycogen molecule that has been degraded to its branch points is called a *limit dextrin.*)
2. **Hydrolysis of the a(1,6) glycosidic bonds at branch points of glycogen.** Amylo-$\alpha(1,6)$-glucosidase, also called debranching enzyme, begins the removal of $\alpha(1,6)$ branch points by transferring the outer three of the four

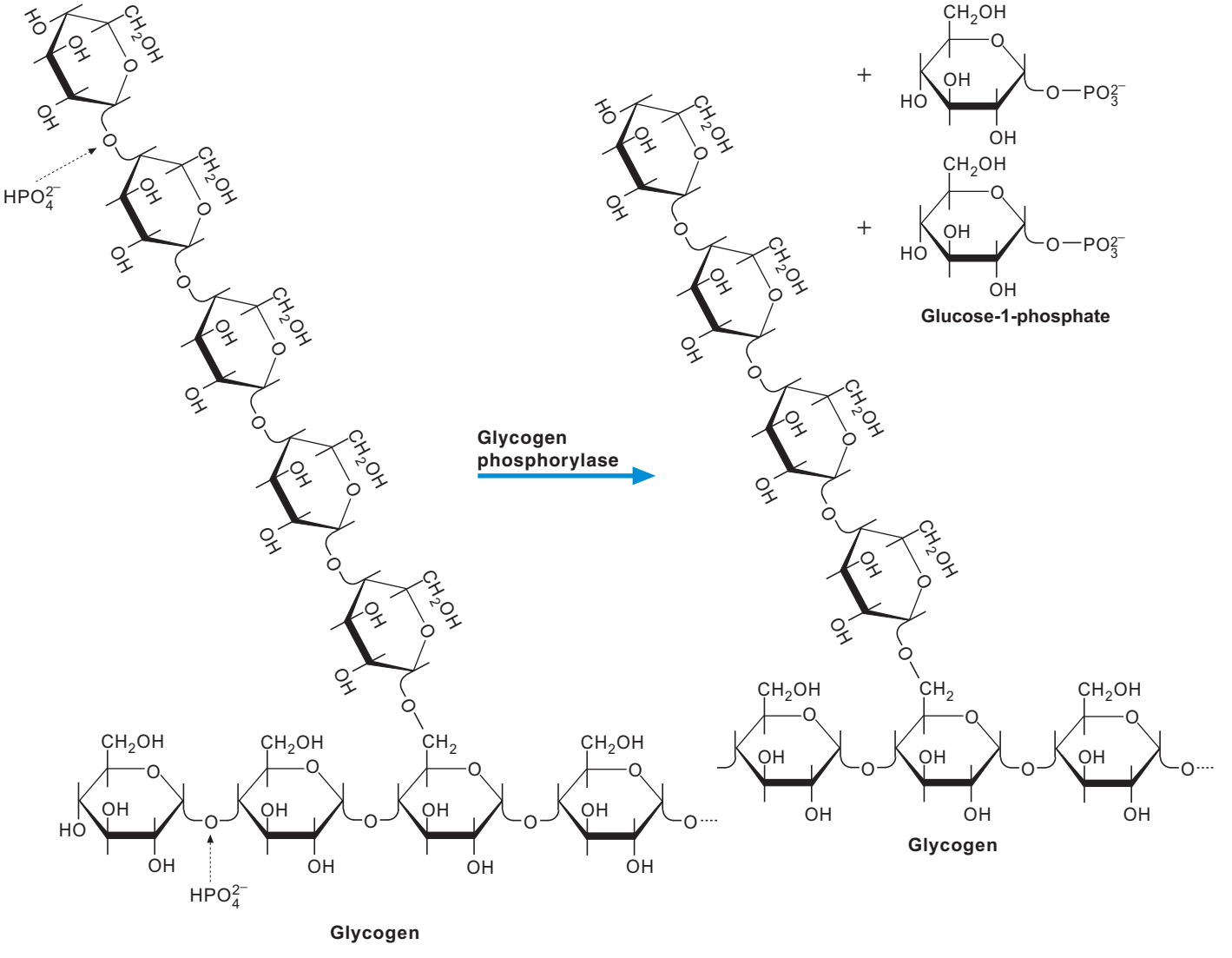


FIGURE 8.18

Glycogen Degradation

Glycogen phosphorylase catalyzes the removal of glucose residues from the nonreducing ends of a glycogen chain to yield glucose-1-phosphate. In this illustration one glucose residue is removed from each of two nonreducing ends. Removal of glucose residues continues until there are four residues at a branch point.

glucose residues attached to the branch point to a nearby nonreducing end. It then removes the single glucose residue attached at each branch point. The product of this latter reaction is free glucose (Figure 8.19).

Glucose-1-phosphate, the major product of glycogenolysis, is diverted to glycolysis in muscle cells to generate energy for muscle contraction. In hepatocytes, glucose-1-phosphate is converted to glucose, by phosphoglucomutase and glucose-6-phosphatase, which is then released into the blood. A summary of glycogenolysis is shown in Figure 8.20.

Regulation of Glycogen Metabolism

Glycogen metabolism is carefully regulated to avoid wasting energy. Both synthesis and degradation are controlled through a complex mechanism involving insulin, glucagon, and epinephrine, as well as allosteric regulators. Glucagon is released from the pancreas when blood glucose levels drop in the hours after a meal. It binds to receptors on hepatocytes and initiates a signal transduction process that elevates intracellular cAMP levels. cAMP amplifies the original glucagon signal and initiates a phosphorylation cascade that leads to the activation of glycogen phosphorylase along with a number of other proteins. Within seconds, glycogenolysis leads to the release of glucose into the bloodstream.

When occupied, the insulin receptor becomes an active tyrosine kinase enzyme that causes a phosphorylation cascade that ultimately has the opposite effect of the glucagon/cAMP system: the enzymes of glycogenolysis are inhibited and the enzymes of glycogenesis are activated. Insulin also increases the rate of glucose uptake into several types of target cells, but not liver or brain cells.

Emotional or physical stress releases the hormone *epinephrine* from the adrenal medulla. Epinephrine promotes glycogenolysis and inhibits glycogenesis. In emergency situations, when epinephrine is released in relatively large quantities, massive production of glucose provides the energy required to manage the situation. This effect is referred to as the flight-or-fight response. Epinephrine initiates the process by activating adenylate cyclase in liver and muscle cells. Calcium ions and inositol trisphosphate (Chapter 16) are also believed to be involved in epinephrine's action.

Glycogen synthase (GS) and glycogen phosphorylase have both active and inactive conformations that are interconverted by covalent modification. The active form of glycogen synthase, known as the I (independent) form, is converted to the inactive or D (dependent) form by phosphorylation. The activity of GS can be finely modulated in response to a range of signal intensities because it is inactivated by phosphorylation reactions catalyzed by a large number of kinases. Physiologically, the most important kinases are glycogen synthase kinase 3 (GSK3) and casein kinase 1 (CS1). In contrast to GS, the inactive form of glycogen phosphorylase (phosphorylase b) is converted to the active form (phosphorylase a) by the phosphorylation of a specific serine residue. The phosphorylating enzyme is called phosphorylase kinase. Phosphorylation of both glycogen synthase (inactivating) and phosphorylase kinase (activating) is catalyzed by PKA a protein kinase activated by cAMP. Glycogen synthesis occurs when glycogen synthase and glycogen phosphorylase have been dephosphorylated. This conversion is catalyzed by phosphoprotein phosphatase 1 (PP1), which also inactivates phosphorylase kinase. It is noteworthy that PP1 is linked to both glycogen synthase and glycogen phosphorylase by an anchor protein (p. xx) called PTG (protein targeting to glycogen). The effects of glucagon, insulin, and epinephine on glycogen metabolism are summarized in Figure 8.21.

Several allosteric regulators also regulate glycogen metabolism. In muscle cells, both calcium ions released during muscle contraction and AMP bind to sites on glycogen phosphorylase b and promote its conversion to phosphorylase a. The reverse process, the conversion of glycogen phosphorylase a to phosphorylase b, is promoted by high levels of ATP and glucose-6-phosphate. Glycogen synthase activity is stimulated by glucose-6-phosphate. In hepatocytes, glucose is an allosteric regulator that promotes the inhibition of glycogen phosphorylase!

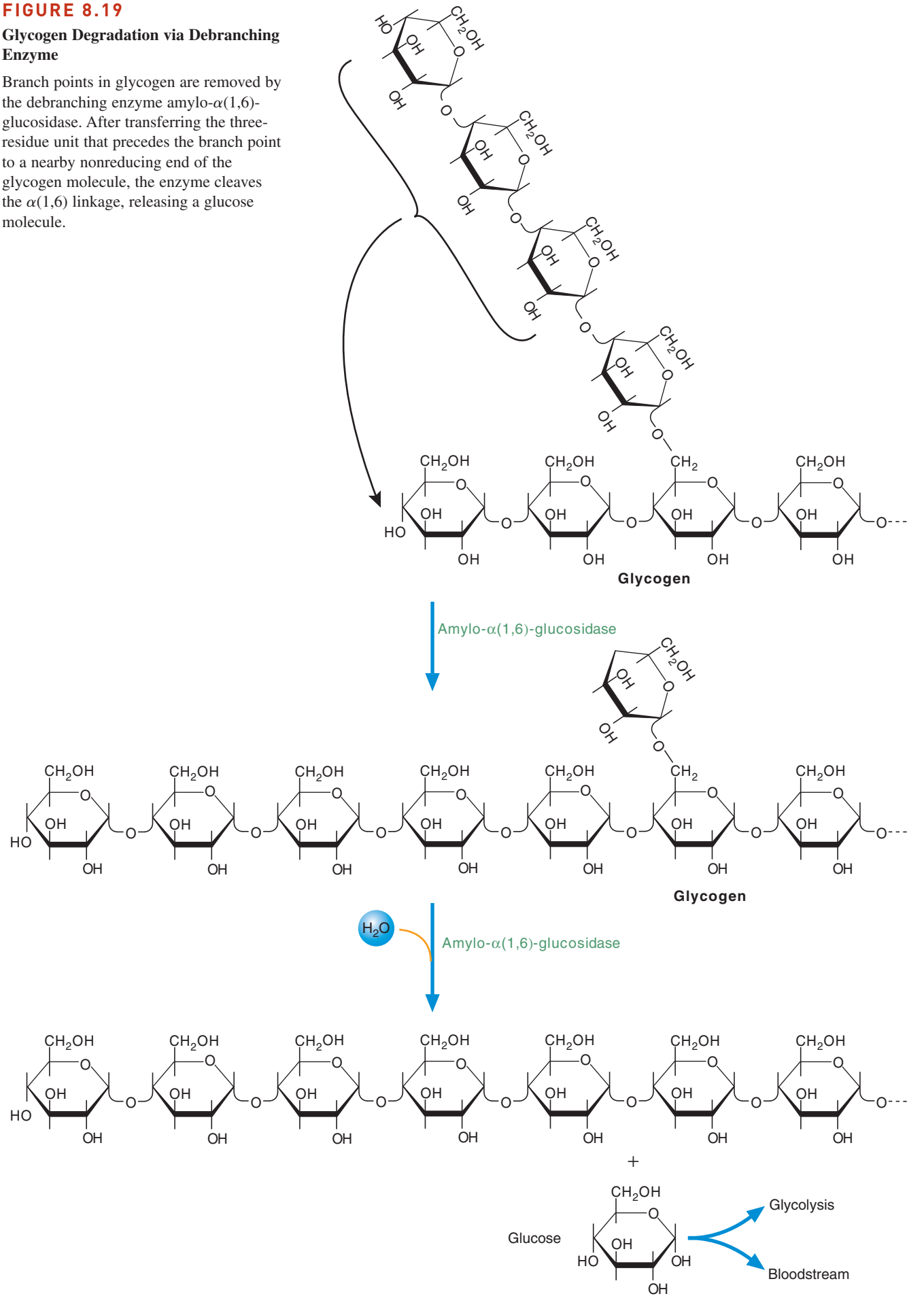
KEY CONCEPTS

- During glycogenesis, glycogen synthase catalyzes the transfer of the glucosyl group of UDP-glucose to the nonreducing ends of glycogen, and glycogen branching enzyme catalyzes the formation of branch points.
- Glycogenolysis requires glycogen phosphorylase and debranching enzyme. Glycogen metabolism is regulated by the actions of three hormones: glucagon, insulin, and epinephrine and several allosteric regulators.

FIGURE 8.19

Glycogen Degradation via Debranching Enzyme

Branch points in glycogen are removed by the debranching enzyme amylo-α(1,6)-glucosidase. After transferring the three-residue unit that precedes the branch point to a nearby nonreducing end of the glycogen molecule, the enzyme cleaves the α(1,6) linkage, releasing a glucose molecule.



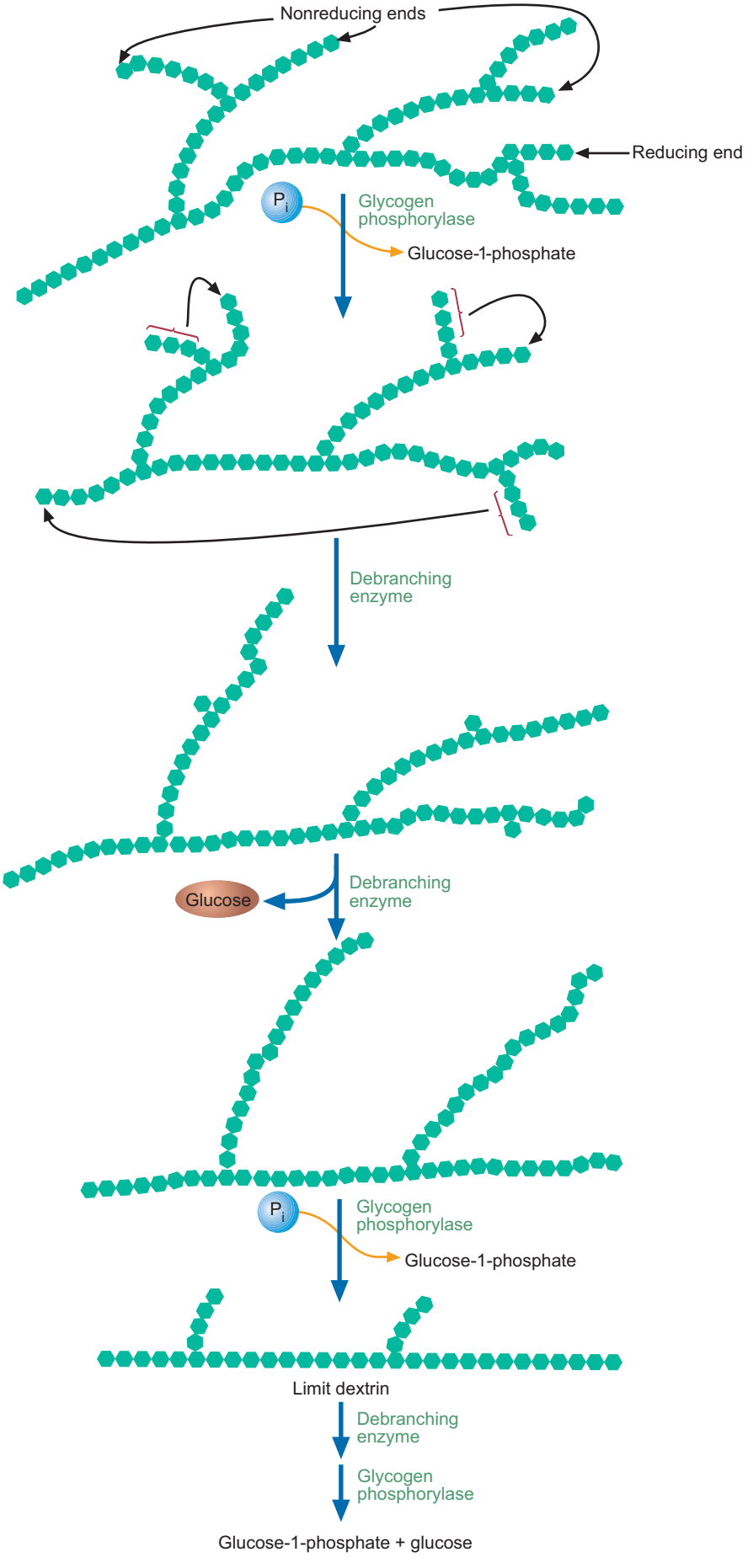


FIGURE 8.20

Glycogen Degradation: Summary

Glycogen phosphorylase cleaves the $\alpha(1,4)$ linkages of glycogen to yield glucose-1-phosphate until it comes within four glucose residues of a branch point. Debranching enzyme transfers three of these residues to a nearby nonreducing end and releases the fourth residue as free glucose. The repeated actions of both enzymes can lead to the complete degradation of glycogen.

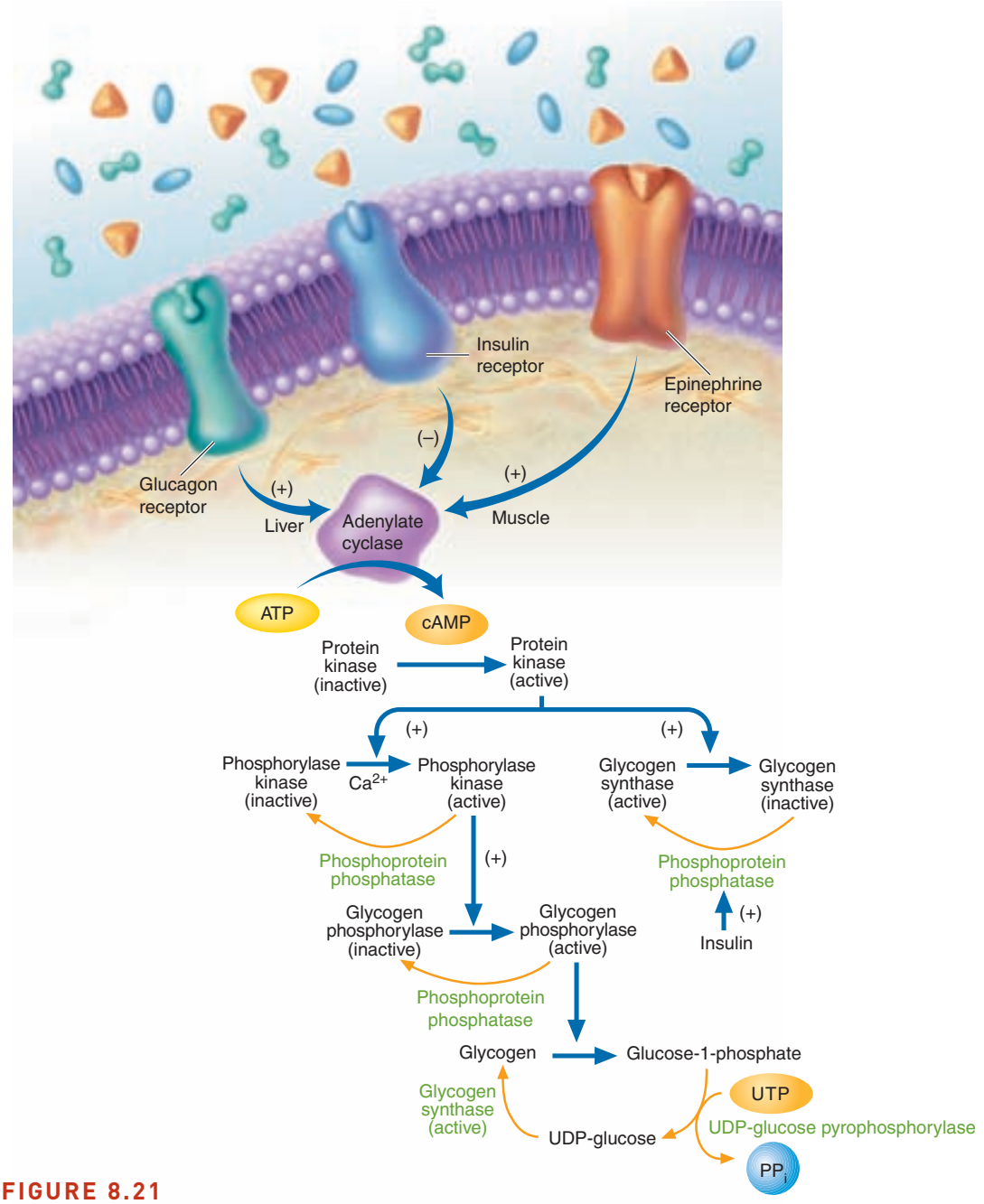


FIGURE 8.21

Major Factors Affecting Glycogen Metabolism

The binding of glucagon (released from the pancreas in response to low blood sugar) and/or epinephrine (released from the adrenal glands in response to stress) to their cognate receptors on the surface of target cells initiates a reaction cascade that converts glycogen to glucose-1-phosphate and inhibits glycogenesis. Insulin inhibits glycogenolysis and stimulates glycogenesis in part by decreasing the synthesis of cAMP and activating phosphoprotein phosphatase. Note that adenylate cyclase is a transmembrane protein with its functional domains protruding into the cytoplasm. In this illutration, for the sake of clarity, adenylate cyclase appears to be a cytoplasmic protien.

Hypoglycemia

QUESTION 8.7

Glycogen storage diseases are caused by inherited defects of one or more enzymes involved in glycogen synthesis or degradation. Patients with *Cori's disease*, caused by a deficiency of debranching enzyme, have enlarged livers (*hepatomegaly*) and low blood sugar concentrations (**hypoglycemia**). Can you suggest what causes these symptoms?

Chapter Summary

1. The metabolism of carbohydrates is dominated by glucose because this sugar is an important fuel molecule in most organisms. If cellular energy reserves are low, glucose is degraded by the glycolytic pathway. Glucose molecules that are not required for immediate energy production are stored as either glycogen (in animals) or starch (in plants).
2. During glycolysis, glucose is phosphorylated and cleaved to form two molecules of glyceraldehyde-3-phosphate. Each glyceraldehyde-3-phosphate is then converted to a molecule of pyruvate. A small amount of energy is captured in two molecules each of ATP and NADH. In anaerobic organisms, pyruvate is converted to waste products. During this process, NAD^+ is regenerated so that glycolysis can continue. In the presence of O_2, aerobic organisms convert pyruvate to acetyl-CoA and then to CO_2 and H_2O. Glycolysis is controlled primarily by allosteric regulation of three enzymes—hexokinase, PFK-1, and pyruvate kinase—and by the hormones glucagon and insulin.
3. During gluconeogenesis, molecules of glucose are synthesized from noncarbohydrate precursors (lactate, pyruvate, glycerol, and certain amino acids). The reaction sequence in gluconeogenesis is largely the reverse of glycolysis. The three irreversible glycolytic reactions (the synthesis of pyruvate, the conversion of fructose-1,6-bisphosphate to fructose-6-phosphate, and the formation of glucose from glucose-6-phosphate) are bypassed by alternate energetically favorable reactions.
4. The pentose phosphate pathway, in which glucose-6-phosphate is oxidized, occurs in two phases. In the oxidative phase, two molecules of NADPH are produced as glucose-6-phosphate is converted to ribulose-5-phosphate. In the nonoxidative phase, ribose-5-phosphate and other sugars are synthesized. If cells need more NADPH than ribose-5-phosphate, a component of nucleotides and the nucleic acids, then metabolites of the nonoxidative phase are converted into glycolytic intermediates.
5. Several sugars other than glucose are important in vertebrate carbohydrate metabolism. These include fructose, galactose, and mannose.
6. The substrate for glycogen synthesis is UDP-glucose, an activated form of the sugar. UDP-glucose pyrophosphorylase catalyzes the formation of UDP-glucose from glucose-1-phosphate and UTP. Glucose-6-phosphate is converted to glucose-1-phosphate by phosphoglucomutase. Glycogen synthesis requires two enzymes: glycogen synthase and branching enzyme. Glycogen degradation requires glycogen phosphorylase and debranching enzyme. The balance between glycogenesis (glycogen synthesis) and glycogenolysis (glycogen breakdown) is carefully regulated by several hormones (insulin, glucagon, and epinephrine) and allosteric regulators.

Suggested Readings

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Key Words

aerobic respiration, *xxx*
aldol cleavage, *xxx*
amphibolic pathway, *xxx*
anaerobic organisms, *xxx*
antioxidant, *xxx*
citric acid cycle, *xxx*
Cori cycle, *xxx*
Crabtree effect, *xxx*
decarboxylation, *xxx*
electron transport system, *xxx*
epinephrine, *xxx*
fermentation, *xxx*
glucagon, *xxx*
gluconeogenesis, *xxx*
glucose-alanine cycle, *xxx*
glycogenesis, *xxx*
glycogenolysis, *xxx*
glycolysis, *xxx*
hypoglycemia, *xxx*
insulin, *xxx*
malate shuttle, *xxx*
Pasteur effect, *xxx*
pentose phosphate pathway, *xxx*
response element, *xxx*
substrate-level phosphorylation, *xxx*
tautomer, *xxx*
tautomerization, *xxx*
transcription factor, *xxx*

Review Questions

These questions are designed to test your knowledge of the key concepts discussed in this chapter, before moving on to the next chapter. You may like to compare your answers to the solutions provided in the back of the book and in the accompanying Study Guide.

1. Define the following terms:
   a. glycolysis
   b. pentose phosphate pathway
   c. gluconeogenesis
   d. glycogenolysis
   e. glycogenesis
2. Define the following terms:
   a. anaerobic organism
   b. aerobic organism
   c. aerobic respiration
   d. aldol cleavage
   e. substrate-level phosphorylation
3. Define the following terms:
   a. tautomerization
   b. tautomer
   c. amphibolic pathway
   d. electron transport system
   e. decarboxylation reaction
4. Define the following terms:
   a. Crabtree effect
   b. transcription factor
   c. response element
   d. insulin
   e. malate shuttle
5. Define the following terms:
   a. Cori cycle
   b. glucagon
   c. glucose-alanine cycle
   d. hypoglycemia
   e. antioxidant
6. Upon entering a cell, glucose is phosphorylated. Give two reasons why this reaction is required.
7. Describe the functions of the following molecules:
   a. insulin
   b. glucagon
   c. fructose-2,6-bisphosphate
   d. UDP-glucose
   e. cAMP
   f. GSSG
   g. NADPH
8. Designate the reactions catalyzed by the following enzymes:
   a. Aldolase
   b. Enolase
   c. Hexokinase
   d. Amylo-α(1,6)-glucosidase
   e. Phosphoglucomutase
9. Why is "turbo design" used in catabolic pathways in living organisms?
10. Identify in which reactions in glycolysis the following functions occur.
    a. ATP consumption
    b. ATP synthesis
    c. NADH synthesis
11. Name the glycolytic enzymes that are allosterically regulated.
12. Name the three unique reactions in gluconeogenesis.
13. Explain and contrast the Pasteur and Crabtree effects.
14. Explain the role of glycogenin in glycogen synthesis.
15. List the three principal hormones that regulate glucose metabolism. Briefly explain the effects these molecules have on carbohydrate metabolism.
16. In which locations in the eukaryotic cell do the following processes occur?
    a. gluconeogenesis
    b. glycolysis
    c. pentose phosphate pathway
17. Compare the entry-level substrates, products, and metabolic purposes of glycolysis and gluconeogenesis.
18. Define substrate-level phosphorylation. Which two reactions in glycolysis are in this category?
19. What is the principal reason that organisms such as yeast produce alcohol?
20. Why is pyruvate not oxidized to CO_2 and H_2O under anaerobic conditions?
21. Describe how epinephrine promotes the conversion of glycogen to glucose.
22. Glycolysis occurs in two stages. Describe what is accomplished in each stage.
23. What effects do the following molecules have on gluconeogenesis?
    a. lactate
    b. ATP
    c. pyruvate
    d. glycerol
    e. AMP
    f. acetyl-CoA
24. Describe the physiological conditions that activate gluconeogenesis.
25. The following two reactions constitute a wasteful cycle:

$$\text{Glucose} + \text{ATP} \rightarrow \text{glucose-6-phosphate}$$
$$\text{Glucose-6-phosphate} + H_2O \rightarrow \text{glucose} + P_i$$

    Suggest how such wasteful cycles are prevented or controlled.
26. Describe the central role of glucose in carbohydrate metabolism.
27. Draw the structure of glucose with the carbons numbered 1 through 6. Number these carbons again as they appear in the two molecules of pyruvate formed during glycolysis.
28. Draw the reactions that convert glucose into ethanol.
29. After reviewing Figure 8.16, draw the reactions that convert galactose into a glycolytic intermediate. Include the Haworth formulas in your work.
30. Draw the reactions that convert fructose into glycolytic intermediates.
31. Why is severe hypoglycemia so dangerous?

32. Explain why ATP hydrolysis occurs so early in glycolysis, an ATP-producing pathway.
33. In which reaction in glycolysis does a dehydration occur?
34. Describe the Cori cycle. What is its physiological function?
35. Describe the effects of insulin and glucagon on glycogen metabolism.
36. Describe the effects of insulin and glucagon on blood glucose.
37. What cells produce insulin, glucagon, epinephrine, and cortisol?
38. Describe the different functions of glycogen in liver and muscle.
39. Describe the fate of pyruvate under anaerobic and aerobic conditions.

Thought Questions

These questions are designed to reinforce your understanding of all of the key concepts discussed in the book so far, including this chapter and all of the chapters before it. They may not have one right answer! The authors have provided possible solutions to these questions in the back of the book and in the accompanying Study Guide, for your reference.

40. In the first stage of glycolysis, fructose-1,6-bisphosphate is cleaved to form glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. The latter molecule can then be converted to glyceraldehyde-3-phosphate. Illustrate the mechanisms whereby these reactions occur.
41. After a carbohydrate-rich meal is consumed and the glucose requirements of all tissues have been met, the liver begins to store excess glucose in glycogen molecules. Explain the role of the hexokinases in this phenomenon.
42. Glucokinase acts as a glucose sensor in hepatocytes (liver cells), α- and β-cells in the pancreas, enterocytes (intestinal wall cells), and the hypothalamus (a control center in the brain of numerous physiological processes). Explain why glucokinase can perform this role.
43. An individual has a genetic deficiency that prevents the production of glucokinase. Following a carbohydrate meal, do you expect blood glucose levels to be high, low, or about normal? What organ accumulates glycogen under these circumstances?
44. Glycogen synthesis requires a short primer chain. Explain how new glycogen molecules are synthesized given this limitation.
45. Why is fructose metabolized more rapidly than glucose?
46. What is the difference between an enol-phosphate ester and a normal phosphate ester that gives PEP such a high phosphoryl group transfer potential?
47. In aerobic oxidation, oxygen is the ultimate oxidizing agent (electron acceptor). Name two common oxidizing agents in anaerobic fermentation.
48. Why is it important that gluconeogenesis is not the exact reverse of glycolysis?
49. Compare the structural formulas of ethanol, acetate, and acetaldehyde. Which molecule is the most oxidized? Which is the most reduced? Explain your answers.
50. *Trypanosoma brucei* is a parasitic protozoan that causes sleeping sickness in humans, Transmitted by the tsetse fly, sleeping sickness is a fatal disease characterized by fever, anemia, inflammation, lethargy, headache, and convulsions. When trypanosomes are present in the human bloodstream, they depend on glycolysis entirely for energy generation. The first seven glycolytic enzymes in these organisms are localized in peroxisome-like organelles called glycosomes, which are only regulated weakly by allosteric regulator molecules. Glycosomes take up glucose and export glycerate-3-phosphate. There are two pools of ADP and ATP (cytoplasmic and glycosomal), and the glycosomal membrane is impermeable to both nucleotides as well as most other glycolytic intermediates. If the glycosomal membrane is compromised, the concentration of phosphoylated glycolytic intermediates rises and the cells die. Explain.
51. The consumption of large amounts of soft drink beverages and processed foods sweetened with high-fructose corn syrup has been linked to obesity. After reviewing Figures 8.1 and 8.15, suggest a likely reason for this phenomenon.
52. How does phosphorylation increase the reactivity of glucose?
53. Examine the structure of phosphoenolpyruvate and explain why it has such a high phosphoryl group transfer potential.
54. Both glycogen and triacylglycerols are energy sources used by the body. Suggest a reason why both are required.
55. Severe dieting results in both the reduction of fat stores and the loss of muscle mass. Use biochemical reactions to trace the conversion of muscle protein to glucose production.
56. Suggest a reason why glycolysis produces NADH and the pentose phosphate pathway produces NADPH.
57. Cells in culture are fed glucose molecules labeled with ^{14}C at carbon 2. Trace the radioactive label through one pass through the pentose phosphate pathway.
58. Ethanol is especially toxic in children for several reasons. For example, ethanol consumption results in elevated levels of NADH in the liver. Suggest a mechanism that explains this phenomenon.