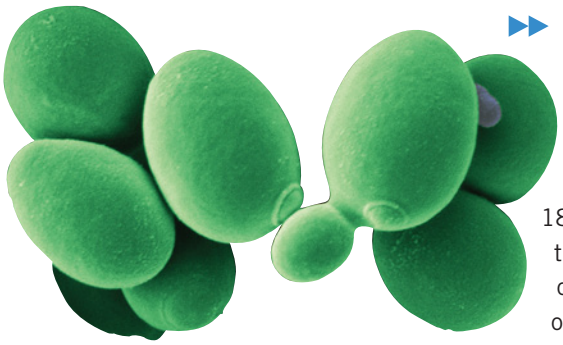
chapter 13

GLUCOSE METABOLISM



[SciMAT/Photo Researchers, Inc.]

▸▸ HOW do yeast transform sugars into other substances?

Yeast, such as the ones pictured here, have been used in brewing and baking for thousands of years. Until relatively recently, their ability to produce bubbles (CO_2 gas) and intoxicants (ethanol) was believed to be a unique property of living things that possessed a "vital force." However, in the mid-1800s, scientists began developing techniques for preparing cell extracts and then for isolating individual enzymes, and it became clear that the conversion of glucose to CO_2, ethanol, and other substances was the result of a series of enzyme-catalyzed chemical reactions. Modern biochemists, who continue to work with model organisms such as yeast, strive to describe each chemical process in detail, revealing a great deal about how yeast—and all organisms—carry out essential metabolic activities.

THIS CHAPTER IN CONTEXT

Do You Remember?

- **Enzymes accelerate chemical reactions using acid–base catalysis, covalent catalysis, and metal ion catalysis (Section 6-2).**
- **Glucose polymers include the fuel-storage polysaccharides starch and glycogen and the structural polysaccharide cellulose (Section 11-2).**
- **Coenzymes such as NAD^+ and ubiquinone collect electrons from compounds that become oxidized (Section 12-2).**
- **A reaction with a large negative change in free energy can be coupled to another unfavorable reaction (Section 12-3).**
- **A reaction that breaks a phosphoanhydride bond in ATP occurs with a large change in free energy (Section 12-3).**
- **Nonequilibrium reactions often serve as metabolic control points (Section 12-3).**

Glucose occupies a central position in the metabolism of most cells. It is a major source of metabolic energy (in some cells, it is the only source), and it provides the precursors for the synthesis of other biomolecules. Recall that glucose is stored in polymeric form as starch in plants and as glycogen in animals (Section 11-2). The breakdown of these polymers provides glucose monomers that can be catabolized to release energy. The conversion of the six-carbon glucose to the three-carbon pyruvate, a pathway we now call **glycolysis,** occurs in ten steps. As a result of many years of research, we know a great deal about the pathway's nine intermediates and the enzymes that mediate their chemical transformations. We have also learned that glycolysis, along with other metabolic pathways, exhibits the following properties:

1. Each step of the pathway is catalyzed by a distinct enzyme.
2. The free energy consumed or released in certain reactions is transferred by molecules such as ATP and NADH.
3. The rate of the pathway can be controlled by altering the activity of individual enzymes.

If metabolic processes did not occur via multiple enzyme-catalyzed steps, cells would have little control over the amount and type of reaction products and no way to manage free energy. For example, the combustion of glucose and O_2 to CO_2 and H_2O—if allowed to occur in one grand explosion—would release about 2850 kJ · mol^{-1} of free energy all at once. In the cell, *glucose combustion requires many steps so that the cell can recover its free energy in smaller, more useful quantities.*

In this chapter, we will examine the major metabolic pathways involving glucose. **Figure 13-1** shows how these pathways relate to the general metabolic scheme outlined in Figure 12-10. The highlighted pathways include the interconversion of the monosaccharide glucose with its polymeric form glycogen, the degradation of glucose to the three-carbon intermediate pyruvate (the glycolytic pathway), the synthesis of glucose from smaller compounds (**gluconeogenesis**), and the conversion of glucose to the five-carbon monosaccharide ribose. For all the pathways, we will present the intermediates and some of the relevant enzymes. We will also examine the thermodynamics of reactions that release or consume large amounts of free energy and discuss how some of these reactions are regulated.

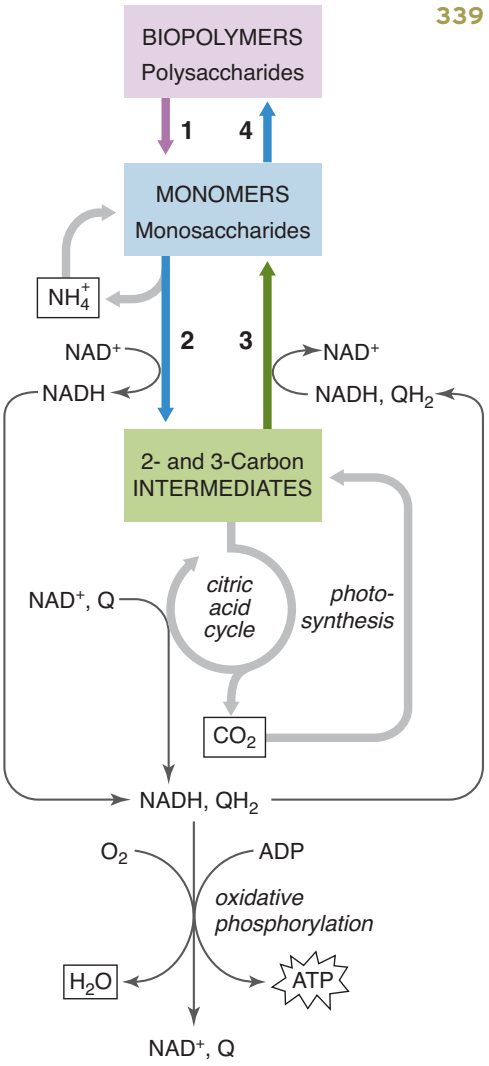


Figure 13-1 Glucose metabolism in context. (1) The polysaccharide glycogen is degraded to glucose, which is then catabolized by the glycolytic pathway (2) to the three-carbon intermediate pyruvate. Gluconeogenesis (3) is the pathway for the synthesis of glucose from smaller precursors. Glucose can then be reincorporated into glycogen (4). The conversion of glucose to ribose, a component of nucleotides, is not shown in this diagram.

13-1 Glycolysis

Glycolysis appears to be an ancient metabolic pathway. The fact that it does not require molecular oxygen suggests that it evolved before photosynthesis increased the level of atmospheric O_2. Overall, glycolysis is a series of 10 enzyme-catalyzed steps in which a six-carbon glucose molecule is broken down into two three-carbon pyruvate molecules. This catabolic pathway is accompanied by the phosphorylation of two molecules of ADP (to produce 2 ATP) and the reduction of two molecules of NAD^+. The net equation for the pathway (ignoring water and protons) is

$$\text{glucose} + 2\,NAD^+ + 2\,ADP + 2\,P_i \rightarrow 2\,\text{pyruvate} + 2\,NADH + 2\,ATP$$

It is convenient to divide the 10 reactions of glycolysis into two phases. In the first (Reactions 1–5), the hexose is phosphorylated and cleaved in half. In the second (Reactions 6–10), the three-carbon molecules are converted to pyruvate (Fig. 13-2).

KEY CONCEPTS

- Glycolysis is a 10-step pathway in which glucose is converted to two molecules of pyruvate.
- Energy is invested in the first half of the pathway, and the second half of the pathway generates 2 ATP and 2 NADH.
- Flux through the pathway is controlled primarily at the phosphofructokinase step.
- Pyruvate can be converted to lactate, acetyl-CoA, or oxaloacetate.

See Guided Exploration. Glycolysis overview.

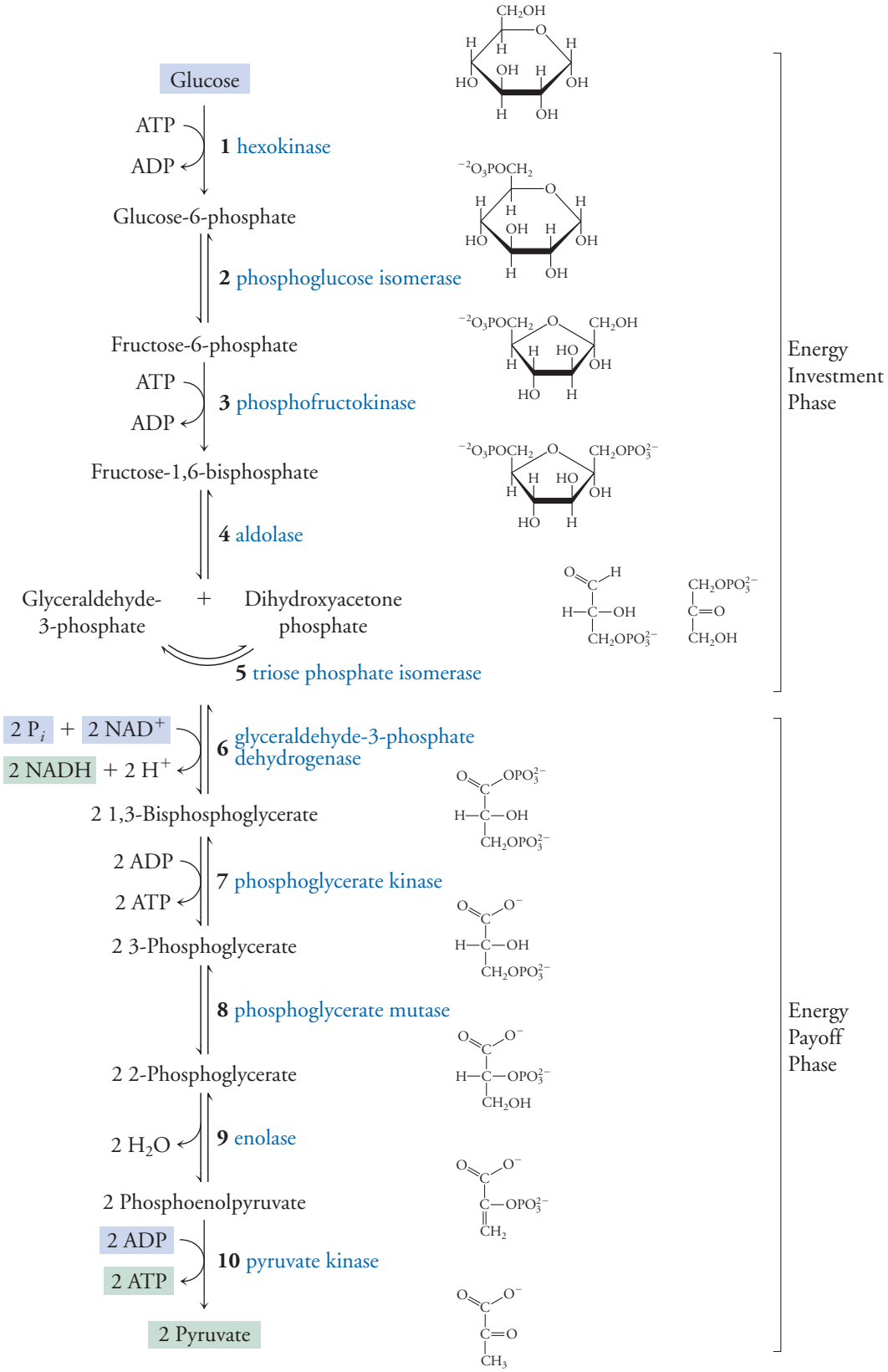


Figure 13-2 The reactions of glycolysis. The substrates, products, and enzymes corresponding to the 10 steps of the pathway are shown. Shading indicates the substrates (purple) and products (green) of the pathway as a whole. ⊕ **See Animated Figure. Overview of glycolysis.**

? Next to each reaction, write the term that describes the type of chemical change that occurs.

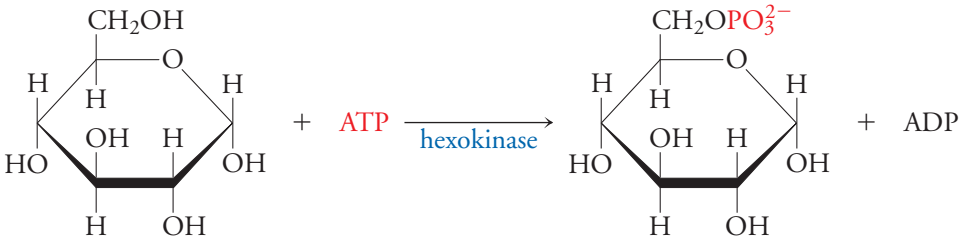
As you examine each of the reactions of glycolysis described in the following pages, note how the reaction substrates are converted to products by the action of an enzyme (and note how the enzyme's name often reveals its purpose). Pay attention also to the free energy change of each reaction.

Reactions 1–5 are the energy-investment phase of glycolysis

The first five reactions of glycolysis can be considered a preparatory phase for the second, energy-producing phase. In fact, the first phase requires the *investment* of free energy in the form of two ATP molecules.

1. Hexokinase

In the first step of glycolysis, the enzyme hexokinase transfers a phosphoryl group from ATP to the C6 OH group of glucose to form glucose-6-phosphate:

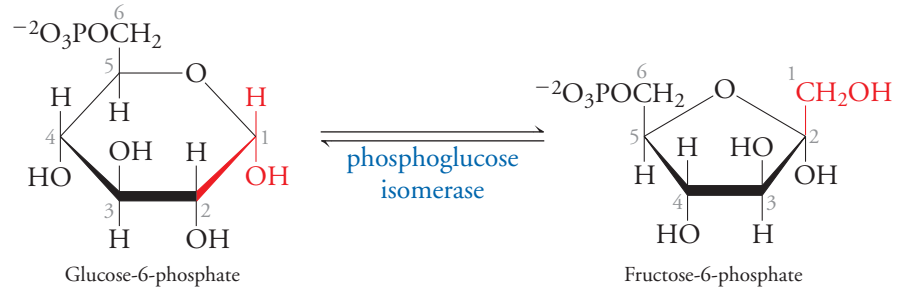


A **kinase** is an enzyme that transfers a phosphoryl group from ATP (or another nucleoside triphosphate) to another substance.

Recall from Section 6-3 that the hexokinase active site closes around its substrates so that a phosphoryl group is efficiently transferred from ATP to glucose. The standard free energy change for this reaction, which cleaves one of ATP's phosphoanhydride bonds, is $-16.7\ \text{kJ} \cdot \text{mol}^{-1}$ (ΔG, the actual free energy change for the reaction inside a cell, has a similar value). The magnitude of this free energy change means that the reaction proceeds in only one direction; the reverse reaction is extremely unlikely since its standard free energy change would be $+16.7\ \text{kJ} \cdot \text{mol}^{-1}$. Consequently, hexokinase is said to catalyze a **metabolically irreversible reaction** that prevents glucose from backing out of glycolysis. *Many metabolic pathways have a similar irreversible step near the start that commits a metabolite to proceed through the pathway.*

2. Phosphoglucose Isomerase

The second reaction of glycolysis is an isomerization reaction in which glucose-6-phosphate is converted to fructose-6-phosphate:



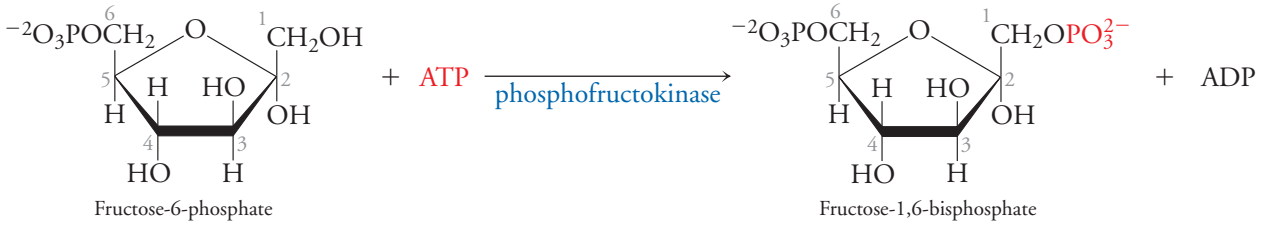
Because fructose is a six-carbon ketose (Section 11-1), it forms a five-membered ring.

The standard free energy change for the phosphoglucose isomerase reaction is $+2.2\ \text{kJ} \cdot \text{mol}^{-1}$, but the reactant concentrations *in vivo* yield a ΔG value of about $-1.4\ \text{kJ} \cdot \text{mol}^{-1}$. A value of ΔG near zero indicates that the reaction operates close to equilibrium (at equilibrium, $\Delta G = 0$). *Such* ***near-equilibrium*** *reactions are considered to be freely reversible, since a slight excess of products can easily drive the*

reaction in reverse by mass action effects. In a metabolically irreversible reaction, such as the hexokinase reaction, the concentration of product could never increase enough to compensate for the reaction's large value of ΔG.

3. Phosphofructokinase

The third reaction of glycolysis consumes a second ATP molecule in the phosphorylation of fructose-6-phosphate to yield fructose-1,6-bisphosphate.

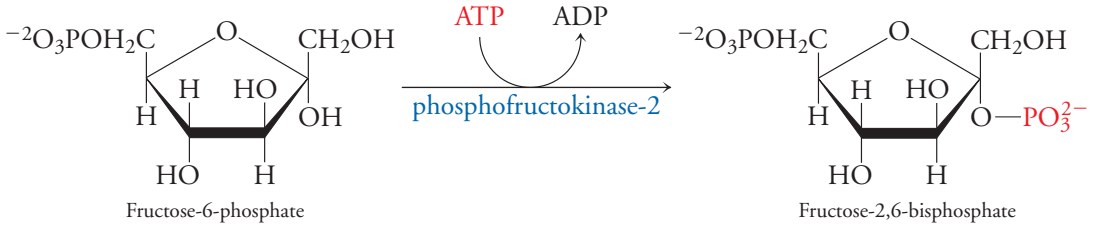


Fructose-6-phosphate — Fructose-1,6-bisphosphate

Phosphofructokinase operates in much the same way as hexokinase, and the reaction it catalyzes is irreversible, with a $\Delta G^{\circ\prime}$ value of -17.2 kJ · mol^{-1}.

In cells, the activity of phosphofructokinase is regulated. We have already seen how the activity of a bacterial phosphofructokinase responds to allosteric effectors (Section 7-3). ADP binds to the enzyme and causes a conformational change that promotes fructose-6-phosphate binding, which in turn promotes catalysis. This mechanism is useful because the concentration of ADP in the cell is a good indicator of the need for ATP, which is a product of glycolysis. Phosphoenolpyruvate, the product of step 9 of glycolysis, binds to bacterial phosphofructokinase and causes it to assume a conformation that destabilizes fructose-6-phosphate binding, thereby diminishing catalytic activity. Thus, when the glycolytic pathway is producing plenty of phosphoenolpyruvate and ATP, the phosphoenolpyruvate can act as a feedback inhibitor to slow the pathway by decreasing the rate of the reaction catalyzed by phosphofructokinase **(Fig. 13-3a)**.

The most potent activator of phosphofructokinase in mammals, however, is the compound fructose-2,6-bisphosphate, which is synthesized from fructose-6-phosphate by an enzyme known as phosphofructokinase-2. (The glycolytic enzyme is therefore sometimes called phosphofructokinase-1).



Fructose-6-phosphate — Fructose-2,6-bisphosphate

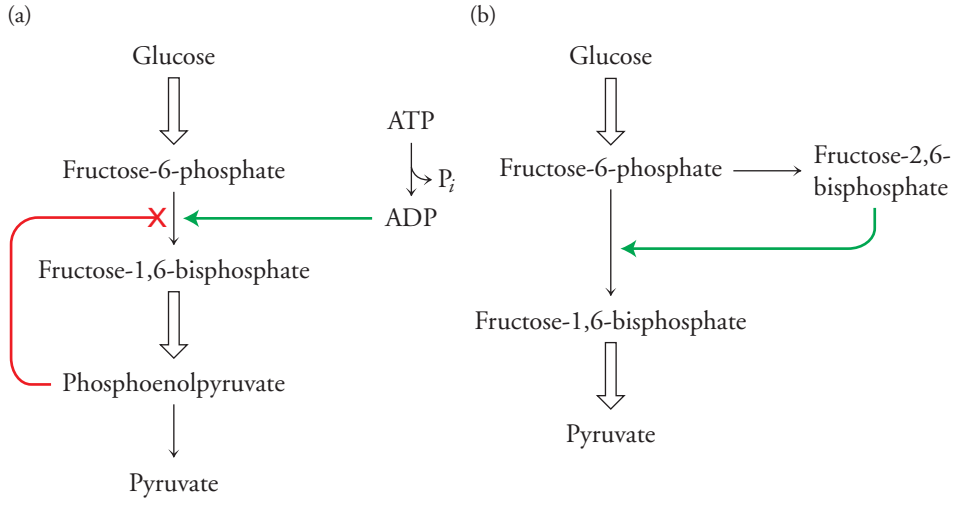


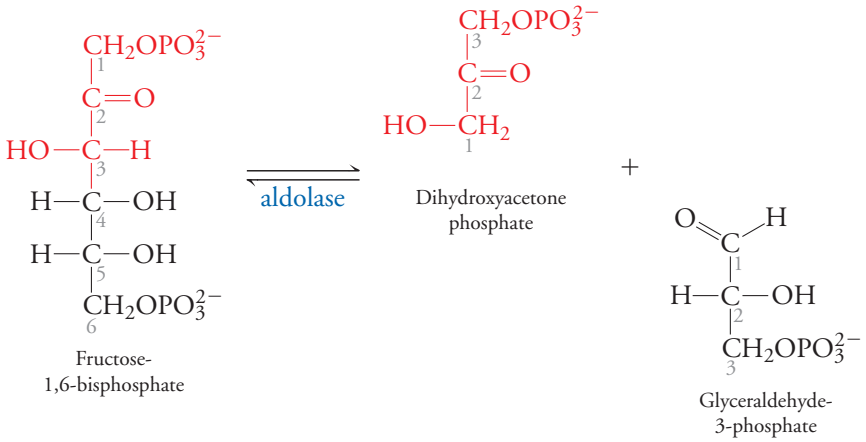
Figure 13-3 Regulation of phosphofructokinase. (a) Regulation in bacteria. ADP, produced when ATP is consumed elsewhere in the cell, stimulates the activity of phosphofructokinase (green arrow). Phosphoenolpyruvate, a late intermediate of glycolysis, inhibits phosphofructokinase (red symbol), thereby decreasing the rate of the entire pathway. (b) Regulation in mammals.

The activity of phosphofructokinase-2 is hormonally stimulated when the concentration of glucose in the blood is high. The resulting increase in fructose-2,6-bisphosphate concentration activates phosphofructokinase to increase the flux of glucose through the glycolytic pathway (Fig. 13-3b).

The phosphofructokinase reaction is the primary control point for glycolysis. It is the slowest reaction of glycolysis, so the rate of this reaction largely determines the flux (rate of flow) of glucose through the entire pathway. In general, a **rate-determining reaction**—such as the phosphofructokinase reaction—operates far from equilibrium; that is, it has a large negative free energy change and is irreversible under metabolic conditions. The rate of the reaction can be altered by allosteric effectors but not by fluctuations in the concentrations of its substrates or products. Thus, it acts as a one-way valve. In contrast, a near-equilibrium reaction—such as the phosphoglucose isomerase reaction—cannot serve as a rate-determining step for a pathway because it can respond to small changes in reactant concentrations by operating in reverse.

4. Aldolase

Reaction 4 converts the hexose fructose-1,6-bisphosphate to two three-carbon molecules, each of which bears a phosphate group.

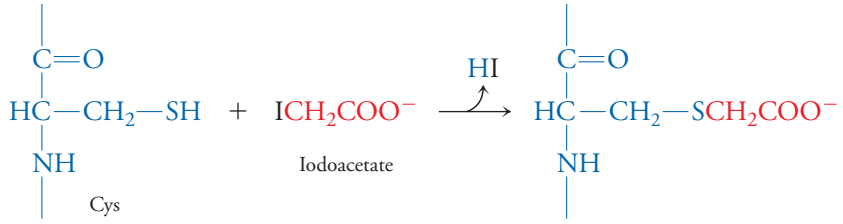


Fructose-1,6-bisphosphate

Glyceraldehyde-3-phosphate

This reaction is the reverse of an aldol (aldehyde–alcohol) condensation, so the enzyme that catalyzes the reaction is called aldolase. It is worth examining its mechanism. The active site of mammalian aldolase contains two catalytically important residues: a Lys residue that forms a Schiff base (imine) with the substrate and an ionized Tyr residue that acts as a base catalyst **(Fig. 13-4)**.

Early studies of aldolase implicated a Cys residue in catalysis because iodoacetate, a reagent that reacts with the Cys side chain, also inactivates the enzyme:



Cys

Iodoacetate

Researchers used iodoacetate to help identify the intermediates of glycolysis: In the presence of iodoacetate, fructose-1,6-bisphosphate accumulates because the next step is blocked. Acetylation of the Cys residue, which turned out not to be part of the active site, probably interferes with a conformational change that is necessary for aldolase activity.

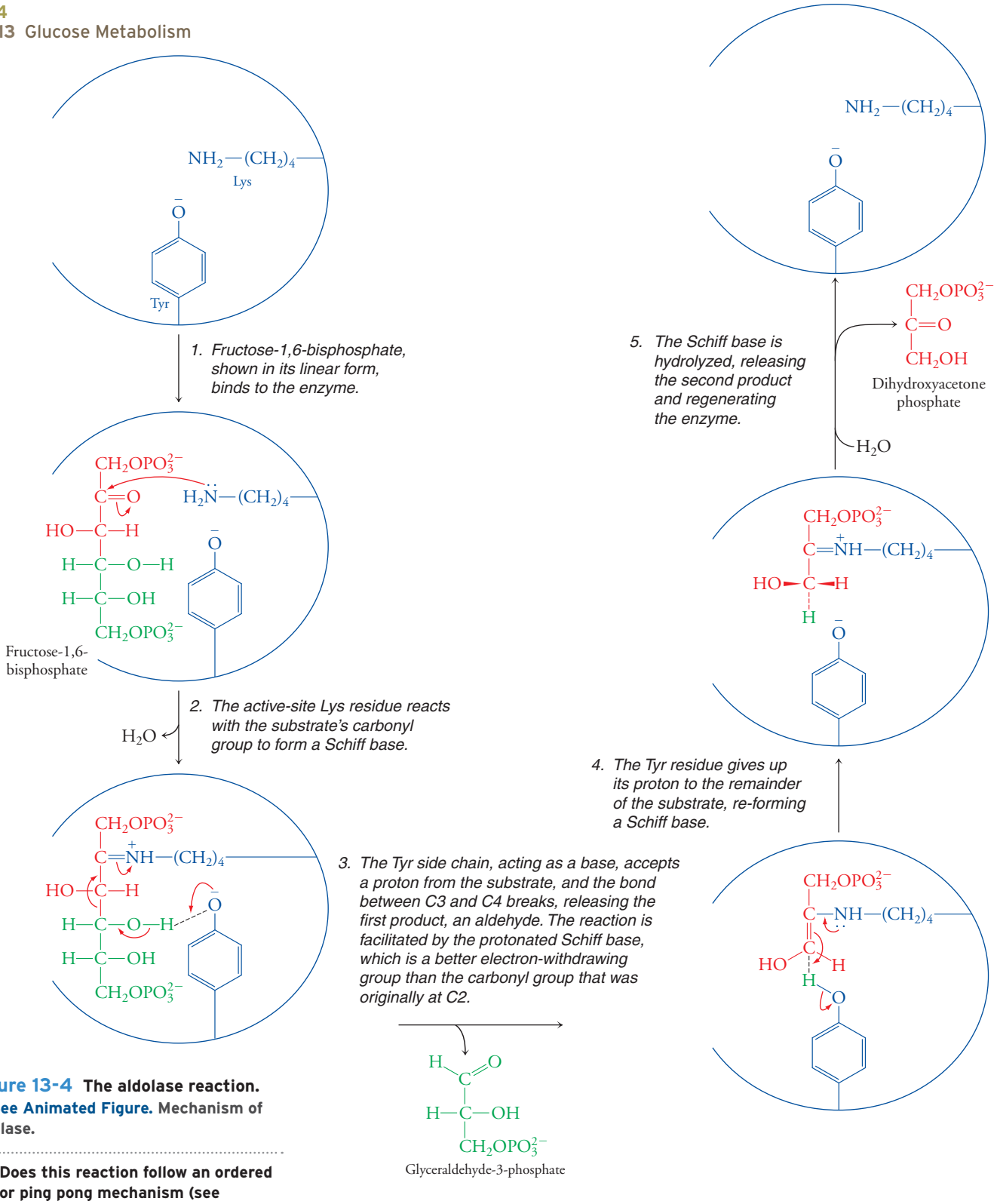


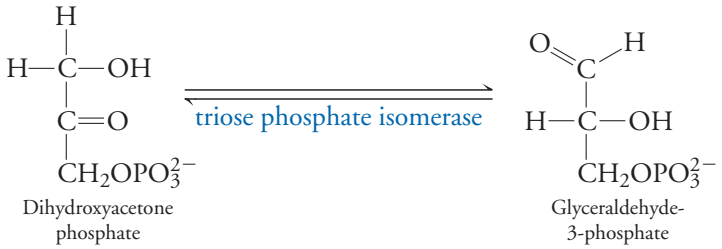
Figure 13-4 **The aldolase reaction.** **See Animated Figure.** **Mechanism of aldolase.**

? Does this reaction follow an ordered or ping pong mechanism (see Section 7-2)?

The $\Delta G^{\circ\prime}$ value for the aldolase reaction is $+22.8$ kJ · mol^{-1}, indicating that the reaction is unfavorable under standard conditions. However, the reaction proceeds *in vivo* (ΔG is actually less than zero) because the products of the reaction are quickly whisked away by subsequent reactions. In essence, the rapid consumption of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate "pulls" the aldolase reaction forward.

5. Triose Phosphate Isomerase

The products of the aldolase reaction are both phosphorylated three-carbon compounds, but only one of them—glyceraldehyde-3-phosphate—proceeds through the remainder of the pathway. Dihydroxyacetone phosphate is converted to glyceraldehyde-3-phosphate by triose phosphate isomerase:

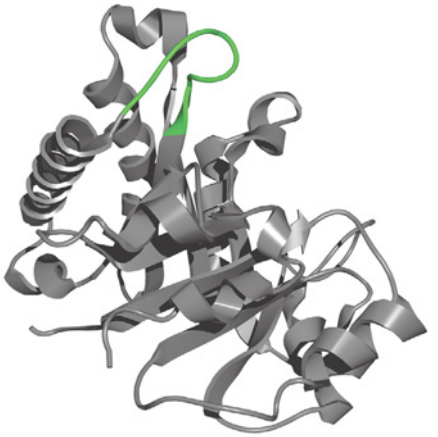


Dihydroxyacetone phosphate

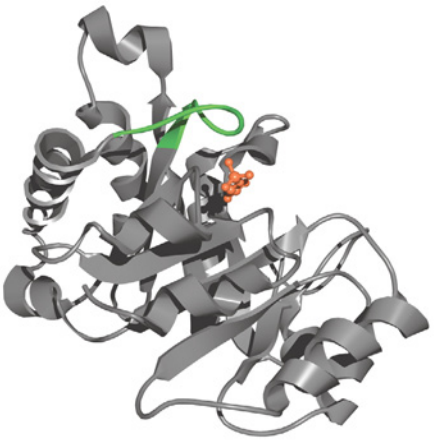
Glyceraldehyde-3-phosphate

Triose phosphate isomerase was introduced in Section 7-2 as an example of a catalytically perfect enzyme, one whose rate is limited only by the rate at which its substrates can diffuse to its active site. The catalytic mechanism of triose phosphate isomerase may involve low-barrier hydrogen bonds (which also help stabilize the transition state in serine proteases; see Section 6-3). In addition, the catalytic power of triose phosphate isomerase depends on a protein loop that closes over the active site **(Fig. 13-5)**.

The standard free energy change for the triose phosphate isomerase reaction is slightly positive, even under physiological conditions ($\Delta G^{\circ\prime} = +7.9 \cdot \text{kJ} \cdot \text{mol}^{-1}$ and $\Delta G = +4.4 \text{ kJ} \cdot \text{mol}^{-1}$), but the reaction proceeds because glyceraldehyde-3-phosphate is quickly consumed in the next reaction, so more dihydroxyacetone phosphate is constantly being converted to glyceraldehyde-3-phosphate.



(a)



(b)

Figure 13-5 Conformational changes in yeast triose phosphate isomerase. (a) One loop of the protein, comprising residues 166–176, is highlighted in green. (b) When a substrate binds to the enzyme, the loop closes over the active site to stabilize the reaction's transition state. In this model, the transition state analog 2-phosphoglycolate (orange) occupies the active site. Triose phosphate isomerase is actually a homodimer; only one subunit is pictured here. [Structure of the enzyme alone (pdb 1YPI) determined by T. Alber, E. Lolis, and G. A. Petsko; structure of the enzyme with the analog (pdb 2YPI) determined by E. Lolis and G. A. Petsko.] **See Interactive Exercise. Triose phosphate isomerase.**

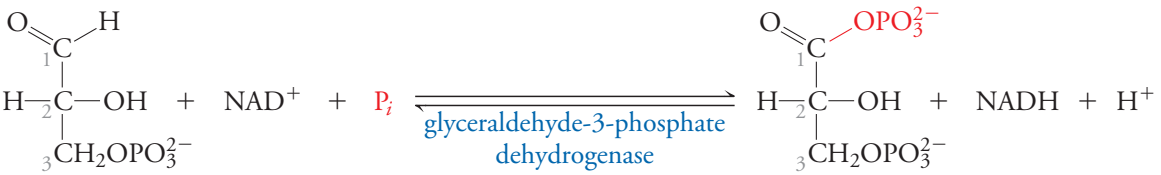
Reactions 6–10 are the energy-payoff phase of glycolysis

So far, the reactions of glycolysis have consumed 2 ATP, but this investment pays off in the second phase of glycolysis when 4 ATP are produced, for a net gain of 2 ATP. All of the reactions of the second phase involve three-carbon intermediates, but keep in mind that *each glucose molecule that enters the pathway yields two of these three-carbon units.*

Some species convert glucose to glyceraldehyde-3-phosphate by different pathways than the one presented above. However, the second phase of glycolysis, which converts glyceraldehyde-3-phosphate to pyruvate, is the same in all organisms. This suggests that glycolysis may have evolved from the "bottom up"; that is, it first evolved as a pathway for extracting free energy from abiotically produced small molecules, before cells developed the ability to synthesize larger molecules such as hexoses.

6. Glyceraldehyde-3-Phosphate Dehydrogenase

In the sixth reaction of glycolysis, glyceraldehyde-3-phosphate is both oxidized and phosphorylated:



Glyceraldehyde-3-phosphate

1,3-Bisphosphoglycerate

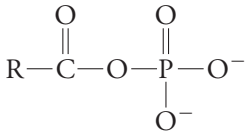
Unlike the kinases that catalyze Reactions 1 and 3, glyceraldehyde-3-phosphate dehydrogenase does not use ATP as a phosphoryl-group donor; it adds inorganic

phosphate to the substrate. This reaction is also an oxidation–reduction reaction in which the aldehyde group of glyceraldehyde-3-phosphate is oxidized and the cofactor NAD^+ is reduced to NADH. In effect, glyceraldehyde-3-phosphate dehydrogenase catalyzes the removal of an H atom (actually, a hydride ion); hence the name "dehydrogenase." Note that the reaction product NADH must eventually be reoxidized to NAD^+, or else glycolysis will come to a halt. In fact, the reoxidation of NADH, which is a form of "energy currency," can generate ATP (Chapter 15).

An active-site Cys residue participates in the glyceraldehyde-3-phosphate dehydrogenase reaction **(Fig. 13-6)**. The enzyme is inhibited by arsenate (AsO_4^{3-}), which competes with P_i (PO_4^{3-}) for binding in the enzyme active site.

7. Phosphoglycerate Kinase

The product of Reaction 6, 1,3-bisphosphoglycerate, is an acyl phosphate.



An acyl phosphate

The subsequent removal of its phosphoryl group releases a large amount of free energy in part because the reaction products are more stable (the same principle con-

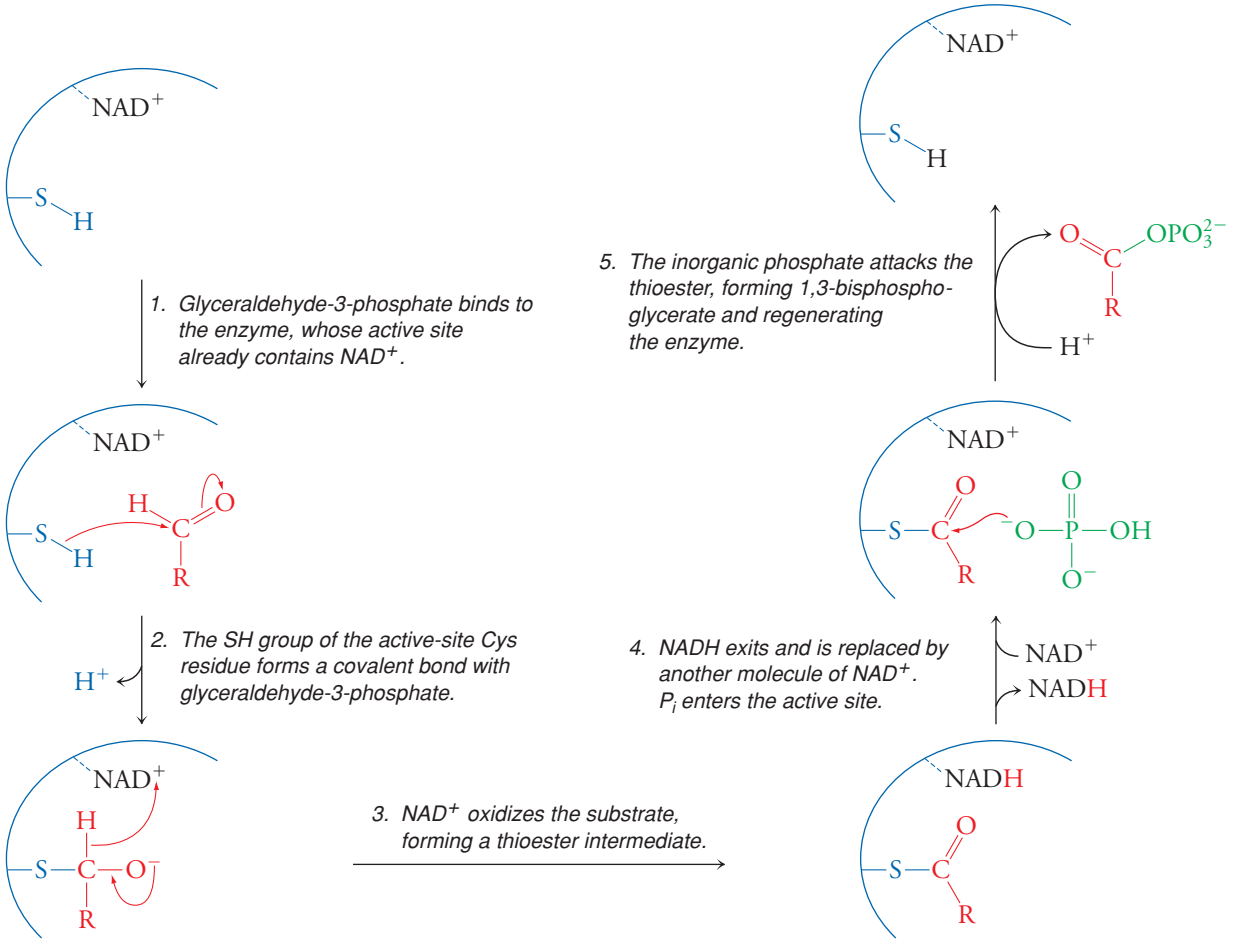
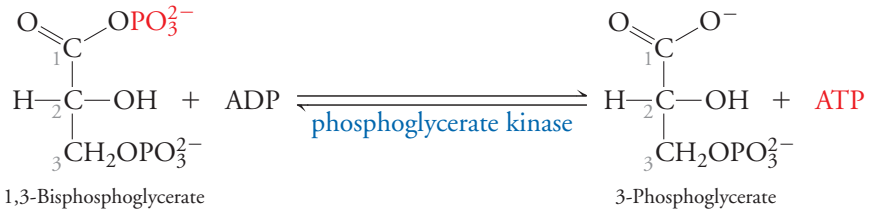


Figure 13-6 **The glyceraldehyde-3-phosphate dehydrogenase reaction.** ⊕ **See Animated Figure. Mechanism of glyceraldehyde-3-phosphate dehydrogenase.**

? **Identify the reactant that undergoes oxidation and the reactant that undergoes reduction.**

tributes to the large negative value of ΔG for reactions involving cleavage of ATP's phosphoanhydride bonds; see Section 12-3). *The free energy released in this reaction is used to drive the formation of ATP, as 1,3-bisphosphoglycerate donates its phosphoryl group to ADP:*



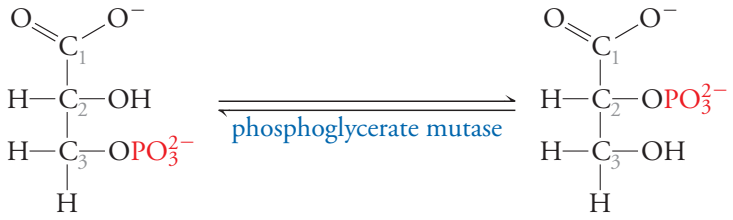
1,3-Bisphosphoglycerate — 3-Phosphoglycerate

Note that the enzyme that catalyzes this reaction is called a kinase since it transfers a phosphoryl group between ATP and another molecule.

The standard free energy change for the phosphoglycerate kinase reaction is $-18.8\ \text{kJ} \cdot \text{mol}^{-1}$. This strongly exergonic reaction helps pull the glyceraldehyde-3-phosphate dehydrogenase reaction forward, since its standard free energy change is greater than zero ($\Delta G^{\circ\prime} = +6.7\ \text{kJ} \cdot \text{mol}^{-1}$). This pair of reactions provides a good example of the coupling of a thermodynamically favorable and unfavorable reaction so that both proceed with a net decrease in free energy: $-18.8\ \text{kJ} \cdot \text{mol}^{-1} + 6.7\ \text{kJ} \cdot \text{mol}^{-1} = -12.1\ \text{kJ} \cdot \text{mol}^{-1}$. Under physiological conditions, ΔG for the paired reactions is close to zero.

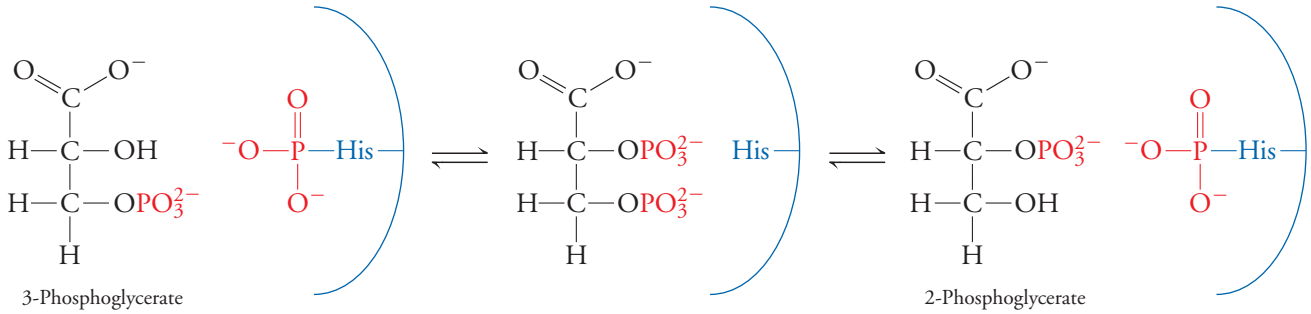
8. Phosphoglycerate Mutase

In the next reaction, 3-phosphoglycerate is converted to 2-phosphoglycerate:



3-Phosphoglycerate — 2-Phosphoglycerate

Although the reaction appears to involve the simple intramolecular transfer of a phosphoryl group, the reaction mechanism is a bit more complicated and requires an enzyme active site that contains a phosphorylated His residue. The phospho-His transfers its phosphoryl group to 3-phosphoglycerate to generate 2,3-bisphosphoglycerate, which then gives a phosphoryl group back to the enzyme, leaving 2-phosphoglycerate and phospho-His:

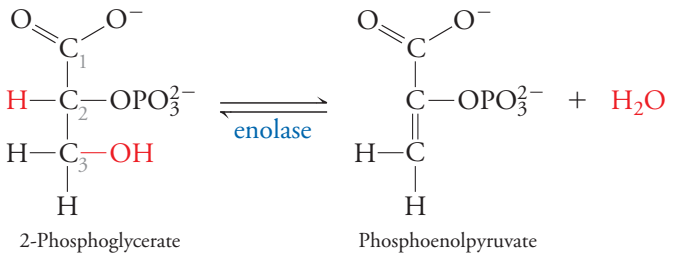


3-Phosphoglycerate — 2-Phosphoglycerate

As can be guessed from its mechanism, the phosphoglycerate mutase reaction is freely reversible *in vivo*.

9. Enolase

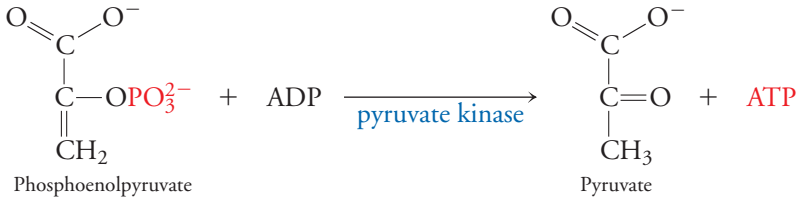
Enolase catalyzes a dehydration reaction, in which water is eliminated:



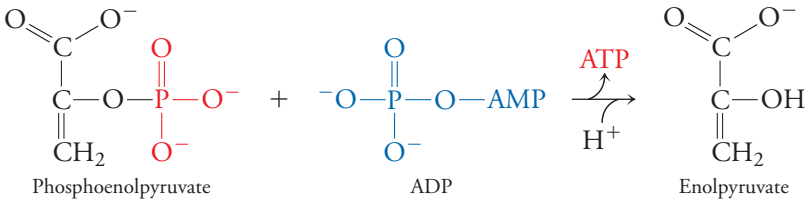
The enzyme active site includes an Mg^{2+} ion that apparently coordinates with the OH group at C3 and makes it a better leaving group. Fluoride ion and P_i can form a complex with the Mg^{2+} and thereby inhibit the enzyme. In early studies demonstrating the inhibition of glycolysis by F^-, 2-phosphoglycerate, the substrate of enolase, accumulated. The concentration of 3-phosphoglycerate also increased in the presence of F^- since phosphoglycerate mutase readily converted the excess 2-phosphoglycerate back to 3-phosphoglycerate.

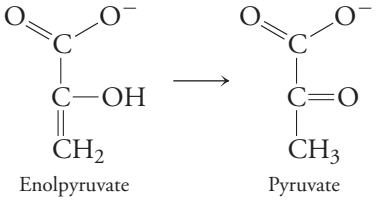
10. Pyruvate Kinase

The tenth reaction of glycolysis is catalyzed by pyruvate kinase, which converts phosphoenolpyruvate to pyruvate and transfers a phosphoryl group to ADP to produce ATP:



The reaction actually occurs in two parts. First, ADP attacks the phosphoryl group of phosphoenolpyruvate to form ATP and enolpyruvate:





Removal of phosphoenolpyruvate's phosphoryl group is not a particularly exergonic reaction: When written as a hydrolytic reaction (transfer of the phosphoryl group to water), the $\Delta G^{\circ\prime}$ value is -16 kJ · mol^{-1}. This is not enough free energy to drive the synthesis of ATP from ADP + P_i (this reaction requires $+30.5$ kJ · mol^{-1}). However, the second half of the pyruvate kinase reaction is highly exergonic. This is the **tautomerization** (isomerization through the shift of an H atom) of enolpyruvate to pyruvate (*left*). $\Delta G^{\circ\prime}$ for this step is -46 kJ · mol^{-1}, so $\Delta G^{\circ\prime}$ for the net reaction (hydrolysis of phosphoenolpyruvate followed by tautomerization of enolpyruvate to pyruvate) is -61.9 kJ · mol^{-1}, more than enough free energy to drive the synthesis of ATP.

Three of the ten reactions of glycolysis (the reactions catalyzed by hexokinase, phosphofructokinase, and pyruvate kinase) have large negative values of ΔG. In theory, any of these far-from-equilibrium reactions could serve as a flux-control point for the pathway. The other seven reactions function near equilibrium ($\Delta G \approx 0$) and can therefore accommodate flux in either direction. The free energy changes for the ten reactions of glycolysis are shown graphically in **Figure 13-7**.

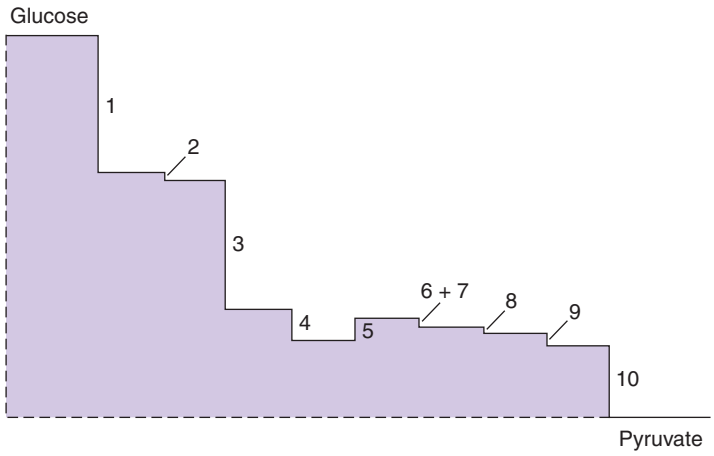


Figure 13-7 Graphical representation of the free energy changes of glycolysis. Three steps have large negative values of ΔG; the remaining steps are near equilibrium ($\Delta G \approx 0$). The height of each step corresponds to its ΔG value in heart muscle, and the numbers correspond to glycolytic enzymes. Keep in mind that ΔG values vary slightly among tissues. [Data from Newsholme, E. A., and Start, C., *Regulation in Metabolism*, p. 97, Wiley (1973).]

We have already discussed the mechanisms for regulating phosphofructokinase activity, the major control point for glycolysis. Hexokinase also catalyzes an irreversible reaction and is subject to inhibition by its product, glucose-6-phosphate. However, hexokinase cannot be the sole control point for glycolysis because glucose can also enter the pathway as glucose-6-phosphate, bypassing the hexokinase reaction. Finally, it would not be efficient for the pyruvate kinase reaction to be the primary regulatory step for glycolysis because it occurs at the very end of the 10-step pathway. Even so, pyruvate kinase activity can be adjusted. In some organisms, fructose-1,6-bisphosphate activates pyruvate kinase at an allosteric site. This is an example of **feed-forward activation:** Once a monosaccharide has entered glycolysis, fructose-1,6-bisphosphate helps ensure rapid flux through the pathway.

To sum up the second phase of glycolysis: Glyceraldehyde-3-phosphate is converted to pyruvate with the synthesis of 2 ATP (in Reactions 7 and 10). Since each molecule of glucose yields two molecules of glyceraldehyde-3-phosphate, the reactions of the second phase of glycolysis must be doubled, so the yield is 4 ATP. Two molecules of ATP are invested in phase 1, bringing the net yield to 2 ATP produced per glucose molecule. Two NADH are also generated for each glucose molecule. Monosaccharides other than glucose are metabolized in a similar fashion to yield ATP (Box 13-A).

BOX 13-A BIOCHEMISTRY NOTE

Catabolism of Other Sugars

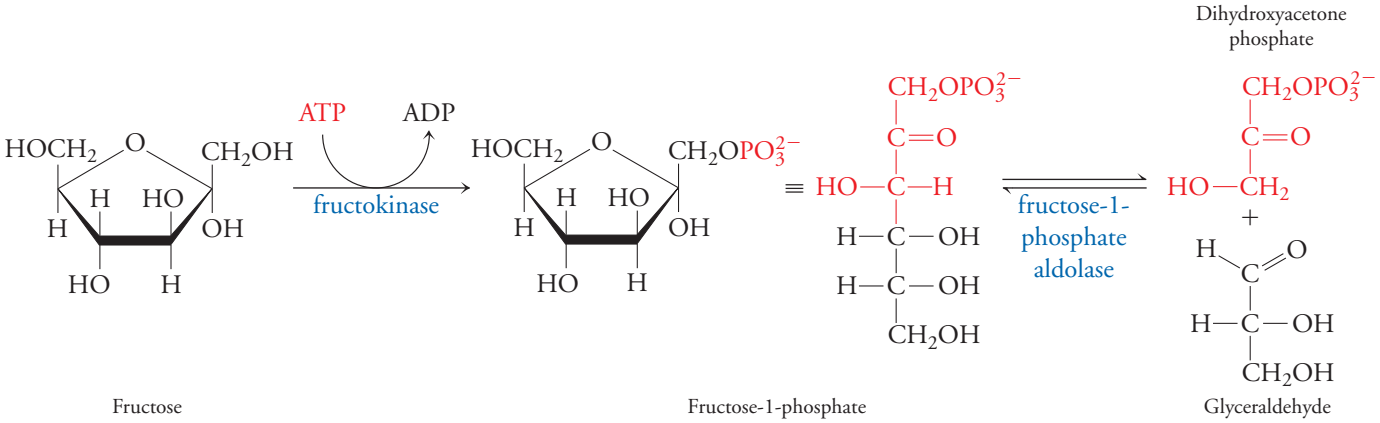
A typical human diet contains many carbohydrates other than glucose and its polymers. For example, lactose, a disaccharide of glucose and galactose, is present in milk and food derived from it (Section 11-2). Lactose is cleaved in the intestine by the enzyme lactase, and the two monosaccharides are absorbed, transported to the liver, and metabolized. Galactose undergoes phosphorylation and isomerization and enters the glycolytic pathway as glucose-6-phosphate, so its energy yield is the same as that of glucose.

Sucrose, the other major dietary disaccharide, is composed of glucose and fructose (Section 11-2); it is present in a variety of foods of plant origin. Like lactose, sucrose is hydrolyzed in the small intestine, and its components glucose and fructose are absorbed. The monosaccharide fructose is also present in many foods, particularly fruits and honey. It tastes sweeter than sucrose, is more soluble, and is inexpensive to produce in the form of high-fructose corn syrup—all of which make fructose attractive to the manufacturers of soft drinks and other processed foods. This is the primary reason why the consumption of fructose in the United States has increased by about 61% over the past 30 years.

The overconsumption of fructose may be contributing to the obesity epidemic. One possible explanation relates to the catabolism of fructose, which differs somewhat from the catabolism of glucose. Fructose is metabolized primarily by the liver, but the form of hexokinase present in the liver (called glucokinase) has very low affinity for fructose. Fructose therefore enters glycolysis by a different route.

(*continued on next page*)

First, fructose is phosphorylated to yield fructose-1-phosphate. The enzyme fructose-1-phosphate aldolase then splits the six-carbon molecule into two three-carbon molecules: glyceraldehyde and dihydroxyacetone phosphate:



Dihydroxyacetone phosphate is converted to glyceraldehyde-3-phosphate by triose phosphate isomerase and can proceed through the second phase of glycolysis. The glyceraldehyde can be phosphorylated to glyceraldehyde-3-phosphate, but it can also be converted to glycerol-3-phosphate, a precursor of the backbone of triacylglycerols. This may contribute to an increase in fat deposition. A second potential hazard of the fructose catabolic pathway is that fructose catabolism bypasses the phosphofructokinase-catalyzed step of glycolysis and thus avoids a major regulatory point. This may disrupt fuel metabolism in such a way that fructose catabolism leads to greater production of lipid than does glucose catabolism. The metabolic consequences of consuming high-fructose foods may therefore go beyond their caloric content.

Question: When its concentration is extremely high, fructose is converted to fructose-1-phosphate much faster than it can be cleaved by the aldolase. How would this affect the cell's ATP supply?

Pyruvate is converted to other substances

What happens to the pyruvate generated by the catabolism of glucose? It can be further broken down to acetyl-CoA or used to synthesize other compounds such as oxaloacetate. The fate of pyruvate depends on the cell type and the need for metabolic free energy and molecular building blocks. Some of the options are diagrammed in Figure 13-8.

During exercise, pyruvate may be temporarily converted to lactate. In a highly active muscle cell, glycolysis rapidly provides ATP to power muscle contraction, but the pathway also consumes NAD^+ at the glyceraldehyde-3-phosphate dehydrogenase step.

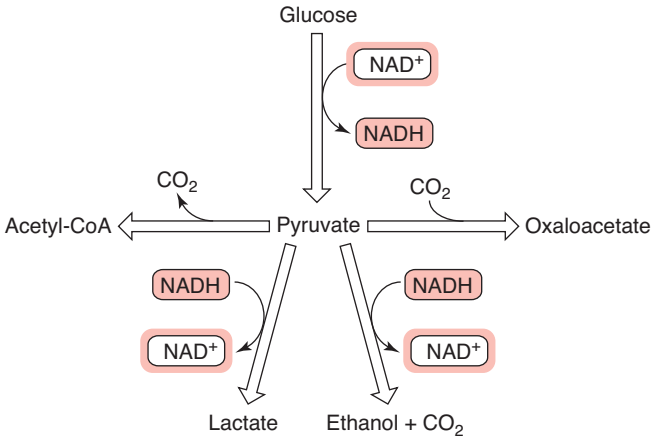
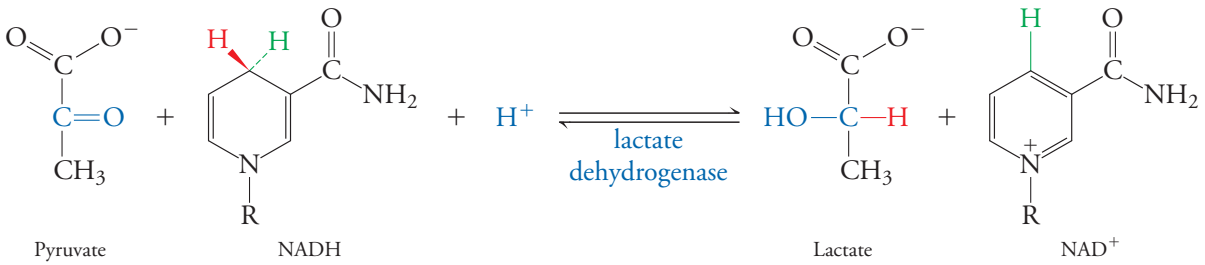


Figure 13-8 Fates of pyruvate. Pyruvate may be converted to a two-carbon acetyl group linked to the carrier coenzyme A. Acetyl-CoA may be further broken down by the citric acid cycle or used to synthesized fatty acids. In muscle, pyruvate is reduced to lactate to regenerate NAD^+ for glycolysis. Yeast degrade pyruvate to ethanol and CO_2. Pyruvate can also be carboxylated to produce the four-carbon oxaloacetate.

? Beside each arrow, write the names of the enzymes that catalyze the process.

The two NADH molecules generated for each glucose molecule catabolized can be reoxidized in the presence of oxygen. However, this process is too slow to replenish the NAD^+ needed for the rapid production of ATP by glycolysis. *To regenerate NAD^+, the enzyme lactate dehydrogenase reduces pyruvate to lactate:*



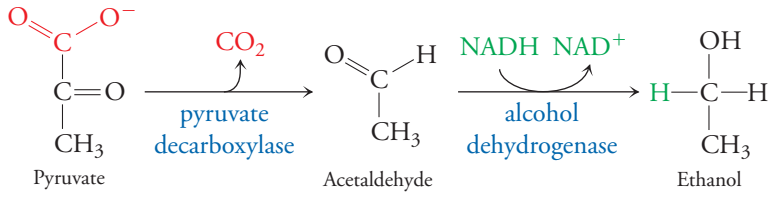
This reaction, sometimes called the eleventh step of glycolysis, allows the muscle to function anaerobically for a minute or so (see Box 12-D). The net reaction for anaerobic glucose catabolism is

$$\text{glucose} + 2\ \text{ADP} + 2\ P_i \rightarrow 2\ \text{lactate} + 2\ \text{ATP}$$

Lactate represents a sort of metabolic dead end: Its only options are to be eventually converted back to pyruvate (the lactate dehydrogenase reaction is reversible) or to be exported from the cell. The liver takes up lactate, oxidizes it back to pyruvate, and then uses it for gluconeogenesis. The glucose produced in this manner may eventually make its way back to the muscle to help fuel continued muscle contraction. When the muscle is functioning aerobically, NADH produced by the glyceraldehyde-3-phosphate dehydrogenase reaction is reoxidized by oxygen and the lactate dehydrogenase reaction is not needed.

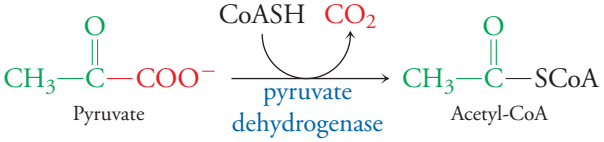
Organisms such as yeast growing under anaerobic conditions can regenerate NAD^+ by producing alcohol. In the mid-1800s, Louis Pasteur called this process **fermentation,** meaning life without air, although yeast also ferment sugars in the presence of O_2. And, to answer the question posed at the start of the chapter, yeast transform sugars to pyruvate by glycolysis, then carry out a two-step fermentation process. First, pyruvate decarboxylase (an enzyme not present in animals) catabolizes the removal of pyruvate's carboxylate group to produce acetaldehyde. Next, alcohol dehydrogenase reduces acetaldehyde to ethanol.

▶▶ HOW do yeast transform sugars into other substances?



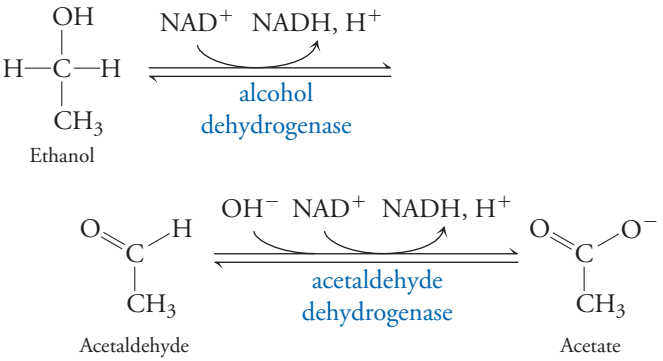
Ethanol is considered to be a waste product of sugar metabolism; its accumulation is toxic to other organisms (Box 13-B), including the yeast that produce it. This is one reason why the alcohol content of yeast-fermented beverages such as wine is limited to about 13%. "Hard" liquor must be distilled to increase its ethanol content.

Although glycolysis is an oxidative pathway, its end product pyruvate is still a relatively reduced molecule. The further catabolism of pyruvate begins with its decarboxylation to form a two-carbon acetyl group linked to coenzyme A.



Alcohol Metabolism

Unlike yeast, mammals do not produce ethanol, although it is naturally present in many foods and is produced in small amounts by intestinal microorganisms. The liver is equipped to metabolize ethanol, a small, weakly polar substance that is readily absorbed from the gastrointestinal tract and transported by the bloodstream. First, alcohol dehydrogenase converts ethanol to acetaldehyde. This is the reverse of the reaction yeast use to produce ethanol. A second reaction converts acetaldehyde to acetate:



Note that both of these reactions require NAD^+, a cofactor used in many other oxidative cellular processes, including glycolysis. The liver uses the same two-enzyme pathway to metabolize the excess ethanol obtained from alcoholic beverages. Ethanol itself is mildly toxic, and the physiological effects of alcohol also reflect the toxicity of acetaldehyde and acetate in tissues such as the liver and brain.

Over the short term and at low doses, alcohol triggers relaxation, often leading to animated movements and talkativeness. Some of these responses may be psychological (resulting from social cues rather than chemical effects), since changes in behavior sometimes occur even before significant amounts of ethanol have been absorbed. Once in the body, ethanol induces vasodilation, apparent as flushing (warming and reddening of the skin due to increased blood flow). At the same time, the heart rate and respiration rate become slightly lower. The kidneys increase the excretion of water as ethanol interferes with the ability of the hypothalamus (a region of the brain) to properly sense osmotic pressure.

Ethanol is considered to be a psychoactive drug because of its effects on the central nervous system. It stimulates signaling from certain neurotransmitter receptors that function as ligand-gated ion channels (Section 9-2) to inhibit neuronal signaling, producing a sedative effect. Sensory, motor, and cognitive functions are impaired, leading to delayed reaction time, loss of balance, and blurred vision. Some of the symptoms of ethanol intoxication can be experienced even at low doses, when the blood alcohol concentration is less than 0.05%. At high doses, usually at blood concentrations above 0.25%, ethanol can cause loss of consciousness, coma, and death. However, there is considerable variation among individuals in their responses to ethanol.

The mostly pleasant responses to moderate ethanol consumption are followed by a period of recovery, when the concentrations of ethanol metabolites are relatively high. The unpleasant symptoms of a hangover in part reflect the chemistry of producing acetaldehyde and acetate. As shown at left, their production in the liver consumes NAD^+, thereby lowering the cell's NAD^+:NADH ratio. Without sufficient NAD^+, the liver's ability to produce ATP by glycolysis is diminished (since NAD^+ is required for the glyceraldehyde-3-phosphate dehydrogenase reaction). Acetaldehyde itself can react with liver proteins, inactivating them. Acetate (acetic acid) production lowers blood pH.

Long-term, excessive alcohol consumption exacerbates the toxic effects of ethanol and its metabolites. For example, a shortage of liver NAD^+ slows fatty acid breakdown (like glycolysis, a process that requires NAD^+) and promotes fatty acid synthesis, leading to fat accumulation in the liver. Over time, cell death causes permanent loss of function in the central nervous system. The death of liver cells and their replacement by fibrous scar tissue causes liver cirrhosis.

Questions:

1. Explain why alcohol consumption is associated with increased risk of developing hypothermia.
2. Drinking a glass of water for each alcoholic drink is a popular hangover-prevention strategy. Explain how increased water consumption might relieve some of the negative effects of alcohol consumption.
3. About 15% of ingested ethanol is metabolized by a cytochrome P450 (see Box 7-A), and chronic alcohol consumption induces the expression of this enzyme. Explain how this would change the effectiveness of therapeutic drugs.
4. Normally, the liver converts lactate, produced mainly by muscles, back to pyruvate so that the pyruvate can be converted to glucose by gluconeogenesis (Section 13-2). How do the activities of alcohol dehydrogenase and acetaldehyde dehydrogenase contribute to hypoglycemia?
5. Acetate can be broken down further but only if it is first converted to acetyl-CoA in a reaction that requires ATP. Explain why this metabolic process is inhibited when the concentration of acetate is high.

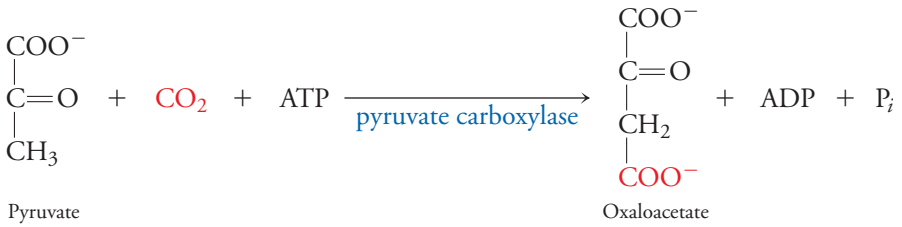
TABLE 13-1 Standard Free Energy Changes for Glucose Catabolism

Catabolic Process	$\Delta G^{\circ\prime}$ (kJ · mol^{-1})
$C_6H_{12}O_6 \rightarrow 2\ C_3H_5O_3^- + 2\ H^+$ (glucose) (lactate)	−196
$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$ (glucose)	−2850

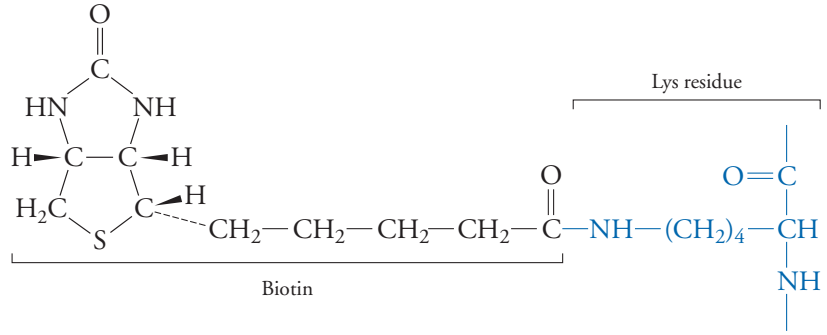
The resulting acetyl-CoA is a substrate for the citric acid cycle (Chapter 14). The complete oxidation of the six glucose carbons to CO_2 releases much more free energy than the conversion of glucose to lactate (Table 13-1). Much of this energy is recovered in the synthesis of ATP by the reactions of the citric acid cycle and oxidative phosphorylation (Chapter 15), pathways that require the presence of molecular oxygen.

Pyruvate is not always destined for catabolism. *Its carbon atoms provide the raw material for synthesizing a variety of molecules,* including, in the liver, more glucose (discussed in the following section). Fatty acids, the precursors of triacylglycerols and many membrane lipids, can be synthesized from the two-carbon units of acetyl-CoA derived from pyruvate. This is how fat is produced from excess carbohydrate.

Pyruvate is also the precursor of oxaloacetate, a four-carbon molecule that is an intermediate in the synthesis of several amino acids. It is also one of the intermediates of the citric acid cycle. Oxaloacetate is synthesized by the action of pyruvate carboxylase:



The pyruvate carboxylase reaction is interesting because of its unusual chemistry. The enzyme has a biotin prosthetic group that acts as a carrier of CO_2. Biotin is considered a vitamin, but a deficiency is rare because it is present in many foods and is synthesized by intestinal bacteria. The biotin group is covalently linked to an enzyme Lys residue:



The Lys side chain and its attached biotin group form a 14-Å-long flexible arm that swings between two active sites in the enzyme. In one active site, a CO_2 molecule is first "activated" by its reaction with ATP, then transferred to the biotin. The second active site transfers the carboxyl group to pyruvate to produce oxaloacetate **(Fig. 13-9)**.

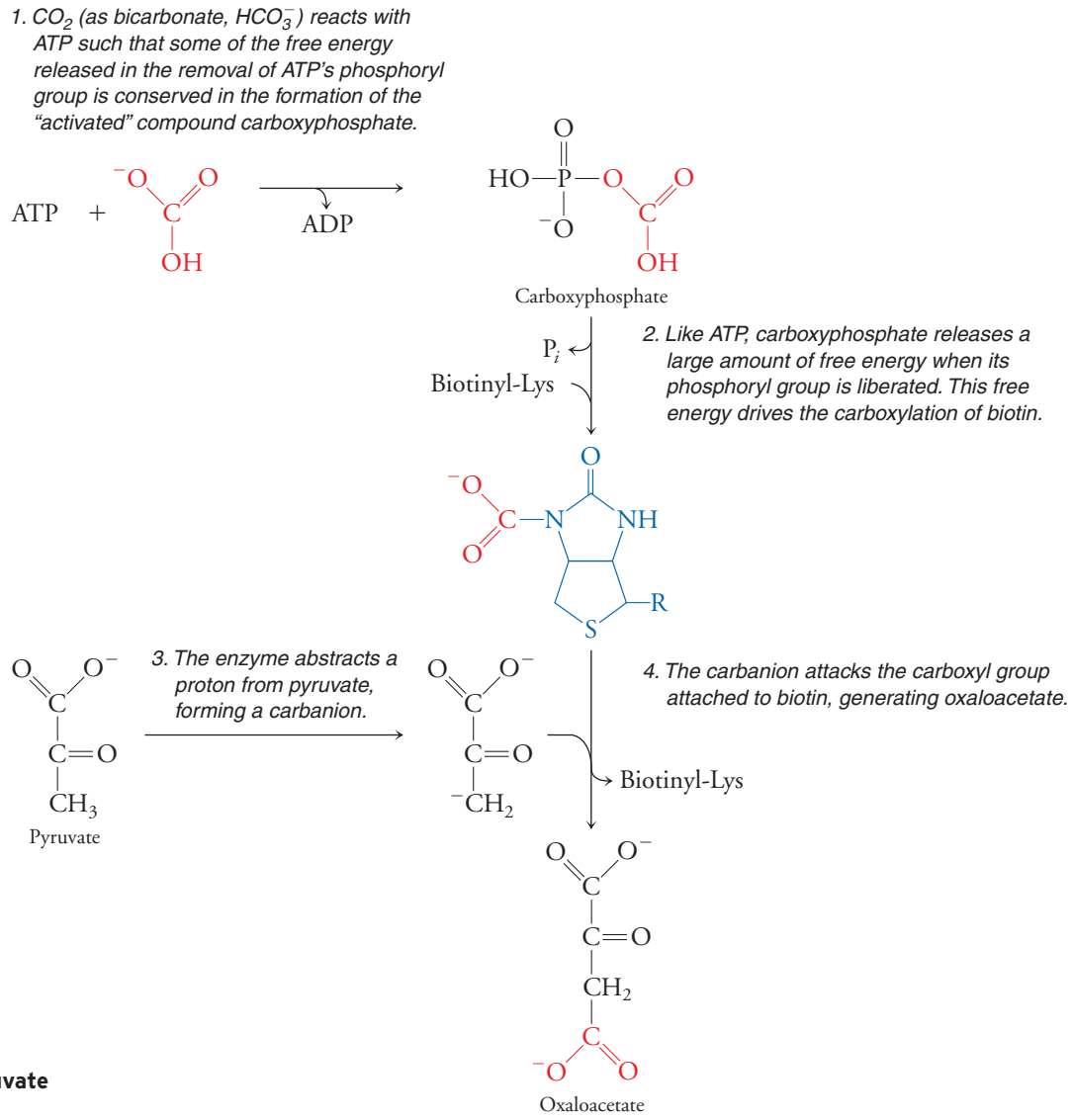


Figure 13-9 **The pyruvate carboxylase reaction.**

CONCEPT REVIEW

- Draw the structures of the substrates and products of the ten glycolytic reactions, name the enzyme that catalyzes each step, and indicate whether ATP or NADH is involved.
- Which glycolytic reactions consume ATP? Which generate ATP?
- What is the net yield of ATP and NADH per glucose molecule?
- Which reactions serve as flux-control points for glycolysis?
- What are the possible metabolic fates of pyruvate?
- What is the metabolic function of lactate dehydrogenase?

13-2 Gluconeogenesis

KEY CONCEPTS

- Pyruvate is converted to glucose by glycolytic enzymes operating in reverse and by enzymes that bypass the irreversible steps of glycolysis.
- Gluconeogenic flux is regulated primarily by fructose-2,6-bisphosphate.

We have already alluded to the ability of the liver to synthesize glucose from noncarbohydrate precursors via the pathway of gluconeogenesis. This pathway, which also occurs to a limited extent in the kidneys, operates when the liver's supply of glycogen is exhausted. Certain tissues, such as the central nervous system and red blood cells, which burn glucose as their primary metabolic fuel, rely on the liver to supply them with newly synthesized glucose.

Gluconeogenesis is considered to be the reversal of glycolysis, that is, the conversion of two molecules of pyruvate to one molecule of glucose. Although some of the steps of gluconeogenesis are catalyzed by glycolytic enzymes operating in reverse, the gluconeogenic

pathway contains several unique enzymes that bypass the three irreversible steps of glycolysis—the steps catalyzed by pyruvate kinase, phosphofructokinase, and hexokinase (Fig. 13-10). This principle applies to all pairs of opposing metabolic pathways: *The pathways may share some near-equilibrium reactions but cannot use the same enzymes to catalyze thermodynamically favorable irreversible reactions.* The three irreversible reactions of glycolysis are clearly visible in the "waterfall" diagram (see Fig. 13-7).

Four gluconeogenic enzymes plus some glycolytic enzymes convert pyruvate to glucose

Pyruvate cannot be converted directly back to phosphoenolpyruvate because pyruvate kinase catalyzes an irreversible reaction (Reaction 10 of glycolysis). To get around this thermodynamic barrier, pyruvate is carboxylated by pyruvate carboxylase to yield

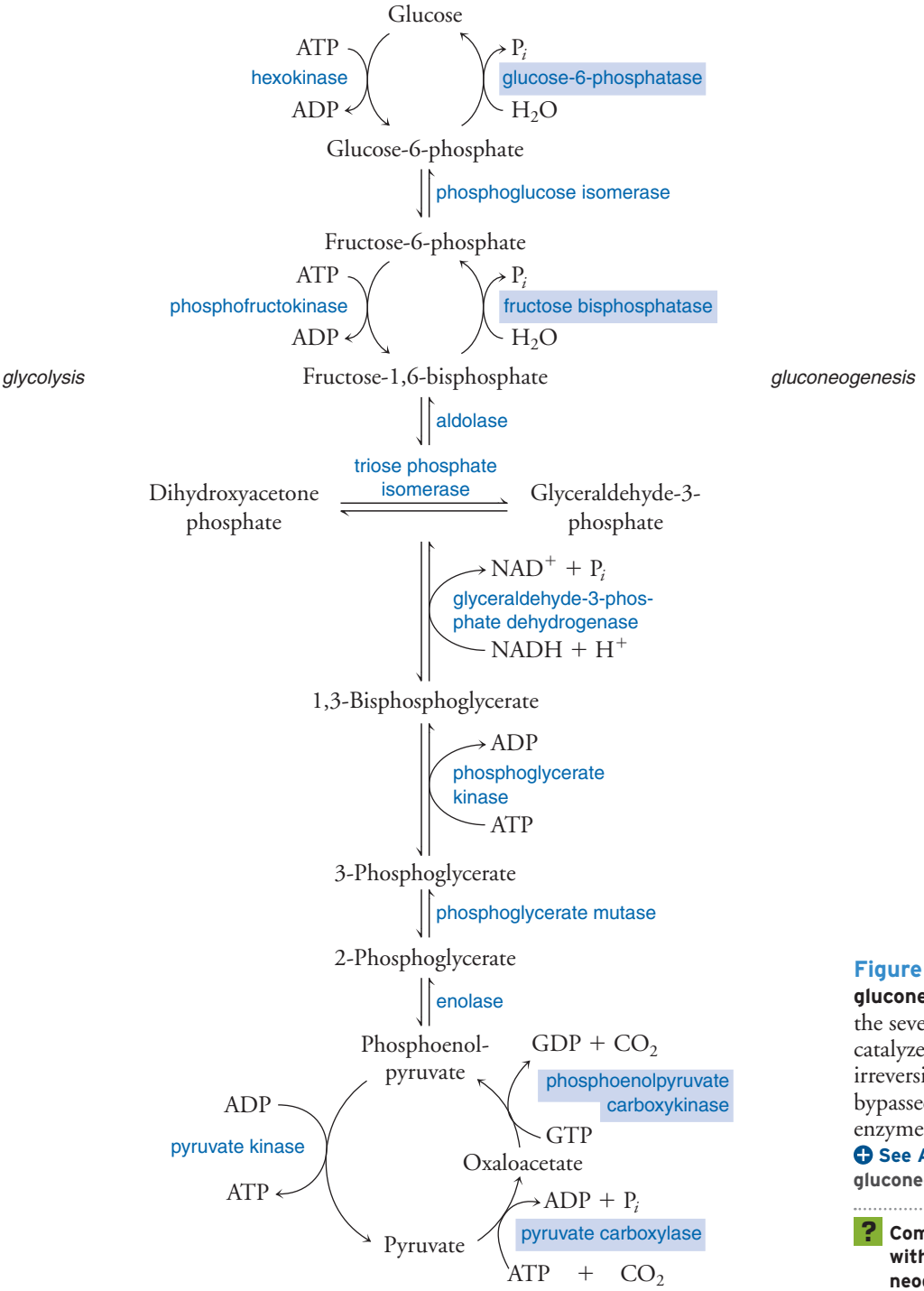
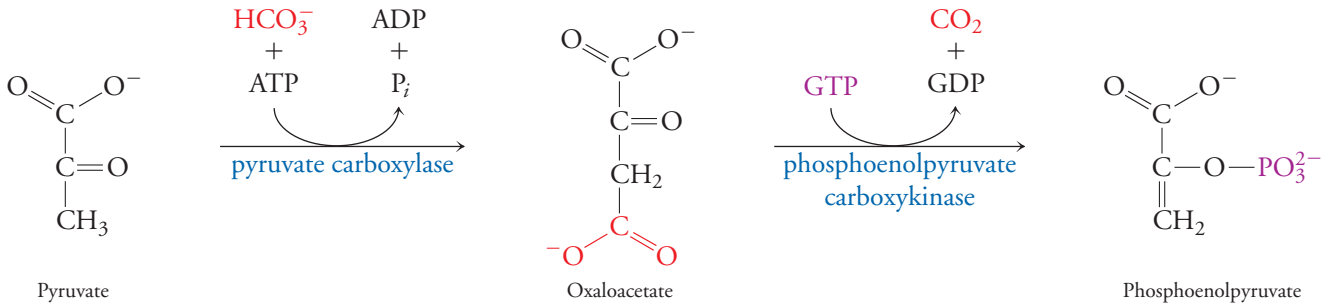


Figure 13-10 The reactions of gluconeogenesis. The pathway uses the seven glycolytic enzymes that catalyze reversible reactions. The three irreversible reactions of glycolysis are bypassed in gluconeogenesis by the four enzymes that are highlighted in blue. **See Animated Figure. Comparison of gluconeogenesis and glycolysis.**

? Compare the ATP yield of glycolysis with the ATP consumption of gluconeogenesis.

the four-carbon compound oxaloacetate (the same reaction shown in Fig. 13-9). Next, phosphoenolpyruvate carboxykinase catalyzes the decarboxylation of oxaloacetate to form phosphoenolpyruvate:



Note that the carboxylate group added in the first reaction is released in the second. The two reactions are energetically costly: Pyruvate carboxylase consumes ATP, and phosphoenolpyruvate carboxykinase consumes GTP (which is energetically equivalent to ATP). Cleavage of two phosphoanhydride bonds is required to supply enough free energy to "undo" the highly exergonic pyruvate kinase reaction.

Amino acids (except for Leu and Lys) are the main sources of gluconeogenic precursors because they can all be converted to oxaloacetate and then to phosphoenolpyruvate. Thus, during starvation, proteins can be broken down and used to produce glucose to fuel the central nervous system. Fatty acids cannot serve as gluconeogenic precursors because they cannot be converted to oxaloacetate. (However, the three-carbon glycerol backbone of triacylglycerols is a gluconeogenic precursor.)

Two molecules of phosphoenolpyruvate are converted to one molecule of fructose-1,6-bisphosphate in a series of six reactions that are all catalyzed by glycolytic enzymes (steps 4–9 in reverse order). These reactions are reversible because they are near equilibrium ($\Delta G \approx 0$), and the direction of flux is determined by the concentrations of substrates and products. Note that the phosphoglycerate kinase reaction consumes ATP when it operates in the direction of gluconeogenesis. NADH is also required to reverse the glyceraldehyde-3-phosphate dehydrogenase reaction.

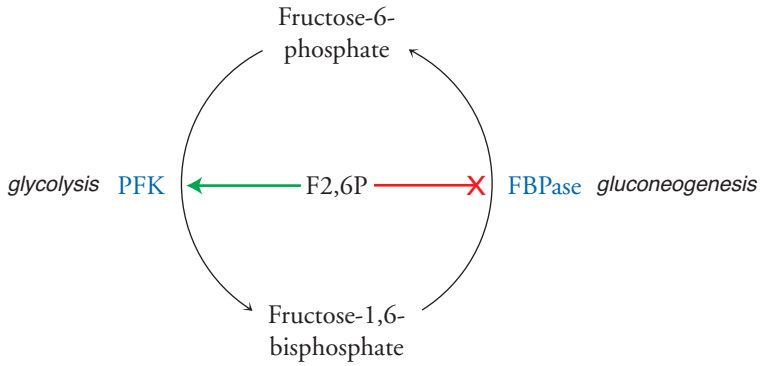
The final three reactions of gluconeogenesis require two enzymes unique to this pathway. The first step undoes the phosphofructokinase reaction, the irreversible reaction that is the major control point of glycolysis. In gluconeogenesis, the enzyme fructose bisphosphatase hydrolyzes the C1 phosphate of fructose-1,6-bisphosphate to yield fructose-6-phosphate. This reaction is thermodynamically favorable, with a ΔG value of -8.6 kJ · mol^{-1}. Next, the glycolytic enzyme phosphoglucose isomerase catalyzes the reverse of step 2 of glycolysis to produce glucose-6-phosphate. Finally, the gluconeogenic enzyme glucose-6-phosphatase catalyzes a hydrolytic reaction that yields glucose and P_i. Note that the hydrolytic reactions catalyzed by fructose bisphosphatase and glucose-6-phosphatase undo the work of two kinases in glycolysis (phosphofructokinase and hexokinase).

Gluconeogenesis is regulated at the fructose bisphosphatase step

Gluconeogenesis is energetically expensive. Producing 1 glucose from 2 pyruvate consumes 6 ATP, 2 each at the steps catalyzed by pyruvate carboxylase, phosphoenolpyruvate carboxykinase, and phosphoglycerate kinase. If glycolysis occurred simultaneously with gluconeogenesis, there would be a net consumption of ATP:

glycolysis	glucose + 2 ADP + 2 P_i → 2 pyruvate + 2 ATP
gluconeogenesis	2 pyruvate + 6 ATP → glucose + 6 ADP + 6 P_i
net	4 ATP → 4 ADP + 4 P_i

To avoid this waste of metabolic free energy, gluconeogenic cells (mainly liver cells) carefully regulate the opposing pathways of glycolysis and gluconeogenesis according to the cell's energy needs. *The major regulatory point is centered on the interconversion of fructose-6-phosphate and fructose-1,6-bisphosphate.* We have already seen that fructose-2,6-bisphosphate is a potent allosteric activator of phosphofructokinase, which catalyzes step 3 of glycolysis. Not surprisingly, fructose-2,6-bisphosphate is a potent *inhibitor* of fructose bisphosphatase, which catalyzes the opposing gluconeogenic reaction.



This mode of allosteric regulation is efficient because *a single compound can control flux through two opposing pathways in a reciprocal fashion.* Thus, when the concentration of fructose-2,6-bisphosphate is high, glycolysis is stimulated and gluconeogenesis is inhibited, and vice versa.

Many cells that do not carry out gluconeogenesis do contain the gluconeogenic enzyme fructose bisphosphatase. What is the reason for this? When both fructose bisphosphatase (FBPase) and phosphofructokinase (PFK) are active, the net result is the hydrolysis of ATP:

PFK	fructose-6-phosphate + ATP $\rightarrow$ fructose-1,6-bisphosphate + ADP
FBPase	fructose-1,6-bisphosphate + H_2O $\rightarrow$ fructose-6-phosphate + P_i
net	ATP + H_2O $\rightarrow$ ADP + P_i

This combination of metabolic reactions is called a **futile cycle** since it seems to have no useful result. However, Eric Newsholme realized that such futile cycles could actually provide a means for fine-tuning the output of a metabolic pathway. For example, flux through the phosphofructokinase step of glycolysis is diminished by the activity of fructose bisphosphatase. An allosteric compound such as fructose-2,6-bisphosphate modulates the activity of both enzymes so that as the activity of one enzyme increases, the activity of the other one decreases. This dual regulatory effect results in a greater possible range of net flux than if the regulator merely activated or inhibited a single enzyme.

CONCEPT REVIEW
- Which reactions of gluconeogenesis are catalyzed by glycolytic enzymes?
- Why are some enzymes unique to gluconeogenesis?
- What is a futile cycle and what is its purpose?

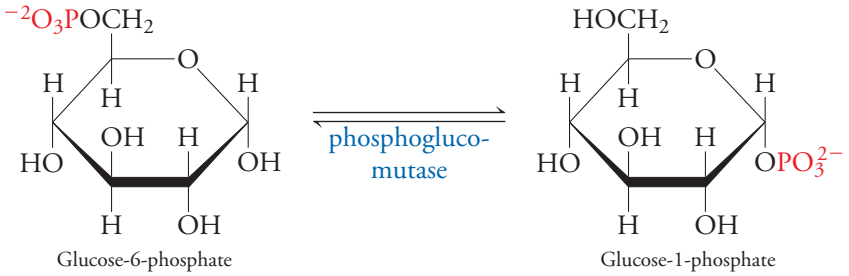
13-3 Glycogen Synthesis and Degradation

Dietary glucose and the glucose produced by gluconeogenesis are stored in the liver and other tissues as glycogen. Later, glucose units can be removed from the glycogen polymer by phosphorolysis (see Section 12-1). Because glycogen degradation is thermodynamically spontaneous, glycogen synthesis requires the input of free energy. The two opposing pathways use different sets of enzymes so that each process can be thermodynamically favorable under cellular conditions.

KEY CONCEPTS
- The substrate for glycogen synthase is UDP–glucose, whose production costs the free energy of one phosphoanhydride bond.
- Glycogen is phosphorolyzed to produce glucose that can exit the cell or be catabolized by glycolysis.

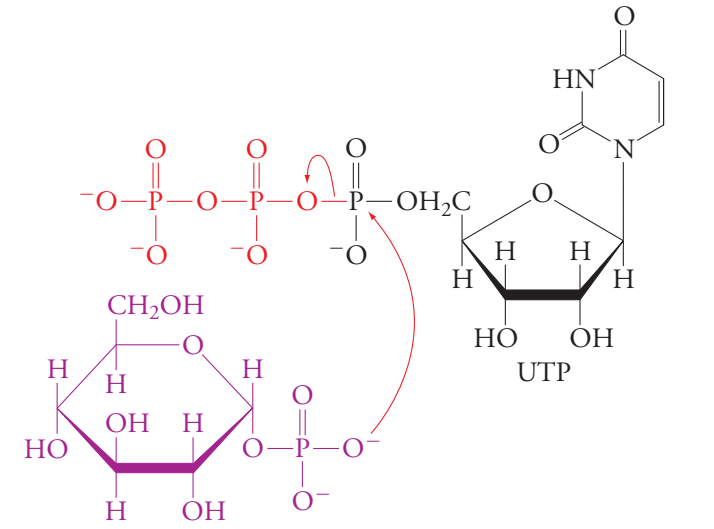
Glycogen synthesis consumes the free energy of UTP

The monosaccharide unit that is incorporated into glycogen is glucose-1-phosphate, which is produced from glucose-6-phosphate (the penultimate product of gluconeogenesis) by the action of the enzyme phosphoglucomutase:



Glucose-6-phosphate Glucose-1-phosphate

In mammalian cells, glucose-1-phosphate is then "activated" by reacting with UTP to form UDP–glucose (like GTP, UTP is energetically equivalent to ATP).

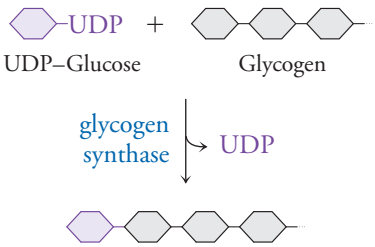


UDP–glucose

This process is a reversible phosphoanhydride exchange reaction ($\Delta G \approx 0$). Note that the two phosphoanhydride bonds of UTP are conserved, one in the product PP_i and one in UDP–glucose. However, the PP_i is rapidly hydrolyzed by inorganic pyrophosphatase to 2 P_i in a highly exergonic reaction ($\Delta G^{\circ\prime} = -19.2$ kJ · mol^{-1}). Thus, cleavage of a phosphoanhydride bond makes the formation of UDP–glucose an exergonic, irreversible process—that is, *PP_i hydrolysis "drives" a reaction that would*

otherwise be near equilibrium. The hydrolysis of PP_i by inorganic pyrophosphatase is a common strategy in biosynthetic reactions; we will see this reaction again in the synthesis of other polymers, namely DNA, RNA, and polypeptides.

Finally, glycogen synthase transfers the glucose unit to the C4 OH group at the end of one of glycogen's branches to extend the linear polymer of α(1 → 4)-linked residues (*right*).



A separate enzyme, called a transglycosylase or branching enzyme, cleaves off a seven-residue segment and reattaches it to a glucose C6 OH group to create an α(1 → 6) branch point.

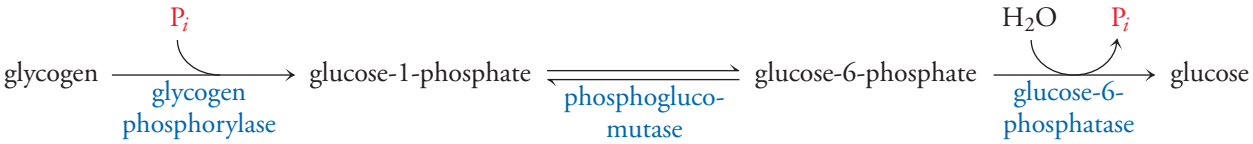
The steps of glycogen synthesis can be summarized as follows:

UDP–glucose pyrophosphorylase	glucose-1-phosphate + UTP ⇌ UDP–glucose + PP_i
pyrophosphatase	$PP_i + H_2O \rightarrow 2\ P_i$
glycogen synthase	UDP–glucose + glycogen (*n* residues) → glycogen (*n* + 1 residues) + UDP
net	glucose-1-phosphate + glycogen + UTP + H_2O → glycogen + UDP + 2 P_i

The energetic price for adding one glucose unit to glycogen is the cleavage of one phosphoanhydride bond of UTP. Nucleotides are also required for the synthesis of other saccharides. For example, lactose is synthesized from glucose and UDP–galactose. In plants, starch is synthesized using ADP–glucose, and cellulose is synthesized using CDP–glucose as starting materials.

Glycogen phosphorylase catalyzes glycogenolysis

Glycogen breakdown follows a different set of steps than glycogen synthesis. *In **glycogenolysis,** glycogen is phosphorolyzed, not hydrolyzed, to yield glucose-1-phosphate.* However, a debranching enzyme can remove α(1 → 6)-linked residues by hydrolysis. In the liver, phosphoglucomutase converts glucose-1-phosphate to glucose-6-phosphate, which is then hydrolyzed by glucose-6-phosphatase to release free glucose.



This glucose leaves the cell and enters the bloodstream. Only gluconeogenic tissues such as the liver can make glucose available to the body at large. Other tissues that store glycogen, such as muscle, lack glucose-6-phosphatase and so break down glycogen only for their own needs. In these tissues, the glucose-1-phosphate liberated by phosphorolysis of glycogen is converted to glucose-6-phosphate, which then enters glycolysis at the phosphoglucose isomerase reaction (step 2). The hexokinase reaction (step 1) is skipped, thereby sparing the consumption of ATP. Consequently, *glycolysis using glycogen-derived glucose has a higher net yield of ATP than glycolysis using glucose supplied by the bloodstream.*

Because the mobilization of glucose must be tailored to meet the energy demands of a particular tissue or the entire body, the activity of glycogen phosphorylase is carefully regulated by a variety of mechanisms linked to hormonal signaling. Likewise, the activity of glycogen synthase is subject to hormonal control. In Chapter 19 we will examine some of the mechanisms for regulating different aspects of fuel metabolism, including glycogen synthesis and degradation. Box 13-C discusses the metabolic disorders known as **glycogen storage diseases.**

CONCEPT REVIEW

- What is the role of UTP in glycogen synthesis?
- What is the advantage of breaking down glycogen by phosphorolysis?
- Why do only some tissues contain glucose-6-phosphatase?

Glycogen Storage Diseases

The glycogen storage diseases are a set of inherited disorders of glycogen metabolism, not all of which result in glycogen accumulation, as the name might suggest. The symptoms of the glycogen storage diseases vary, depending on whether the affected tissue is liver or muscle or both. In general, the disorders that affect the liver cause hypoglycemia (too little glucose in the blood) and an enlarged liver. Glycogen storage diseases that affect primarily muscle are characterized by muscle weakness and cramps. The incidence of glycogen storage diseases is estimated to be as high as 1 in 20,000 births, although some disorders are not apparent until adulthood. Twelve types of glycogen storage diseases have been described, and the defect in each is listed in the table on the next page. The following discussion focuses on the most common of these conditions.

A defect of glucose-6-phosphatase (type I glycogen storage disease, also called von Gierke's disease) affects both gluconeogenesis and glycogenolysis, since glucose-6-phosphatase catalyzes the final step of gluconeogenesis and makes free glucose available from glycogenolysis. The enlarged liver and hypoglycemia can lead to a host of other symptoms, including irritability, lethargy, and, in severe cases, death. A related defect is the deficiency of the transport protein that imports glucose-6-phosphate into the endoplasmic reticulum, where the phosphatase is located.

Type III glycogen storage disease, or Cori's disease, results from a deficiency of the glycogen debranching enzyme. This condition accounts for about one-quarter of all cases of glycogen storage disease and usually affects both liver and muscle. The symptoms include muscle weakness and liver enlargement due to the accumulation of glycogen that cannot be efficiently broken down. The symptoms of type III glycogen storage disease often improve with age and disappear by early adulthood.

The most common type of glycogen storage disease is type IX. In this disorder, the kinase that activates glycogen phosphorylase is defective. Symptoms range from severe to mild and may fade with time. The complexity of this disease reflects the fact that the phosphorylase kinase consists of four subunits, with isoforms that are differentially expressed in the liver and other tissues. Genes for the α chain (the kinase catalytic subunit) are located on the X chromosome, so one form of disease (type VIII glycogen storage disease) is inherited in a sex-linked manner (more males than females are affected). Genes for the β, γ, and δ subunits of the kinase, which have regulatory functions, are on other chromosomes, so defects in these genes affect males and females equally.

Type II glycogen storage disease, the deficiency of a muscle glucosidase, is not common, but it causes death within the first year. The missing enzyme is a lysosomal hydrolase that does not participate in the main pathways of glycogen degradation but, like many lysosomal enzymes, apparently plays a role in recycling cellular materials. Glycogen accumulates in the lysosomes, eventually killing the cell.

In the past, glycogen storage diseases were diagnosed on the basis of symptoms, blood tests, and painful biopsies of liver or muscle to assess its glycogen content. Current diagnostic methods are centered on analyzing the relevant genes for mutations, a noninvasive approach. Treatment of glycogen storage diseases typically includes a regimen of frequent, small, carbohydrate-rich meals to alleviate hypoglycemia. However, because dietary therapy does not completely eliminate the symptoms of some glycogen storage diseases, and because the metabolic abnormalities, such as chronic hypoglycemia and liver damage, can severely impair physical growth as well as cognitive development, liver transplant has proved to be an effective treatment. At lease one disorder, the type II glycogen storage disease, has been treated with infusions of the missing enzyme. The glycogen storage diseases are single-gene defects, which makes them attractive targets for gene therapy (see Section 3-4).

Type	Enzyme Deficiency
I	Glucose-6-phosphatase
II	α-1,4-Glucosidase
III	Amylo-1,6-glucosidase (debranching enzyme)
IV	Amylo-(1,4 → 1,6)-transglycosylase (branching enzyme)
V	Muscle glycogen phosphorylase
VI	Liver glycogen phosphorylase
VII	Phosphofructokinase
VIII, IX, X	Phosphorylase kinase
XI	GLUT2 transporter
0	Glycogen synthase

Questions:

1. Most patients with moderate to severe glycogen storage disease experience some growth retardation. What feature of the glycogen storage diseases would account for this?
2. Patients with type I glycogen storage disease sometimes have enlarged kidneys. Explain.
3. Would frequent small feedings of cornstarch relieve the symptoms of type 0 glycogen storage disease?
4. Would a liver transplant cure all the symptoms of type III glycogen storage disease? Explain.
5. The symptoms of type XI glycogen storage disease include hypoglycemia and hypergalactosemia. What does this tell you about the function of the GLUT2 glucose transport protein?

13-4 The Pentose Phosphate Pathway

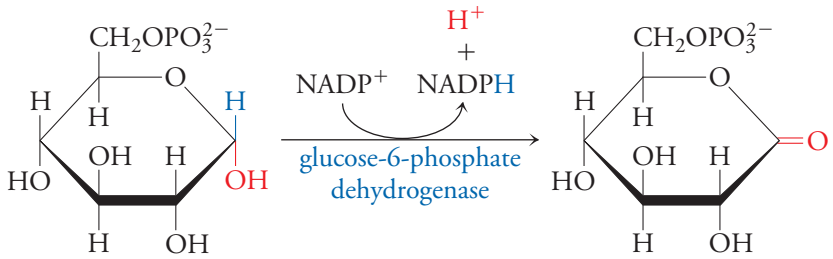
KEY CONCEPTS

- The pentose phosphate pathway is an oxidative pathway for producing NADPH and converting glucose to ribose.
- The reversible reactions of the pathway allow the interconversion of ribose and intermediates of glycolysis and gluconeogenesis.

We have already seen that glucose catabolism can lead to pyruvate, which can be further oxidized to generate more ATP or used to synthesize amino acids and fatty acids. Glucose is also a precursor of the ribose groups used for nucleotide synthesis. The **pentose phosphate pathway,** which converts glucose-6-phosphate to ribose-5-phosphate, is an oxidative pathway that occurs in all cells. But unlike glycolysis, the pentose phosphate pathway generates NADPH rather than NADH. The two cofactors are not interchangeable and are easily distinguished by degradative enzymes (which generally use NAD^+) and biosynthetic enzymes (which generally use $NADP^+$). The pentose phosphate pathway is by no means a minor feature of glucose metabolism. As much as 30% of glucose in the liver may be catabolized by the pentose phosphate pathway. This pathway can be divided into two phases: a series of oxidative reactions followed by a series of reversible interconversion reactions.

The oxidative reactions of the pentose phosphate pathway produce NADPH

The starting point of the pentose phosphate pathway is glucose-6-phosphate, which can be derived from free glucose, from the glucose-1-phosphate produced by glycogen phosphorolysis, or from gluconeogenesis. In the first step of the pathway, glucose-6-phosphate dehydrogenase catalyzes the metabolically irreversible transfer of a hydride ion from glucose-6-phosphate to $NADP^+$, forming a lactone and NADPH:

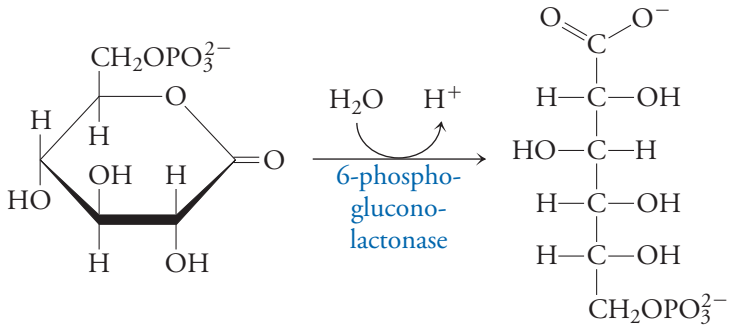


Glucose-6-phosphate

6-Phosphoglucono-δ-lactone

A deficiency of glucose-6-phosphate dehydrogenase is the most common human enzyme deficiency. This defect, which decreases the cellular production of NADPH, interferes with the normal function of certain oxidation–reduction processes and makes the cells more susceptible to oxidative damage. However, individuals with glucose-6-phosphate dehydrogenase deficiency are also more resistant to malaria. Thus, the gene for the defective enzyme (like the gene for sickle cell hemoglobin described in Box 5-C) persists because it confers a selective advantage.

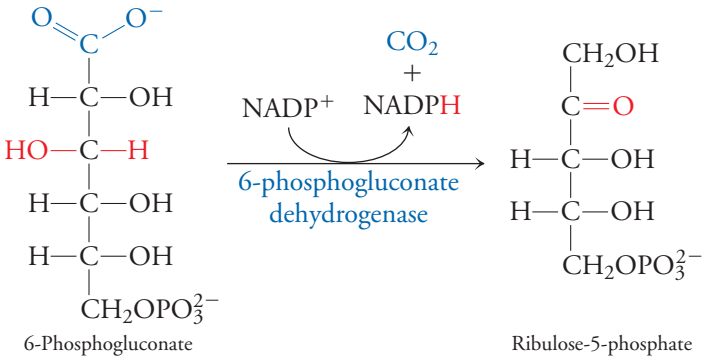
The lactone intermediate is hydrolyzed to 6-phosphogluconate by the action of 6-phosphogluconolactonase, although this reaction can also occur in the absence of the enzyme:



6-Phosphoglucono-δ-lactone

6-Phosphogluconate

In the third step of the pentose phosphate pathway, 6-phosphogluconate is oxidatively decarboxylated in a reaction that converts the six-carbon sugar to a five-carbon sugar and reduces a second $NADP^+$ to NADPH:

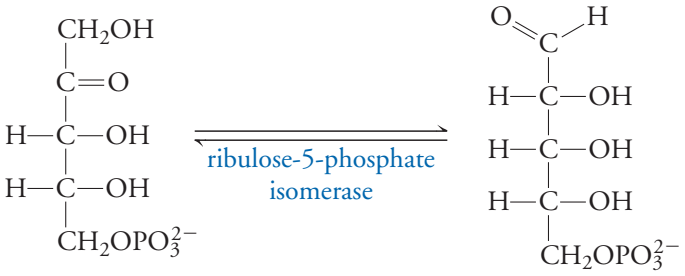


6-Phosphogluconate — Ribulose-5-phosphate

The two molecules of NADPH produced for each glucose molecule that enters the pathway are used primarily for biosynthetic reactions, such as fatty acid synthesis and the synthesis of deoxynucleotides.

Isomerization and interconversion reactions generate a variety of monosaccharides

The ribulose-5-phosphate product of the oxidative phase of the pentose phosphate pathway can isomerize to ribose-5-phosphate:

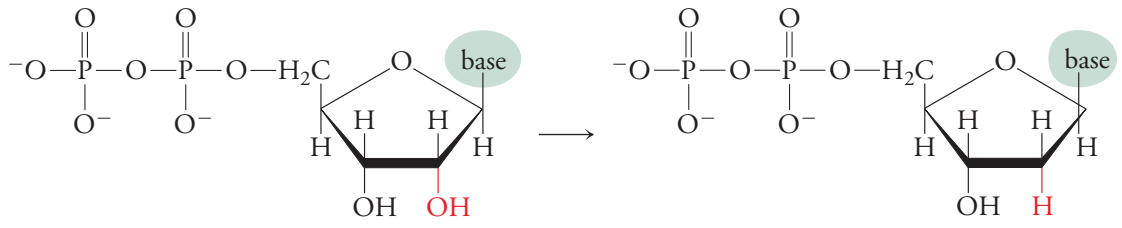


Ribulose-5-phosphate — Ribose-5-phosphate

Ribose-5-phosphate is the precursor of the ribose unit of nucleotides. In many cells, this marks the end of the pentose phosphate pathway, which has the net equation

$$\text{glucose-6-phosphate} + 2\ NADP^+ + H_2O \rightarrow \text{ribose-5-phosphate} + 2\ NADPH + CO_2 + 2\ H^+$$

Not surprisingly, the activity of the pentose phosphate pathway is high in rapidly dividing cells that must synthesize large amounts of DNA. In fact, the pentose phosphate pathway not only produces ribose, it also provides a reducing agent (NADPH) required for the reduction of ribose to deoxyribose. Ribonucleotide reductase carries out the reduction of nucleotide diphosphates (NDPs):



NDP — dNDP

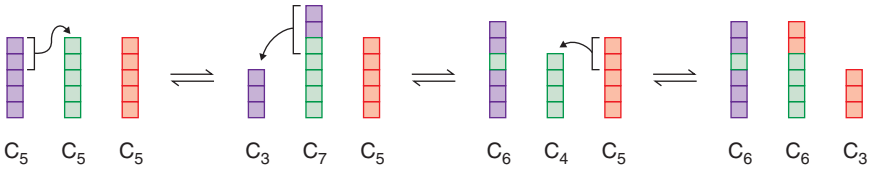


Figure 13-11 Rearrangements of the products of the pentose phosphate pathway. Three of the five-carbon products of the oxidative phase of the pentose phosphate pathway are converted to two fructose-6-phosphate and one glyceraldehyde-3-phosphate by reversible reactions involving the transfer of two- and three-carbon units. Each square represents a carbon atom in a monosaccharide. This pathway also allows ribose carbons to be used in glycolysis and gluconeogenesis.

The enzyme, which is oxidized in the process, is restored to its original state by a series of reactions in which NADPH is reduced.

In some cells, however, the need for NADPH for other biosynthetic reactions is greater than the need for ribose-5-phosphate. In this case, *the excess carbons of the pentose are recycled into intermediates of the glycolytic pathway so that they can be degraded to pyruvate or used in gluconeogenesis,* depending on the cell type and its metabolic needs.

A set of reversible reactions transform five-carbon ribulose units into six-carbon units (fructose-6-phosphate) and three-carbon units (glyceraldehyde-3-phosphate). These transformations are accomplished mainly by the enzymes transketolase and transaldolase, which transfer two- and three-carbon units among various intermediates to produce a set of sugars containing three, four, five, six, or seven carbons (the reaction catalyzed by transketolase was introduced in Section 7-2). **Figure 13-11** is a schematic view of this process. Because all these interconversions are reversible, *glycolytic intermediates can also be siphoned from glycolysis or gluconeogenesis to synthesize ribose-5-phosphate.* Thus, the cell can use some or all of the steps of the pentose phosphate pathway to generate NADPH, to produce ribose, and to interconvert other monosaccharides.

CONCEPT REVIEW

- What are the main products of the pentose phosphate pathway and how does the cell use them?
- How does the cell catabolize excess ribose groups?

A summary of glucose metabolism

The central position of glucose metabolism in all cells warrants its close study. Indeed, the enzymes of glycogen metabolism, glycolysis, gluconeogenesis, and the pentose phosphate pathway are among the best-studied proteins. In nearly all cases, detailed knowledge of their molecular structures has provided insight into their catalytic mechanisms and mode of regulation.

Although our coverage of glucose metabolism is far from exhaustive, this chapter describes quite a few enzymes and reactions, which are compiled in **Figure 13-12**. As you examine this diagram, keep in mind the following points, which also apply to the metabolic pathways we will encounter in subsequent chapters:

1. A metabolic pathway is a series of enzyme-catalyzed reactions, so the pathway's substrate is converted to its product in discrete steps.
2. A monomeric compound such as glucose is interconverted with its polymeric form (glycogen), with other monosaccharides (fructose-6-phosphate and ribose-5-phosphate, for example), and with smaller metabolites such as the three-carbon pyruvate.
3. Although anabolic and catabolic pathways may share some steps, their irreversible steps are catalyzed by enzymes unique to each pathway.
4. Certain reactions consume or produce free energy in the form of ATP. In most cases, these are phosphoryl-group transfer reactions.
5. Some steps are oxidation–reduction reactions that require or generate a reduced cofactor such as NADH or NADPH.

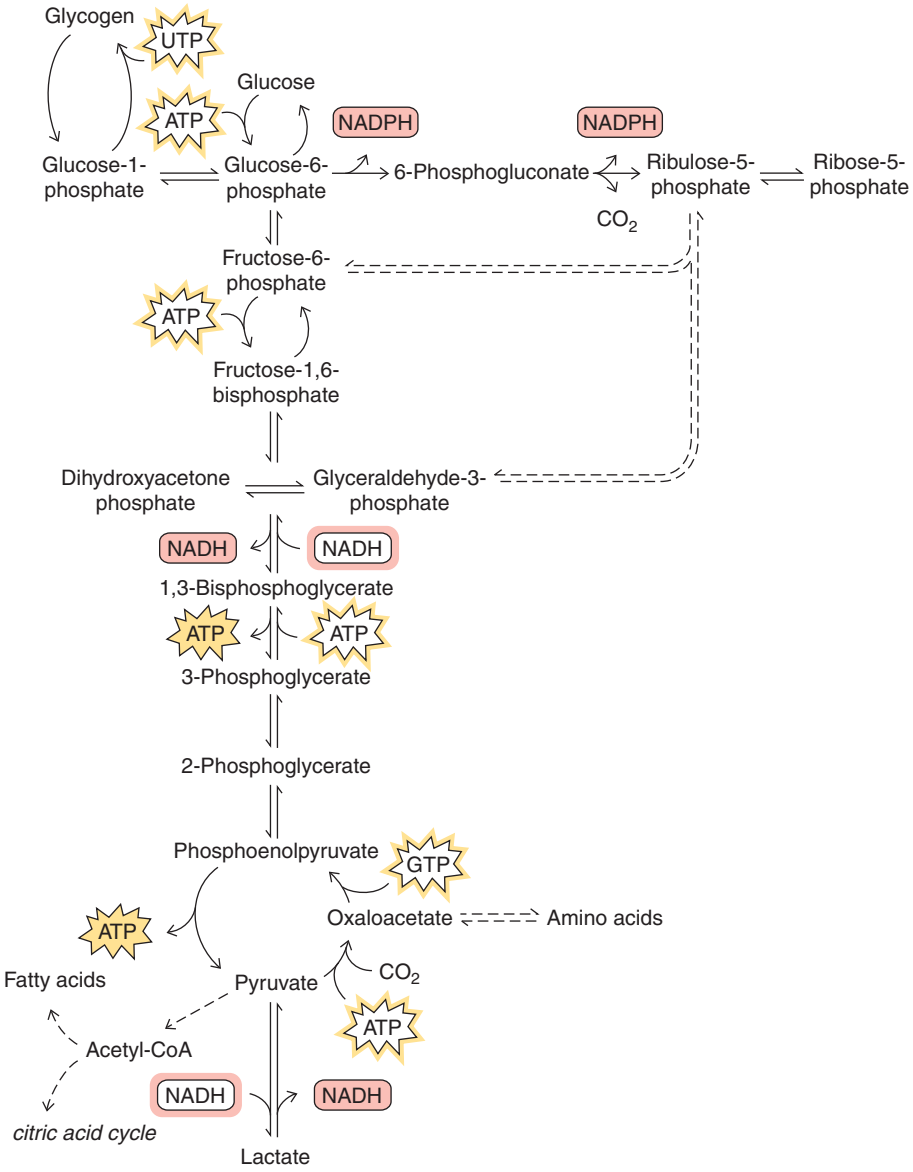


Figure 13-12 Summary of glucose metabolism. This diagram includes the pathways of glycogen synthesis and degradation, glycolysis, gluconeogenesis, and the pentose phosphate pathway. Dotted lines are used where the individual reactions are not shown. Filled gold symbols indicate ATP production; shadowed gold symbols indicate ATP consumption. Filled and shadowed red symbols represent the production and consumption of the reduced cofactors NADH and NADPH. ⊕ **See Animated Figure. Overview of glucose metabolism.**

SUMMARY

13-1 Glycolysis

- The pathway of glucose catabolism, or glycolysis, is a series of enzyme-catalyzed steps in which free energy is conserved as ATP or NADH.
- The 10 reactions of glycolysis convert the six-carbon glucose to two molecules of pyruvate and produce two molecules of NADH and two molecules of ATP. The first phase (reactions catalyzed by hexokinase, phosphoglucose isomerase, phosphofructokinase, aldolase, and triose phosphate isomerase) requires the investment of two ATP. The irreversible reaction catalyzed by phosphofructokinase is the rate-determining step and the major control point for glycolysis. The second phase of the pathway (reactions catalyzed by glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglycerate mutase, enolase, and pyruvate kinase) generates four ATP per glucose.
- Pyruvate may be reduced to lactate or ethanol, further oxidized by the citric acid cycle, or converted to other compounds.

13-2 Gluconeogenesis

- The pathway of gluconeogenesis converts two molecules of pyruvate to one molecule of glucose at a cost of six ATP. The pathway uses seven glycolytic enzymes, and the activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose bisphosphatase, and glucose-6-phosphatase bypass the three irreversible steps of glycolysis.
- A futile cycle involving phosphofructokinase and fructose bisphosphatase helps regulate the flux through glycolysis and gluconeogenesis.

13-3 Glycogen Synthesis and Degradation

- Glucose residues are incorporated into glycogen after first being activated by attachment to UDP.
- Phosphorolysis of glycogen produces phosphorylated glucose that can enter glycolysis. In the liver, this glucose is dephosphorylated and exported.

13-4 The Pentose Phosphate Pathway

- The pentose phosphate catabolic pathway for glucose yields NADPH and ribose groups. The five-carbon sugar intermediates can be converted to glycolytic intermediates.

GLOSSARY TERMS

glycolysis
gluconeogenesis
kinase
metabolically irreversible reaction
near-equilibrium reaction
rate-determining reaction
tautomerization
feed-forward activation
fermentation
futile cycle
glycogenolysis
glycogen storage disease
pentose phosphate pathway

PROBLEMS

13-1 Glycolysis

1. Which of the 10 reactions of glycolysis are **(a)** phosphorylations, **(b)** isomerizations, **(c)** oxidation–reductions, **(d)** dehydrations, and **(e)** carbon–carbon bond cleavages?

2. Which reactions of glycolysis can be reversed? Which reactions are irreversible? What is the significance of the metabolically irreversible reactions?

3. The $\Delta G^{\circ\prime}$ value for the hexokinase reaction is $-16.7\ \text{kJ} \cdot \text{mol}^{-1}$, while the ΔG value under cellular conditions is similar.
(a) What is the ratio of glucose-6-phosphate to glucose under standard conditions if the ratio of [ATP] to [ADP] is 10:1?
(b) How high would the ratio of glucose-6-phosphate to glucose have to be in order to reverse the hexokinase reaction by mass action?

4. What is the ratio of fructose-6-phosphate to glucose-6-phosphate under **(a)** standard conditions and **(b)** cellular conditions? In which direction does the reaction proceed under cellular conditions?

5. Except during starvation, the brain burns glucose as its sole metabolic fuel and consumes up to 40% of the body's circulating glucose.
(a) Why would hexokinase be the primary rate-determining step of glycolysis in the brain? (In tissues such as muscle, phosphofructokinase rather than hexokinase catalyzes the rate-determining step.)
(b) Brain hexokinase has a K_M for glucose that is 100 times lower than the concentration of circulating glucose (5 mM). What is the advantage of this low K_M?

6. Glucose is frequently administered intravenously (injected directly into the bloodstream) to patients as a food source. A new resident at a hospital where you are doing one of your rotations suggests administering glucose-6-phosphate instead. You recall from biochemistry class that the transformation of glucose to glucose-6-phosphate requires ATP and you consider the possibility that administering glucose-6-phosphate might save the patient energy. Should you use the resident's suggestion?

7. ADP stimulates the activity of phosphofructokinase (PFK), yet it is a product of the reaction, not a reactant. Explain this apparently contradictory regulatory strategy.

8. We can apply the T and R nomenclature used to describe the low- and high-affinity forms of hemoglobin (see Section 5-1) to allosteric enzymes like PFK. Allosteric inhibitors stabilize the T form, which has a low affinity for its substrate, while activators stabilize the high-affinity R form. Do the following allosteric effectors stabilize the T form of PFK or the R form?
(a) ADP (bacteria)
(b) PEP (bacteria)
(c) fructose-2,6-bisphosphate (mammals)

9. PFK isolated from the bacterium *Bacillus stearothermophilus* is a tetramer that binds fructose-6-phosphate with hyperbolic kinetics and a K_M of 23 μM. What happens to the K_M in the presence of phosphoenolpyruvate (PEP; see Figure 7-15)? Use the T $\rightleftharpoons$ R terminology to explain what happens.

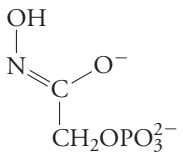
10. Refer to Figure 7-16. Why does the conformational change that results when Arg 162 changes places with Glu 161 result in a form of the enzyme that has a low affinity for its substrate?

11. Researchers isolated a yeast mutant that was deficient in the enzyme phosphofructokinase. The mutant yeast was able to grow on glycerol as an energy source, but not glucose. Explain why.

12. Researchers isolated a yeast PFK mutant in which a serine at the fructose-2,6-bisphosphate binding site was replaced with an aspartate residue. The amino acid substitution completely abolished the binding of fructose-2,6-bisphosphate (F26BP) to PFK. There was a dramatic decline in glucose consumption and ethanol production in the mutant compared to control yeast.
(a) Propose a hypothesis that explains why the mutant PFK cannot bind fructose-2,6-bisphosphate.
(b) What does the decline of glucose consumption and ethanol production in the yeast reveal about the role of fructose-2,6-bisphosphate in glycolysis?

13. Explain why iodoacetate was useful for determining the order of intermediates in glycolysis but provided misleading information about an enzyme's active site.

14. Biochemists use transition state analogs to determine the structure of a short-lived intermediate in an enzyme-catalyzed reaction. Because an enzyme binds tightly to the transition state, a compound that resembles the transition state should be a potent competitive inhibitor. Phosphoglycohydroxamate binds 150 times more tightly than dihydroxyacetone phosphate to triose phosphate isomerase. Based on this information, propose a structure for the intermediate of the triose phosphate isomerase reaction.



Phosphoglycohydroxamate

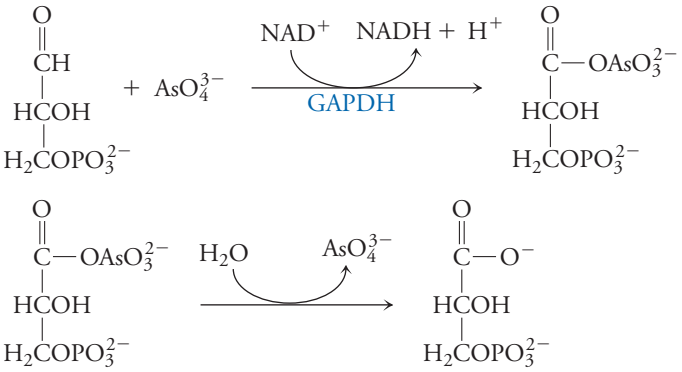
15. What is the ratio of glyceraldehyde-3-phosphate (GAP) to dihydroxyacetone phosphate (DHAP) in cells at 37°C under nonequilibrium conditions? Considering your answer to this question, how do you account for the fact that the conversion of DHAP to GAP occurs readily in cells?

16. Cancer cells have elevated levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which may account for the high rate of glycolysis seen in cancer cells. The compound methylglyoxal has been shown to inhibit GAPDH in cancer cells but not in normal cells. This observation may lead to the development of rapid screening assays for cancer cells and to the development of drugs for treatment of cancerous tumors.

(a) What mechanisms might be responsible for the elevated levels of GAPDH in cancer cells?

(b) Why might methylglyoxal inhibit GAPDH in cancer cells but not in normal cells?

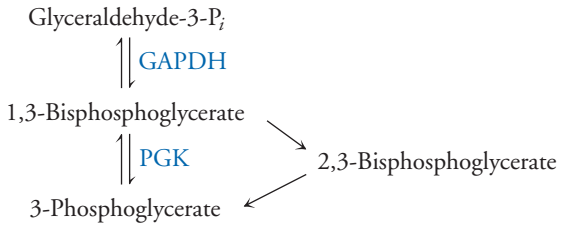
17. Arsenate, AsO_4^{3-}, acts as a phosphate analog and can replace phosphate in the GAPDH reaction. The product of this reaction is 1-arseno-3-phosphoglycerate. It is unstable and spontaneously hydrolyzes to form 3-phosphoglycerate, as shown below. What is the effect of arsenate on cells undergoing glycolysis?



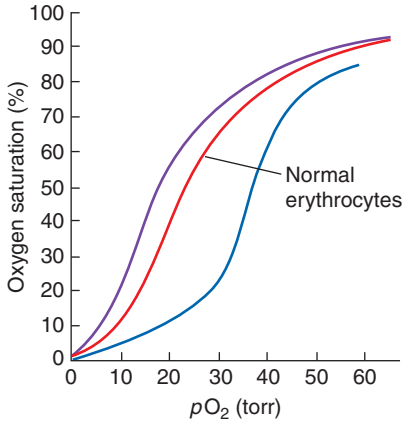
18. In several species of bacteria, activity of GADPH is controlled by the NADH/NAD^+ ratio. Does the activity of GAPDH increase or decrease when the NADH/NAD^+ ratio increases? Explain. Assume that only the forward direction of the reaction is relevant.

19. Phosphoglycerate kinase in red blood cells is bound to the plasma membrane. This allows the kinase reaction to be coupled to the Na,K-ATPase pump. How does the proximity of the enzyme to the membrane facilitate the action of the pump?

20. Red blood cells synthesize and degrade 2,3-bisphosphoglycerate (2,3-BPG) as a detour from the glycolytic pathway, as shown in the figure.



2,3-BPG decreases the oxygen affinity of hemoglobin by binding in the central cavity of the deoxygenated form of hemoglobin. This encourages delivery of oxygen to tissues. A defect in one of the glycolytic enzymes may affect levels of 2,3-BPG. The plot above right shows oxygen-binding curves for normal erythrocytes and for hexokinase- and pyruvate kinase–deficient erythrocytes. Identify which curve corresponds to which enzyme deficiency.



21. Vanadate, VO_4^{3-}, inhibits GAPDH, not by acting as a phosphate analog, but by interacting with essential —SH groups on the enzyme. What happens to cellular levels of phosphate, ATP, and 2,3-bisphosphoglycerate (see Problem 20) when red blood cells are incubated with vanadate?

22. The mechanism of plant phosphoglycerate mutase is different from the mechanism of mammalian phosphoglycerate mutase presented in the text. 3-Phosphoglycerate (3PG) binds to the plant enzyme, transfers its phosphate to the enzyme, and then the enzyme transfers the phosphate group back to the substrate to form 2-phosphoglycerate (2PG). When [^{32}P]-labeled 3PG is added to cultured **(a)** hepatocytes or **(b)** plant cells, what is the fate of the [^{32}P] label?

23. Which intermediates of glycolysis accumulate if fluoride ions are present?

24. Assuming a standard free energy change of 30.5 kJ · mol^{-1} for the synthesis of ATP from ADP and P_i, how many molecules of ATP can be theoretically produced by the catabolism of glucose to **(a)** lactate or **(b)** CO_2 (see Table 13-1)?

25. What happens to the [ADP]/[ATP] and [NAD^+]/[NADH] ratios in red blood cells with a pyruvate kinase deficiency (see Problem 20)?

26. One of the symptoms of a pyruvate kinase deficiency (see Problem 25) is hemolytic anemia, in which red blood cells swell and eventually lyse. Explain why the deficiency of the enzyme brings about this symptom.

27. Organisms such as yeast growing under anaerobic conditions can convert pyruvate to alcohol in a process called fermentation, as described in the text. Instead of being converted to lactate, pyruvate is converted to ethanol in a two-step reaction. Why is the second step of this process essential to the yeast cell?

28. Several studies have shown that aluminum inhibits PFK in liver cells.

(a) Compare the production of pyruvate by perfused livers in control and aluminum-treated rats using fructose as an energy source.

(b) What would the experimental results be if glucose was used instead of fructose?

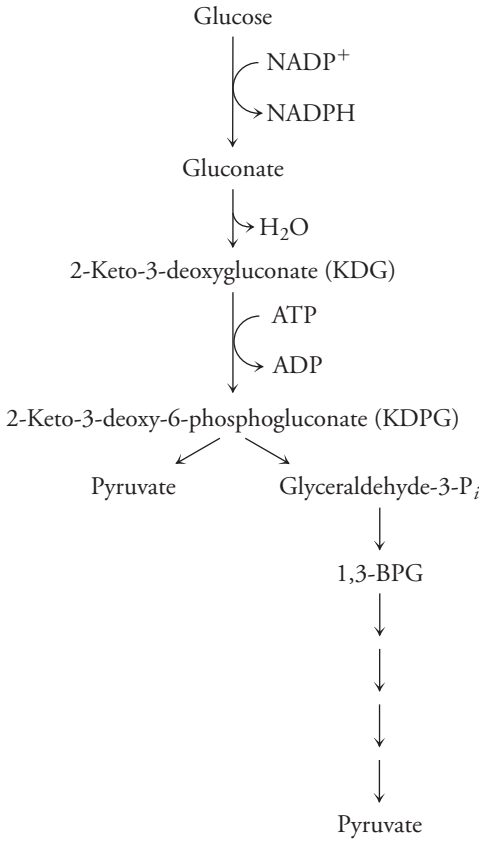
29. Drinking methanol can cause blindness and death, depending on the dosage. The causative agent is formaldehyde derived from methanol.

(a) Draw the balanced chemical reaction for the conversion of methanol to formaldehyde.

(b) Why would administering whiskey (ethanol) to a person poisoned with methanol be a good antidote?

30. The term *turbo design* has been used to describe pathways such as glycolysis that have one or more ATP-consuming steps followed by one or more ATP-producing steps with a net yield of ATP production for the pathway overall. Mathematical models have shown that "turbo" pathways have the risk of substrate-accelerated death unless there is a "guard at the gate," that is, a mechanism for inhibiting an early step of the pathway. In yeast, hexokinase is inhibited by a complex mechanism mediated by trehalose-6-phosphate synthase (TPSI). Mutant yeast in which TPSI is defective (there is no "guard at the gate") die if grown under conditions of high glucose concentration. Explain why.

31. Recent studies have shown that the halophilic organism *Haloccus saccharolyticus* degrades glucose via the Entner–Doudoroff pathway rather than by the glycolytic pathway presented in this chapter. A modified scheme of the Entner–Doudoroff pathway is shown here.

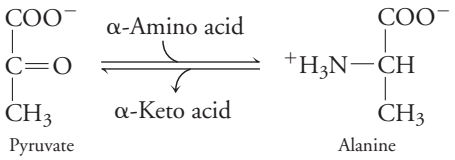


(a) What is the ATP yield per mole of glucose for this pathway?
(b) Describe (in general) what kinds of reactions would need to follow the Entner–Doudoroff pathway in this organism.

32. Trypanosomes living in the bloodstream obtain all their free energy from glycolysis. They take up glucose from the host's blood and excrete pyruvate as a waste product. In this part of their life cycle, trypanosomes do not carry out any oxidative phosphorylation, but they do use another oxygen-dependent pathway, which is absent in mammals, to oxidize NADH.
(a) Why is this other pathway necessary?
(b) Would the pathway be necessary if the trypanosome excreted lactate rather than pyruvate?
(c) Why would this pathway be a good target for antiparasitic drugs?

13-2 Gluconeogenesis

33. Flux through the opposing pathways of glycolysis and gluconeogenesis is controlled in several ways.
(a) Explain how the activation of pyruvate carboxylase by acetyl-CoA affects glucose metabolism.
(b) Pyruvate can undergo a reversible amino-group transfer reaction to yield alanine (see Section 12-2). Alanine is an allosteric effector of pyruvate kinase. Would you expect alanine to stimulate or inhibit pyruvate kinase? Explain.



34. A liver biopsy of a four-year-old boy indicated that the fructose-1,6-bisphosphatase enzyme activity was 20% of normal. The patient's blood glucose levels were normal at the beginning of a fast but then decreased suddenly. Pyruvate and alanine concentrations were also elevated, as was the glyceraldehyde-3-phosphate/dihydroxyacetone phosphate ratio. Explain the reason for these symptoms.

35. Insulin is one of the major hormones that regulates gluconeogenesis. Insulin acts in part by decreasing the transcription of genes coding for certain gluconeogenic enzymes. For which genes would you expect insulin to suppress transcription?

36. Type 2 diabetes is characterized by insulin resistance, in which insulin is unable to perform its many functions. What symptom would you expect in a type 2 diabetic patient if insulin is unable to perform the function described in Problem 35?

37. The concentration of fructose-2,6-bisphosphate (F26BP) is regulated in the cell by a homodimeric enzyme with two catalytic activities: a kinase that phosphorylates fructose-6-phosphate on the C2 hydroxyl group to form fructose-2,6-bisphosphate and a phosphatase that removes the phosphate group.
(a) Which enzyme activity, the kinase or the phosphatase, would you expect to be active under fasting conditions? Explain.
(b) Which hormone is likely to be responsible for inducing this activity?
(c) Consult Section 10-2 and propose a mechanism for this induction.

38. The "carbon skeletons" of most amino acids can be converted to glucose, a process that may require many enzymatic steps. Which amino acids can enter the gluconeogenic pathway directly after undergoing deamination (a reaction in which the carbon with the amino group becomes a ketone)?

39. Brazilin, a compound found in aqueous extracts of sappan wood, has been used to treat diabetics in Korea. Brazilin increases the activity of the enzyme that produces fructose-2,6-bisphosphate, and the compound also stimulates the activity of pyruvate kinase.
(a) What is the effect of adding brazilin to hepatocytes (liver cells) in culture?
(b) Why would brazilin be an effective treatment for diabetes?

40. Metformin is a drug that decreases the expression of phosphoenolpyruvate carboxykinase. Explain why metformin would be helpful in treating diabetes.

41. Draw a diagram that illustrates how lactate released from the muscle is converted back to glucose in the liver. What is the cost (in ATP) of running this cycle?

42. Draw a diagram that illustrates how alanine (see Problem 33b) released from the muscle is converted back to glucose in the liver. What is the physiological cost if this cycle runs for a prolonged period of time?

13-3 Glycogen Synthesis and Degradation

43. Beer is produced from raw materials such as wheat and barley. Explain why the grains are allowed to sprout, a process in which their starch is broken down to glucose, before fermentation begins.

44. Some bread manufacturers add amylase to bread dough prior to the fermentation process. What role does this enzyme (see Section 12-1) play in the bread-making process?

45. Glycogen is degraded via a phosphorolysis process, which produces glucose-1-phosphate. What advantage does this process have over a simple hydrolysis, which would produce glucose instead of phosphorylated glucose?

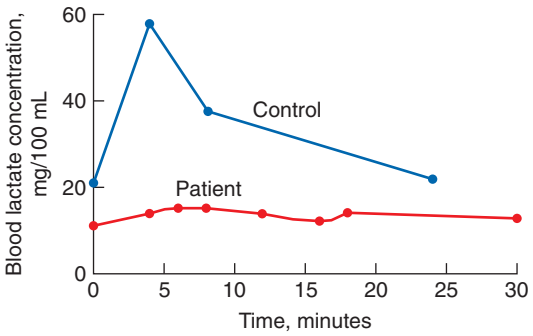
46. The equation for the degradation of glycogen is shown below.
(a) What is the ratio of $[P_i]/[G1P]$ under standard conditions?
(b) What is the value of ΔG under cellular conditions when the $[P_i]/[G1P]$ ratio is 50:1?

$$\text{glycogen } (n \text{ residues}) + P_i \xrightleftharpoons{\text{phosphorylase}} \text{glycogen } (n-1 \text{ residues}) + G1P \quad \Delta G^{\circ\prime} = +3.1 \text{ kJ} \cdot \text{mol}^{-1}$$

47. During even mild exertion, individuals with McArdle's disease experience painful muscle cramps due to a genetic defect in glycogen phosphorylase, the enzyme that breaks down glycogen. Yet the muscles in these individuals contain normal amounts of glycogen. What did this observation tell researchers about the pathways for glycogen degradation and glycogen synthesis?

48. Patients with McArdle's disease have normal liver glycogen content and structure. Identify the type of glycogen storage disease as listed in the table in Box 13-C.

49. A patient with McArdle's disease performs ischemic (anaerobic) exercise for as long as he is able to do so. Blood is withdrawn from the patient every few minutes during the exercise period and tested for lactate. The patient's samples are compared with control samples from a patient who does not suffer from a glycogen storage disease. The results are shown in the figure. Why does the lactate concentration increase in the normal patient? Why is there no corresponding increase in the patient's lactate concentration?

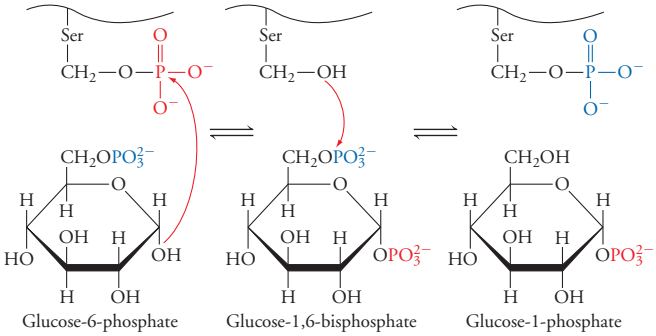


50. Does a patient with McArdle's disease (see Problems 47–49) suffer from hypoglycemia, hyperglycemia, or neither?

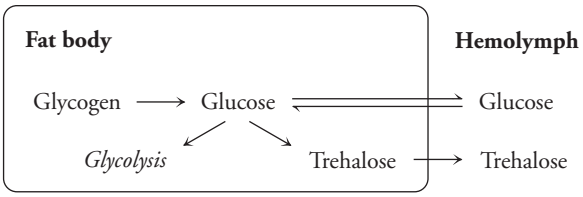
51. Patients with von Gierke's disease (type I glycogen storage disease) have a deficiency of glucose-6-phosphatase. One of the most prominent symptoms of the disease is a protruding abdomen due to an enlarged liver. Explain why the liver is enlarged in patients with von Gierke's disease.

52. Does a patient with von Gierke's disease (see Problem 51) suffer from hypoglycemia, hyperglycemia, or neither?

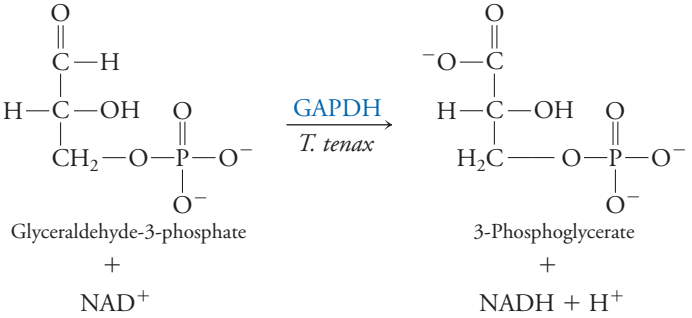
53. The mechanism of the phosphoglucomutase enzyme is similar to that of the plant mutase described in Problem 22 and is shown here. On occasion, the glucose-1,6-bisphosphate dissociates from the enzyme. Why does the dissociation of glucose-1,6-bisphosphate inhibit the enzyme?



54. Trehalose is one of the major sugars in the insect hemolymph (the fluid that circulates through the insect's body). It is a disaccharide consisting of two linked glucose residues. In the hemolymph, trehalose serves as a storage form of glucose and also helps protect the insect from desiccation and freezing. Its concentration in the hemolymph must be closely regulated. Trehalose is synthesized in the insect fat body, which plays a role in metabolism analogous to the vertebrate liver. Recent studies on the insect *Manduca sexta* have shown that during starvation, hemolymph glucose concentration decreases, which results in an increase in fat body glycogen phosphorylase activity and a decrease in the concentration of fructose-2,6-bisphosphate. What effect do these changes have on hemolymph trehalose concentration in the fasted insect?



55. The glycolytic pathway in the thermophilic archaebacterium *Thermoproteus tenax* differs from the pathway presented in this chapter. The phosphofructokinase reaction in *T. tenax* is reversible and depends on pyrophosphate rather than ATP. In addition, *T. tenax* has two glyceraldehyde-3-phosphate dehydrogenase (GADPH) isozymes. The "phosphorylating GAPDH" is similar to the enzyme described in this chapter. The second isozyme is the irreversible "nonphosphorylating GAPDH," which catalyzes the reaction shown below. *T. tenax* relies on glycogen stores as a source of energy. What is the ATP yield for one mole of glucose oxidized by the pathway that uses the nonphosphorylating GAPDH enzyme?



56. Individuals with fructose intolerance lack fructose-1-phosphate aldolase, a liver enzyme essential for catabolizing fructose. In the absence of fructose-1-phosphate aldolase, fructose-1-phosphate accumulates in the liver and inhibits glycogen phosphorylase and fructose-1,6-bisphosphatase.

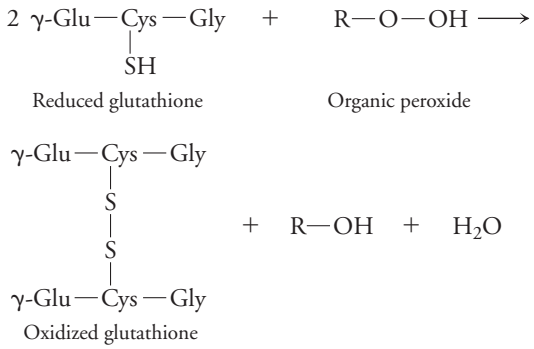
(a) Explain why individuals with fructose intolerance exhibit hypoglycemia (low blood sugar).
(b) Administering glycerol and dihydroxyacetone phosphate does not alleviate the hypoglycemia, but administering galactose does relieve the hypoglycemia. Explain.

13-4 The Pentose Phosphate Pathway

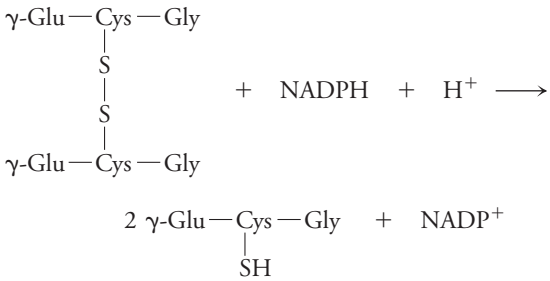
57. Most metabolic pathways include an enzyme-catalyzed reaction that commits a metabolite to continue through the pathway.
(a) Identify the first committed step of the pentose phosphate pathway. Explain your reasoning.
(b) Hexokinase catalyzes an irreversible reaction at the start of glycolysis. Does this step commit glucose to continue through glycolysis?

58. A given metabolite may follow more than one metabolic pathway. List all the possible fates of glucose-6-phosphate in **(a)** a liver cell and **(b)** a muscle cell.

59. Reduced glutathione, a tripeptide containing a Cys residue, is found in red blood cells, where it reduces organic peroxides formed in cellular structures exposed to high concentrations of reactive oxygen.



Reduced glutathione also plays a role in maintaining normal red blood cell structure and keeping the iron ion of hemoglobin in the +2 oxidation state. Glutathione is regenerated as shown in the following reaction:



Use this information to predict the physiological effects of a glucose-6-phosphate dehydrogenase deficiency.

60. Experiments were carried out in cultured cells to determine the relationship between glucose-6-phosphate dehydrogenase (G6PDH) activity and rates of cell growth. Cells were cultured in a medium supplemented with serum, which contains growth factors that stimulate G6PDH activity. Predict how the cellular NADPH/$NADP^+$ ratio would change under the following circumstances:
(a) Serum is withdrawn from the medium.
(b) DHEA, an inhibitor of glucose-6-phosphate dehydrogenase, is added.
(c) The oxidant H_2O_2 is added.
(d) Serum is withdrawn and H_2O_2 is added.

61. Write a mechanism for the nonenzymatic hydrolysis of 6-phosphogluconolactone to 6-phosphogluconate.

62. Enzymes in the soil fungus *Aspergillus nidulans* use NADPH as a coenzyme when converting nitrate to the ammonium ion. When the fungus was cultured in a growth medium containing nitrate, it was discovered that the activities of several enzymes involved in glucose metabolism increased. What enzymes are good candidates for regulation under these conditions? Explain.

63. Several studies have shown that the metabolite glucose-1,6-bisphosphate (G16BP) regulates several pathways of carbohydrate metabolism by inhibiting or activating key enzymes. The effect of G16BP on several important enzymes is summarized in the table below. What pathways are active when G16BP is present? What pathways are inactive? What is the overall effect? Explain.

Enzyme	Effect of G16BP
Hexokinase	Inhibits
Phosphofructokinase (PFK)	Activates
Pyruvate kinase (PK)	Activates
Phosphoglucomutase	Activates
6-Phosphogluconate dehydrogenase	Inhibits

64. Xylulose-5-phosphate acts as an intracellular signaling molecule that activates kinases and phosphatases in liver cells. As a result of this signaling, there is an increase in the activity of the enzyme that produces fructose-2,6-bisphosphate, and the expression of genes for lipid synthesis is increased. What is the net effect of these responses?

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chapter 14

THE CITRIC ACID CYCLE

[Tom Merton/OJO Images/Getty Images, Inc.]

▸▸ WHERE does exhaled CO_2 come from?

Inflating a toy balloon is one way to capture exhaled breath, which contains CO_2. It is tempting to believe that in air-breathing animals, the oxygen that is inhaled is transformed into carbon dioxide that is exhaled. In fact, the two types of molecules never directly interact inside the body. In this chapter we will see that exhaled CO_2, a waste product of cellular metabolism, is generated mostly by operation of the citric acid cycle. This metabolic pathway converts the carbons of metabolic fuels into CO_2, saving their energy for ATP synthesis.

THIS CHAPTER IN CONTEXT

Part 1 Foundations

Part 2 Molecular Structure and Function

Part 3 Metabolism

14 The Citric Acid Cycle

Part 4 Genetic Information

Do You Remember?

- **Enzymes accelerate chemical reactions using acid–base catalysis, covalent catalysis, and metal ion catalysis (Section 6-2).**
- **Coenzymes such as NAD^+ and ubiquinone collect electrons from compounds that become oxidized (Section 12-2).**
- **Metabolic pathways in cells are connected and are regulated (Section 12-2).**
- **Many vitamins, substances that humans cannot synthesize, are components of coenzymes (Section 12-2).**
- **Pyruvate can be converted to lactate, acetyl-CoA, or oxaloacetate (Section 13-1).**