

GLYCOLYSIS, GLUCONEOGENESIS, AND THE PENTOSE PHOSPHATE PATHWAY

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The problem of alcoholic fermentation, of the origin and nature of that mysterious and apparently spontaneous change, which converted the insipid juice of the grape into stimulating wine, seems to have exerted a fascination over the minds of natural philosophers from the very earliest times.

—Arthur Harden, *Alcoholic Fermentation*, 1923

Glucose occupies a central position in the metabolism of plants, animals, and many microorganisms. It is relatively rich in potential energy, and thus a good fuel; the complete oxidation of glucose to carbon dioxide and water proceeds with a standard free-energy change of $-2,840 \text{ kJ/mol}$. By storing glucose as a high molecular weight polymer such as starch or glycogen, a cell can stockpile large quantities of hexose units while maintaining a relatively low cytosolic osmolarity. When energy demands increase, glucose can be released from these intracellular storage polymers and used to produce ATP either aerobically or anaerobically.

Glucose is not only an excellent fuel, it is also a remarkably versatile precursor, capable of supplying a huge array of metabolic intermediates for biosynthetic reactions. A bacterium such as *Escherichia coli* can obtain from glucose the carbon skeletons for every amino acid, nucleotide, coenzyme, fatty acid, or other metabolic intermediate it needs for growth. A comprehensive study of the metabolic fates of glucose would encompass hundreds or thousands of transformations. In animals and vascular plants, glucose has three major fates: it may be stored (as a polysaccharide or as sucrose); oxidized to a three-carbon compound (pyruvate) via glycolysis to provide ATP and metabolic intermediates; or oxidized via the pentose phosphate (phosphogluconate) pathway to yield ribose 5-phosphate for nucleic acid synthesis and NADPH for reductive biosynthetic processes (Fig. 14–1).

Organisms that do not have access to glucose from other sources must make it. Photosynthetic organisms make glucose by first reducing atmospheric CO_2 to trioses, then converting the trioses to glucose. Non-photosynthetic cells make glucose from simpler three- and four-carbon precursors by the process of gluconeogenesis, effectively reversing glycolysis in a pathway that uses many of the glycolytic enzymes.

In this chapter we describe the individual reactions of glycolysis, gluconeogenesis, and the pentose phosphate pathway and the functional significance of each pathway. We also describe the various fates of the pyruvate produced by glycolysis; they include the fermentations that are used by many organisms in anaerobic niches to produce ATP and that are exploited industrially as sources of ethanol, lactic acid, and other

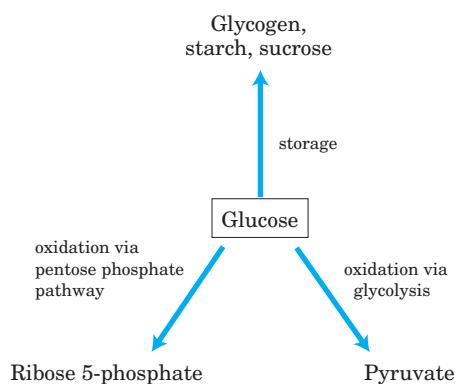


FIGURE 14-1 Major pathways of glucose utilization. Although not the only possible fates for glucose, these three pathways are the most significant in terms of the amount of glucose that flows through them in most cells.

commercially useful products. And we look at the pathways that feed various sugars from mono-, di-, and polysaccharides into the glycolytic pathway. The discussion of glucose metabolism continues in Chapter 15, where we describe the opposing anabolic and catabolic pathways that connect glucose and glycogen, and use the processes of carbohydrate synthesis and degradation as examples of the many mechanisms by which organisms regulate metabolic pathways.

14.1 Glycolysis

In **glycolysis** (from the Greek *glykys*, meaning “sweet,” and *lysis*, meaning “splitting”), a molecule of glucose is degraded in a series of enzyme-catalyzed reactions to yield two molecules of the three-carbon compound pyruvate. During the sequential reactions of glycolysis, some of the free energy released from glucose is conserved in the form of ATP and NADH. Glycolysis was the first metabolic pathway to be elucidated and is probably the best understood. From Eduard Buchner’s discovery in 1897 of fermentation in broken extracts of yeast cells until the elucidation of the whole pathway in yeast (by Otto Warburg and Hans von Euler-Chelpin)

and in muscle (by Gustav Embden and Otto Meyerhof) in the 1930s, the reactions of glycolysis in extracts of yeast and muscle were a major focus of biochemical research. The philosophical shift that accompanied these discoveries was announced by Jacques Loeb in 1906:

Through the discovery of Buchner, Biology was relieved of another fragment of mysticism. The splitting up of sugar into CO_2 and alcohol is no more the effect of a “vital principle” than the splitting up of cane sugar by invertase. The history of this problem is instructive, as it warns us against considering problems as beyond our reach because they have not yet found their solution.

The development of methods of enzyme purification, the discovery and recognition of the importance of coenzymes such as NAD, and the discovery of the pivotal metabolic role of ATP and other phosphorylated compounds all came out of studies of glycolysis. The glycolytic enzymes of many species have long since been purified and thoroughly studied.

Glycolysis is an almost universal central pathway of glucose catabolism, the pathway with the largest flux of carbon in most cells. The glycolytic breakdown of glucose is the sole source of metabolic energy in some mammalian tissues and cell types (erythrocytes, renal medulla, brain, and sperm, for example). Some plant tissues that are modified to store starch (such as potato tubers) and some aquatic plants (watercress, for example) derive most of their energy from glycolysis; many anaerobic microorganisms are entirely dependent on glycolysis.

Fermentation is a general term for the *anaerobic* degradation of glucose or other organic nutrients to obtain energy, conserved as ATP. Because living organisms first arose in an atmosphere without oxygen, anaerobic breakdown of glucose is probably the most ancient biological mechanism for obtaining energy from organic fuel molecules. In the course of evolution, the chemistry of this reaction sequence has been completely conserved; the glycolytic enzymes of vertebrates are closely

similar, in amino acid sequence and three-dimensional structure, to their homologs in yeast and spinach. Glycolysis differs among species only in the details of its regulation and in the subsequent metabolic fate of the pyruvate formed. The thermodynamic principles and the types of regulatory mechanisms that govern glycolysis are common to all pathways of cell metabolism. A study of glycolysis can therefore serve as a model for many aspects of the pathways discussed throughout this book.



Hans von Euler-Chelpin,
1873–1964



Gustav Embden,
1874–1933



Otto Meyerhof,
1884–1951

Before examining each step of the pathway in some detail, we take a look at glycolysis as a whole.

An Overview: Glycolysis Has Two Phases

The breakdown of the six-carbon glucose into two molecules of the three-carbon pyruvate occurs in ten steps, the first five of which constitute the *preparatory phase* (Fig. 14–2a). In these reactions, glucose is first phosphorylated at the hydroxyl group on C-6 (step ①). The D-glucose 6-phosphate thus formed is converted to D-fructose 6-phosphate (step ②), which is again phosphorylated, this time at C-1, to yield D-fructose 1,6-bisphosphate (step ③). For both phosphorylations, ATP is the phosphoryl group donor. As all sugar derivatives in glycolysis are the D isomers, we will usually omit the D designation except when emphasizing stereochemistry.

Fructose 1,6-bisphosphate is split to yield two three-carbon molecules, dihydroxyacetone phosphate and glyceraldehyde 3-phosphate (step ④); this is the “lysis” step that gives the pathway its name. The dihydroxyacetone phosphate is isomerized to a second molecule of glyceraldehyde 3-phosphate (step ⑤), ending the first phase of glycolysis. From a chemical perspective, the isomerization in step ② is critical for setting up the phosphorylation and C—C bond cleavage reactions in steps ③ and ④, as detailed later. Note that two molecules of ATP are invested before the cleavage of glucose into two three-carbon pieces; later there will be a good return on this investment. To summarize: in the preparatory phase of glycolysis the energy of ATP is invested, raising the free-energy content of the intermediates, and the carbon chains of all the metabolized hexoses are converted into a common product, glyceraldehyde 3-phosphate.

The energy gain comes in the *payoff phase* of glycolysis (Fig. 14–2b). Each molecule of glyceraldehyde 3-phosphate is oxidized and phosphorylated by inorganic phosphate (*not* by ATP) to form 1,3-bisphosphoglycerate (step ⑥). Energy is then released as the two molecules of 1,3-bisphosphoglycerate are converted to two molecules of pyruvate (steps ⑦ through ⑩). Much of this energy is conserved by the coupled phosphorylation of four molecules of ADP to ATP. The net yield is two molecules of ATP per molecule of glucose used, because two molecules of ATP were invested in the preparatory phase. Energy is also conserved in the payoff phase in the formation of two molecules of NADH per molecule of glucose.

In the sequential reactions of glycolysis, three types of chemical transformations are particularly noteworthy: (1) degradation of the carbon skeleton of glucose to yield pyruvate, (2) phosphorylation of ADP to ATP by high-energy phosphate compounds formed during glycolysis, and (3) transfer of a hydride ion to NAD^+ , forming NADH.

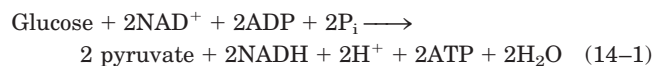
Fates of Pyruvate With the exception of some interesting variations in the bacterial realm, the pyruvate formed by glycolysis is further metabolized via one of three catabolic routes. In aerobic organisms or tissues, under aerobic conditions, glycolysis is only the first stage in the complete degradation of glucose (Fig. 14–3). Pyruvate is oxidized, with loss of its carboxyl group as CO_2 , to yield the acetyl group of acetyl-coenzyme A; the acetyl group is then oxidized completely to CO_2 by the citric acid cycle (Chapter 16). The electrons from these oxidations are passed to O_2 through a chain of carriers in the mitochondrion, to form H_2O . The energy from the electron-transfer reactions drives the synthesis of ATP in the mitochondrion (Chapter 19).

The second route for pyruvate is its reduction to lactate via **lactic acid fermentation**. When vigorously contracting skeletal muscle must function under low-oxygen conditions (**hypoxia**), NADH cannot be reoxidized to NAD^+ , but NAD^+ is required as an electron acceptor for the further oxidation of pyruvate. Under these conditions pyruvate is reduced to lactate, accepting electrons from NADH and thereby regenerating the NAD^+ necessary for glycolysis to continue. Certain tissues and cell types (retina and erythrocytes, for example) convert glucose to lactate even under aerobic conditions, and lactate is also the product of glycolysis under anaerobic conditions in some microorganisms (Fig. 14–3).

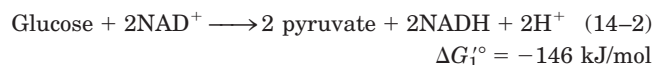
The third major route of pyruvate catabolism leads to ethanol. In some plant tissues and in certain invertebrates, protists, and microorganisms such as brewer's yeast, pyruvate is converted under hypoxic or anaerobic conditions into ethanol and CO_2 , a process called **ethanol (alcohol) fermentation** (Fig. 14–3).

The oxidation of pyruvate is an important catabolic process, but pyruvate has anabolic fates as well. It can, for example, provide the carbon skeleton for the synthesis of the amino acid alanine. We return to these anabolic reactions of pyruvate in later chapters.

ATP Formation Coupled to Glycolysis During glycolysis some of the energy of the glucose molecule is conserved in ATP, while much remains in the product, pyruvate. The overall equation for glycolysis is



For each molecule of glucose degraded to pyruvate, two molecules of ATP are generated from ADP and P_i . We can now resolve the equation of glycolysis into two processes—the conversion of glucose to pyruvate, which is exergonic:



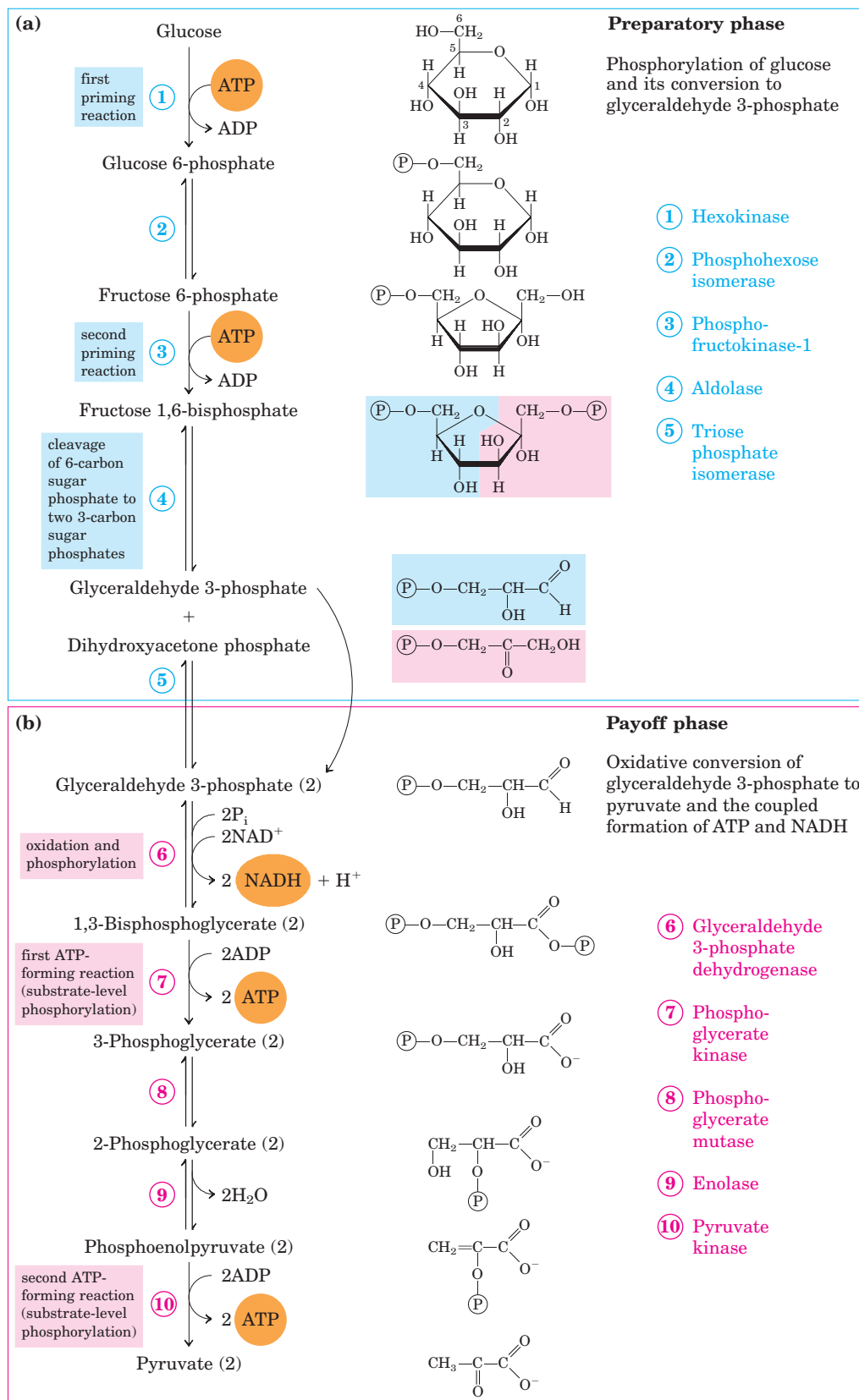
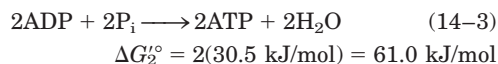


FIGURE 14-2 The two phases of glycolysis. For each molecule of glucose that passes through the preparatory phase (a), two molecules of glyceraldehyde 3-phosphate are formed; both pass through the payoff phase (b). Pyruvate is the end product of the second phase of glycolysis. For each glucose molecule, two ATP are consumed in the preparatory phase and four ATP are produced in the payoff phase, giving a

net yield of two ATP per molecule of glucose converted to pyruvate. The numbered reaction steps are catalyzed by the enzymes listed on the right, and also correspond to the numbered headings in the text discussion. Keep in mind that each phosphoryl group, represented here as (P), has two negative charges ($-\text{PO}_3^{2-}$).

and the formation of ATP from ADP and P_i , which is endergonic:



The sum of Equations 14-2 and 14-3 gives the overall standard free-energy change of glycolysis, ΔG_s° :

$$\Delta G_s^{\circ} = \Delta G_1^{\circ} + \Delta G_2^{\circ} = -146 \text{ kJ/mol} + 61.0 \text{ kJ/mol}$$

$$= -85 \text{ kJ/mol}$$

Under standard conditions and in the cell, glycolysis is an essentially irreversible process, driven to completion by a large net decrease in free energy. At the actual intracellular concentrations of ATP, ADP, and P_i (see Box 13-1) and of glucose and pyruvate, the energy released in glycolysis (with pyruvate as the end product) is recovered as ATP with an efficiency of more than 60%.

Energy Remaining in Pyruvate Glycolysis releases only a small fraction of the total available energy of the glucose molecule; the two molecules of pyruvate formed by glycolysis still contain most of the chemical potential energy of glucose, energy that can be extracted by oxidative reactions in the citric acid cycle (Chapter 16) and oxidative phosphorylation (Chapter 19).

Importance of Phosphorylated Intermediates Each of the nine glycolytic intermediates between glucose and pyruvate is phosphorylated (Fig. 14-2). The phosphoryl groups appear to have three functions.

1. Because the plasma membrane generally lacks transporters for phosphorylated sugars, the phosphorylated glycolytic intermediates cannot leave the cell. After the initial phosphorylation, no further energy is necessary to retain phosphorylated intermediates in the cell, despite the large difference in their intracellular and extracellular concentrations.
2. Phosphoryl groups are essential components in the enzymatic conservation of metabolic energy. Energy released in the breakage of phosphoanhydride bonds (such as those in ATP) is partially conserved in the formation of phosphate esters such as glucose 6-phosphate. High-energy phosphate compounds formed in glycolysis (1,3-bisphoglycerate and phosphoenolpyruvate) donate phosphoryl groups to ADP to form ATP.
3. Binding energy resulting from the binding of phosphate groups to the active sites of enzymes lowers the activation energy and increases the specificity of the enzymatic reactions (Chapter 6). The phosphate groups of ADP, ATP, and the glycolytic intermediates form complexes with Mg^{2+} , and the substrate binding sites of many glycolytic enzymes are specific for these Mg^{2+} complexes. Most glycolytic enzymes require Mg^{2+} for activity.

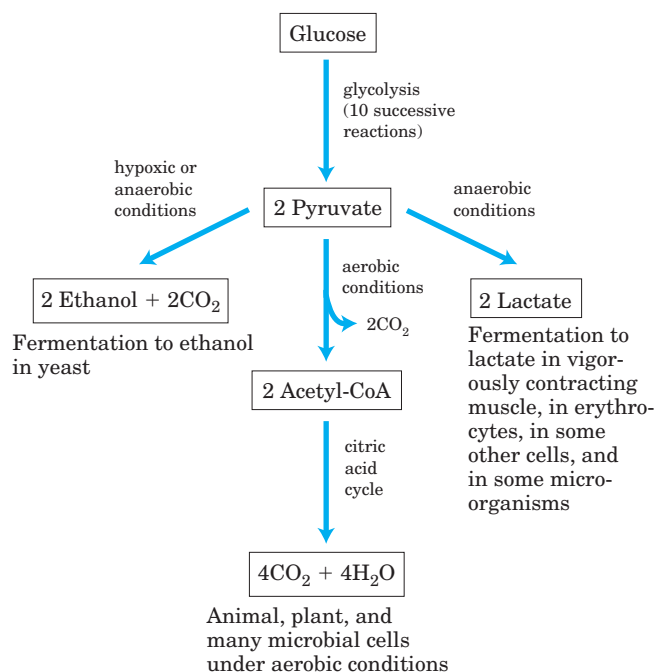


FIGURE 14-3 Three possible catabolic fates of the pyruvate formed in glycolysis. Pyruvate also serves as a precursor in many anabolic reactions, not shown here.

The Preparatory Phase of Glycolysis Requires ATP

In the preparatory phase of glycolysis, two molecules of ATP are invested and the hexose chain is cleaved into two triose phosphates. The realization that *phosphorylated* hexoses were intermediates in glycolysis came slowly and serendipitously. In 1906, Arthur Harden and William Young tested their hypothesis that inhibitors of proteolytic enzymes would stabilize the glucose-fermenting enzymes in yeast extract. They added blood serum (known to contain inhibitors of proteolytic enzymes) to yeast extracts and observed the predicted stimulation of glucose metabolism. However, in a control experiment intended to show that boiling the serum destroyed the stimulatory activity, they discovered that boiled serum was just as effective at stimulating glycolysis. Careful examination and testing of the contents of



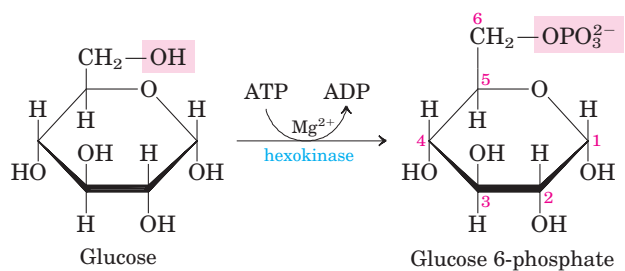
Arthur Harden,
1865–1940



William Young,
1878–1942

the boiled serum revealed that inorganic phosphate was responsible for the stimulation. Harden and Young soon discovered that glucose added to their yeast extract was converted to a hexose biphosphate (the “Harden-Young ester,” eventually identified as fructose 1,6-bisphosphate). This was the beginning of a long series of investigations on the role of organic esters of phosphate in biochemistry, which has led to our current understanding of the central role of phosphoryl group transfer in biology.

① Phosphorylation of Glucose In the first step of glycolysis, glucose is activated for subsequent reactions by its phosphorylation at C-6 to yield **glucose 6-phosphate**, with ATP as the phosphoryl donor:



$$\Delta G'^{\circ} = -16.7 \text{ kJ/mol}$$

This reaction, which is irreversible under intracellular conditions, is catalyzed by **hexokinase**. Recall that kinases are enzymes that catalyze the transfer of the terminal phosphoryl group from ATP to an acceptor nucleophile (see Fig. 13–10). Kinases are a subclass of transferases (see Table 6–3). The acceptor in the case of hexokinase is a hexose, normally D-glucose, although hexokinase also catalyzes the phosphorylation of other common hexoses, such as D-fructose and D-mannose.

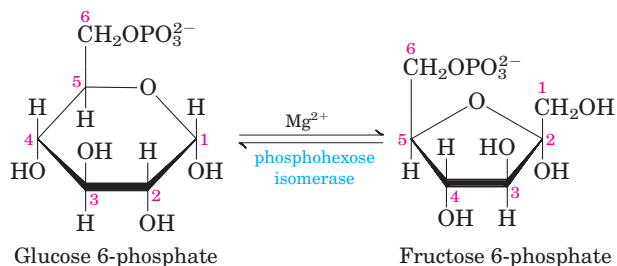
Hexokinase, like many other kinases, requires Mg^{2+} for its activity, because the true substrate of the enzyme is not ATP^{4-} but the MgATP^{2-} complex (see Fig. 13–2). Mg^{2+} shields the negative charges of the phosphoryl groups in ATP, making the terminal phosphorus atom an easier target for nucleophilic attack by an —OH of glucose. Hexokinase undergoes a profound change in shape, an induced fit, when it binds glucose; two domains of the protein move about 8 Å closer to each other when ATP binds (see Fig. 6–22). This movement brings bound ATP closer to a molecule of glucose also bound to the enzyme and blocks the access of water (from the solvent), which might otherwise enter the active site and attack (hydrolyze) the phosphoanhydride bonds of ATP. Like the other nine enzymes of glycolysis, hexokinase is a soluble, cytosolic protein.

Hexokinase is present in all cells of all organisms. Hepatocytes also contain a form of hexokinase called hexokinase IV or glucokinase, which differs from other forms of hexokinase in kinetic and regulatory properties (see Box 15–2). Two enzymes that catalyze the

same reaction but are encoded in different genes are called **isozymes**.

② Conversion of Glucose 6-Phosphate to Fructose 6-Phosphate

The enzyme **phosphohexose isomerase (phosphoglucose isomerase)** catalyzes the reversible isomerization of glucose 6-phosphate, an aldose, to **fructose 6-phosphate**, a ketose:

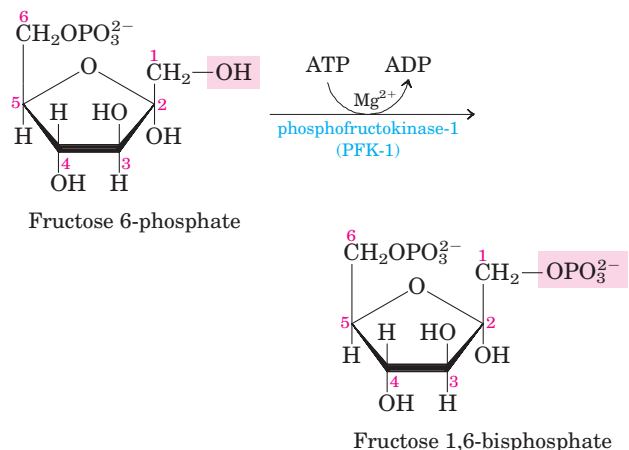


$$\Delta G'^{\circ} = 1.7 \text{ kJ/mol}$$

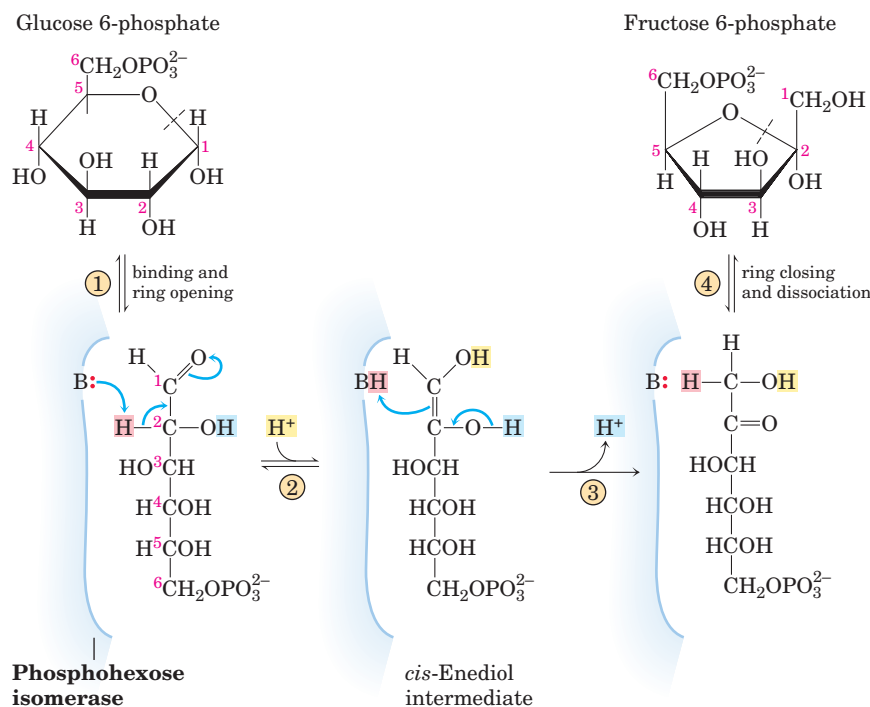
The mechanism for this reaction is shown in Figure 14–4. The reaction proceeds readily in either direction, as might be expected from the relatively small change in standard free energy. This isomerization has a critical role in the overall chemistry of the glycolytic pathway, as the rearrangement of the carbonyl and hydroxyl groups at C-1 and C-2 is a necessary prelude to the next two steps. The phosphorylation that occurs in the next reaction (step ③) requires that the group at C-1 first be converted from a carbonyl to an alcohol, and in the subsequent reaction (step ④) cleavage of the bond between C-3 and C-4 requires a carbonyl group at C-2 (p. 485).

③ Phosphorylation of Fructose 6-Phosphate to Fructose 1,6-Bisphosphate

In the second of the two priming reactions of glycolysis, **phosphofructokinase-1 (PFK-1)** catalyzes the transfer of a phosphoryl group from ATP to fructose 6-phosphate to yield **fructose 1,6-bisphosphate**:



$$\Delta G'^{\circ} = -14.2 \text{ kJ/mol}$$

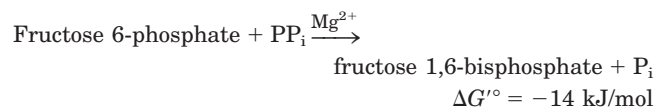


MECHANISM FIGURE 14-4 The phosphohexose isomerase reaction. The ring opening and closing reactions (steps ① and ④) are catalyzed by an active-site His residue, by mechanisms omitted here for simplicity. The movement of the proton between C-2 and C-1 (steps ② and ③) is base-catalyzed by an active-site Glu residue (shown as B:). The proton (pink) initially at C-2 is made more easily abstractable by electron withdrawal by the adjacent carbonyl and the nearby hydroxyl group. After its transfer from C-2 to the active-site Glu residue, the proton is freely exchanged with the surrounding solution; that is, the proton abstracted from C-2 in step ② is not necessarily the same one that is added to C-1 in step ③. (The additional exchange of protons (yellow and blue) between the hydroxyl groups and solvent is shown for completeness. The hydroxyl groups are weak acids and can exchange protons with the surrounding water whether the isomerization reaction is underway or not.)

Phosphohexose Isomerase Mechanism

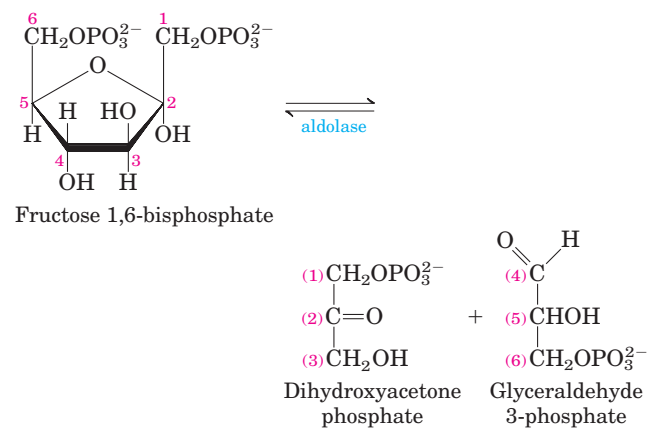
This enzyme is called PFK-1 to distinguish it from a second enzyme (PFK-2) that catalyzes the formation of fructose 2,6-bisphosphate from fructose 6-phosphate in a separate pathway. The PFK-1 reaction is essentially irreversible under cellular conditions, and it is the first “committed” step in the glycolytic pathway; glucose 6-phosphate and fructose 6-phosphate have other possible fates, but fructose 1,6-bisphosphate is targeted for glycolysis.

Some bacteria and protists and perhaps all plants have a phosphofructokinase that uses pyrophosphate (PP_i), not ATP, as the phosphoryl group donor in the synthesis of fructose 1,6-bisphosphate:



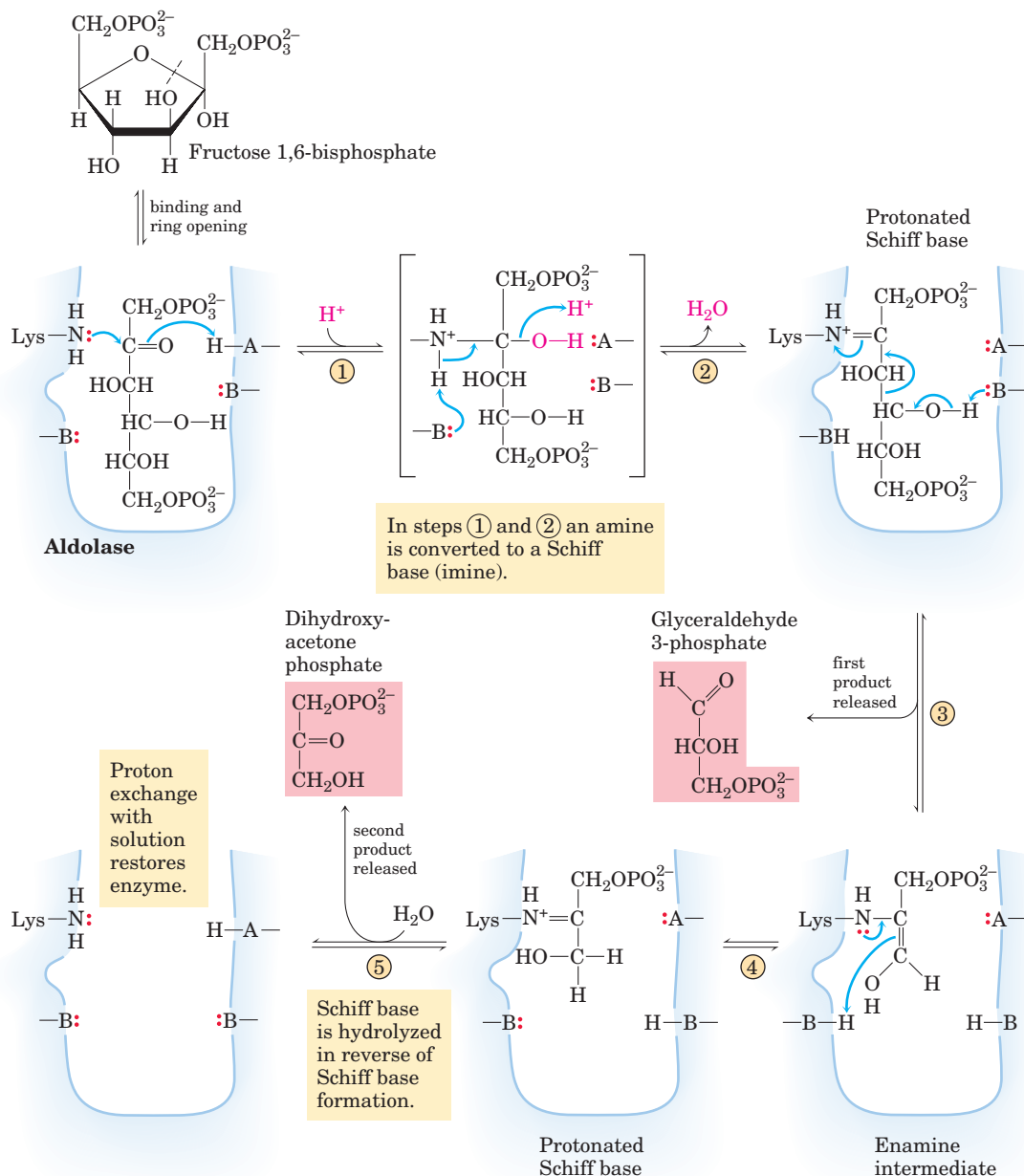
Phosphofructokinase-1 is a regulatory enzyme (Chapter 6), one of the most complex known. It is the major point of regulation in glycolysis. The activity of PFK-1 is increased whenever the cell's ATP supply is depleted or when the ATP breakdown products, ADP and AMP (particularly the latter), are in excess. The enzyme is inhibited whenever the cell has ample ATP and is well supplied by other fuels such as fatty acids. In some organisms, fructose 2,6-bisphosphate (not to be confused with the PFK-1 reaction product, fructose 1,6-bisphosphate) is a potent allosteric activator of PFK-1. The regulation of this step in glycolysis is discussed in greater detail in Chapter 15.

④ **Cleavage of Fructose 1,6-Bisphosphate** The enzyme **fructose 1,6-bisphosphate aldolase**, often called simply **aldolase**, catalyzes a reversible aldol condensation (p. 485). Fructose 1,6-bisphosphate is cleaved to yield two different triose phosphates, **glyceraldehyde 3-phosphate**, an aldose, and **dihydroxyacetone phosphate**, a ketose:



$$\Delta G'^{\circ} = 23.8 \text{ kJ/mol}$$

There are two classes of aldolases. Class I aldolases, found in animals and plants, use the mechanism shown in Figure 14-5. Class II enzymes, in fungi and bacteria, do not form the Schiff base intermediate. Instead, a zinc ion at the active site is coordinated with the carbonyl oxygen at C-2; the Zn^{2+} polarizes the carbonyl group



MECHANISM FIGURE 14-5 The class I aldolase reaction. The reaction shown here is the reverse of an aldol condensation. Note that cleavage between C-3 and C-4 depends on the presence of the carbonyl group at C-2. ① and ② The carbonyl reacts with an active-site Lys residue to form an imine, which stabilizes the carbanion generated by the bond cleavage—an imine delocalizes electrons even better than

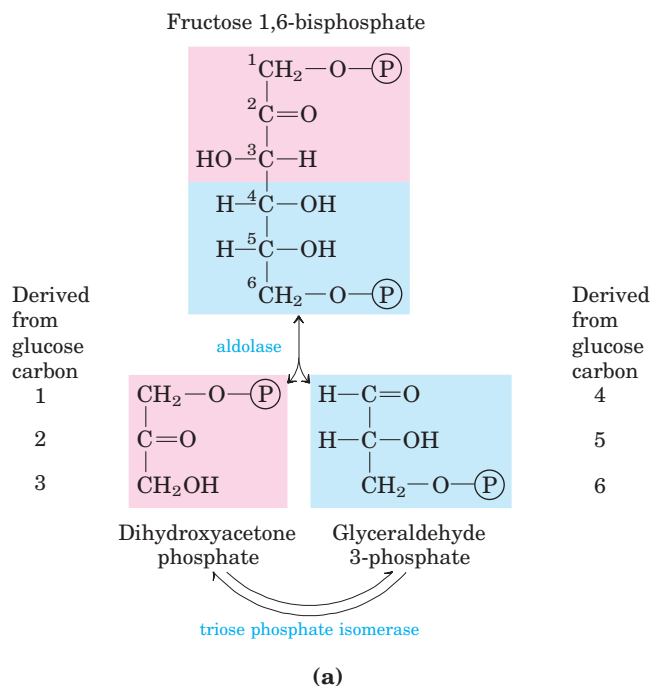
does a carbonyl. ③ Bond cleavage releases glyceraldehyde 3-phosphate as the first product. ④ The resulting enamine covalently linked to the enzyme is isomerized to a protonated Schiff base, and ⑤ hydrolysis of the Schiff base generates dihydroxyacetone phosphate as the second product. A and B represent amino acid residues that serve as general acid (A) or base (B).

and stabilizes the enolate intermediate created in the C—C bond cleavage step.

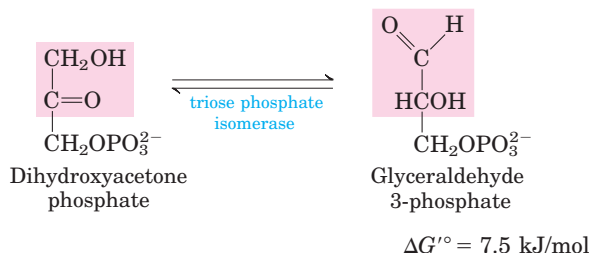
Although the aldolase reaction has a strongly positive standard free-energy change in the direction of fructose 1,6-bisphosphate cleavage, at the lower concentrations of reactants present in cells, the actual free-energy change is small and the aldolase reaction is readily reversible. We shall see later that aldolase acts in the re-

verse direction during the process of gluconeogenesis (see Fig. 14-16).

⑤ Interconversion of the Triose Phosphates Only one of the two triose phosphates formed by aldolase, glyceraldehyde 3-phosphate, can be directly degraded in the subsequent steps of glycolysis. The other product, dihydroxyacetone phosphate, is rapidly and reversibly



converted to glyceraldehyde 3-phosphate by the fifth enzyme of the sequence, **triose phosphate isomerase**:



The reaction mechanism is similar to the reaction promoted by phosphohexose isomerase in step ② of glycolysis (Fig. 14-4). After the triose phosphate isomerase reaction, C-1, C-2, and C-3 of the starting glucose are chemically indistinguishable from C-6, C-5, and C-4, respectively (Fig. 14-6), setting up the efficient metabolism of the entire six-carbon glucose molecule.

This reaction completes the preparatory phase of glycolysis. The hexose molecule has been phosphorylated at C-1 and C-6 and then cleaved to form two molecules of glyceraldehyde 3-phosphate.

The Payoff Phase of Glycolysis Yields ATP and NADH

The payoff phase of glycolysis (Fig. 14-2b) includes the energy-conserving phosphorylation steps in which some of the free energy of the glucose molecule is conserved in the form of ATP. Remember that one molecule of glucose yields two molecules of glyceraldehyde 3-phosphate; both halves of the glucose molecule follow the

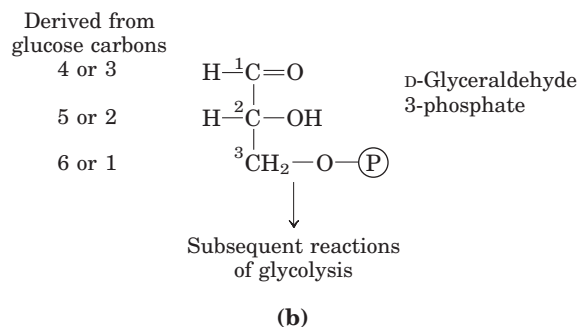
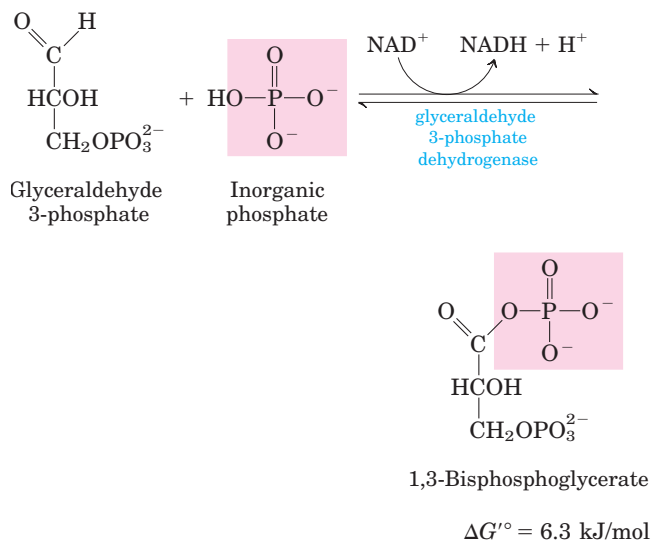


FIGURE 14-6 Fate of the glucose carbons in the formation of glyceraldehyde 3-phosphate. (a) The origin of the carbons in the two three-carbon products of the aldolase and triose phosphate isomerase reactions. The end product of the two reactions is glyceraldehyde 3-phosphate (two molecules). (b) Each carbon of glyceraldehyde 3-phosphate is derived from either of two specific carbons of glucose. Note that the numbering of the carbon atoms of glyceraldehyde 3-phosphate differs from that of the glucose from which it is derived. In glyceraldehyde 3-phosphate, the most complex functional group (the carbonyl) is specified as C-1. This numbering change is important for interpreting experiments with glucose in which a single carbon is labeled with a radioisotope. (See Problems 3 and 5 at the end of this chapter.)

same pathway in the second phase of glycolysis. The conversion of two molecules of glyceraldehyde 3-phosphate to two molecules of pyruvate is accompanied by the formation of four molecules of ATP from ADP. However, the net yield of ATP per molecule of glucose degraded is only two, because two ATP were invested in the preparatory phase of glycolysis to phosphorylate the two ends of the hexose molecule.

⑥ Oxidation of Glyceraldehyde 3-Phosphate to 1,3-Bisphosphoglycerate

The first step in the payoff phase is the oxidation of glyceraldehyde 3-phosphate to **1,3-bisphosphoglycerate**, catalyzed by **glyceraldehyde 3-phosphate dehydrogenase**:



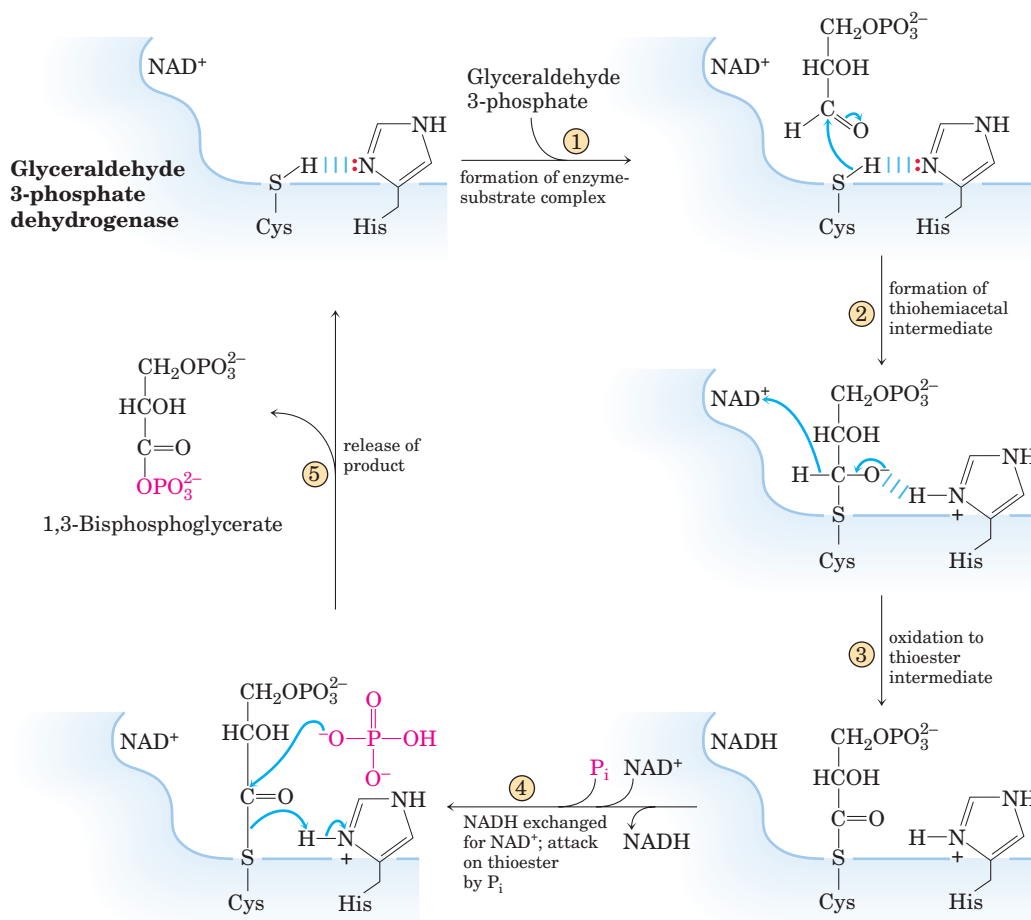
This is the first of the two energy-conserving reactions of glycolysis that eventually lead to the formation of ATP. The aldehyde group of glyceraldehyde 3-phosphate is oxidized, not to a free carboxyl group but to a carboxylic acid anhydride with phosphoric acid. This type of anhydride, called an **acyl phosphate**, has a very high standard free energy of hydrolysis ($\Delta G'^{\circ} = -49.3$ kJ/mol; see Fig. 13-4, Table 13-6). Much of the free energy of oxidation of the aldehyde group of glyceraldehyde 3-phosphate is conserved by formation of the acyl phosphate group at C-1 of 1,3-bisphosphoglycerate.

The acceptor of hydrogen in the glyceraldehyde 3-phosphate dehydrogenase reaction is NAD^+ (see Fig. 13-15), bound to a Rossmann fold as shown in Figure 13-16. The reduction of NAD^+ proceeds by the enzymatic transfer of a hydride ion (:H^-) from the aldehyde group of glyceraldehyde 3-phosphate to the nicoti-

namide ring of NAD^+ , yielding the reduced coenzyme NADH. The other hydrogen atom of the substrate molecule is released to the solution as H^+ .

Glyceraldehyde 3-phosphate is covalently bound to the dehydrogenase during the reaction (Fig. 14-7). The aldehyde group of glyceraldehyde 3-phosphate reacts with the —SH group of an essential Cys residue in the active site, in a reaction analogous to the formation of a hemiacetal (see Fig. 7-5), in this case producing a *thio*-hemiacetal. Reaction of the essential Cys residue with a heavy metal such as Hg^{2+} irreversibly inhibits the enzyme.

Because cells maintain only limited amounts of NAD^+ , glycolysis would soon come to a halt if the NADH formed in this step of glycolysis were not continuously reoxidized. The reactions in which NAD^+ is regenerated anaerobically are described in detail in Section 14.3, in our discussion of the alternative fates of pyruvate.

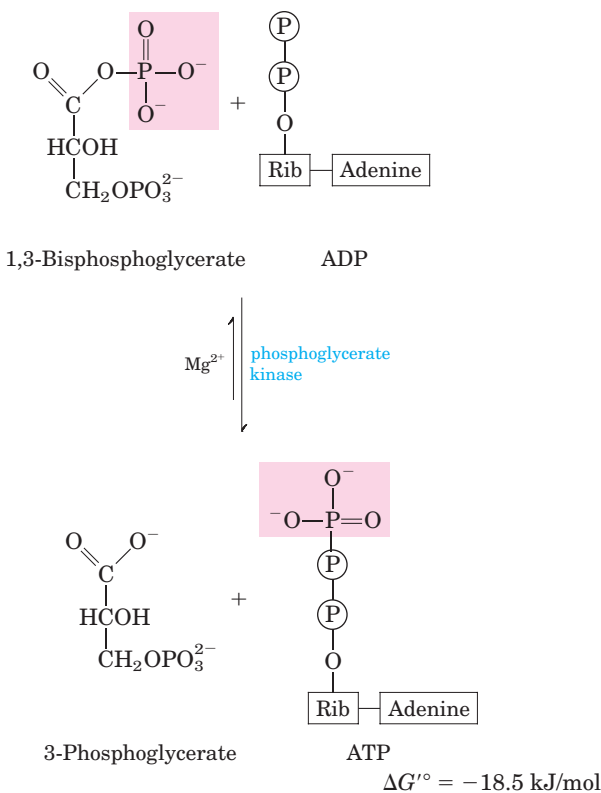


MECHANISM FIGURE 14-7 The glyceraldehyde 3-phosphate dehydrogenase reaction. After ① formation of the enzyme-substrate complex, ② a covalent thiohemiacetal linkage forms between the substrate and the —SH group of a Cys residue—facilitated by acid-base catalysis with a neighboring base catalyst, probably a His residue. ③ This enzyme-substrate intermediate is oxidized by NAD^+ bound to the active site, forming a covalent acyl-enzyme intermediate, a

thioester. ④ The newly formed NADH leaves the active site and is replaced by another NAD^+ molecule. The bond between the acyl group and the thiol group of the enzyme has a very high standard free energy of hydrolysis. ⑤ This bond undergoes phosphorolysis (attack by P_i), releasing the acyl phosphate product, 1,3-bisphosphoglycerate. Formation of this product conserves much of the free energy liberated during oxidation of the aldehyde group of glyceraldehyde 3-phosphate.

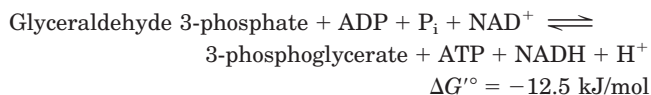
⑦ Phosphoryl Transfer from 1,3-Bisphosphoglycerate to ADP

The enzyme **phosphoglycerate kinase** transfers the high-energy phosphoryl group from the carboxyl group of 1,3-bisphosphoglycerate to ADP, forming ATP and **3-phosphoglycerate**:



Notice that phosphoglycerate kinase is named for the reverse reaction. Like all enzymes, it catalyzes the reaction in both directions. This enzyme acts in the direction suggested by its name during gluconeogenesis (see Fig. 14–16) and during photosynthetic CO_2 assimilation (see Fig. 20–4).

Steps ⑥ and ⑦ of glycolysis together constitute an energy-coupling process in which 1,3-bisphosphoglycerate is the common intermediate; it is formed in the first reaction (which would be endergonic in isolation), and its acyl phosphate group is transferred to ADP in the second reaction (which is strongly exergonic). The sum of these two reactions is



Thus the overall reaction is exergonic.

Recall from Chapter 13 that the actual free-energy change, ΔG , is determined by the standard free-energy change, $\Delta G'^{\circ}$, and the mass-action ratio, Q , which is the ratio [products]/[reactants] (see Eqn 13–3). For step ⑥

$$\Delta G = \Delta G'^{\circ} + RT \ln Q$$

$$= \Delta G'^{\circ} + RT \ln \frac{[\text{1,3-bisphosphoglycerate}][\text{NADH}]}{[\text{glyceraldehyde 3-phosphate}][\text{P}_i][\text{NAD}^+]}$$

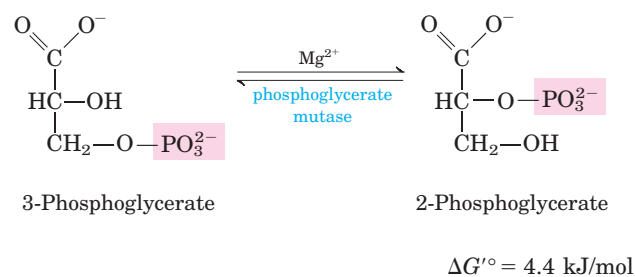
Notice that $[\text{H}^+]$ is not included in Q . In biochemical calculations, $[\text{H}^+]$ is assumed to be a constant (10^{-7} M), and this constant is included in the definition of $\Delta G'^{\circ}$ (p. 491).

When the mass-action ratio is less than 1.0, its natural logarithm has a negative sign. Step ⑦, by consuming the product of step ⑥ (1,3-bisphosphoglycerate), keeps [1,3-bisphosphoglycerate] relatively low in the steady state and thereby keeps Q for the overall energy-coupling process small. When Q is small, the contribution of $\ln Q$ can make ΔG strongly negative. This is simply another way of showing how the two reactions, steps ⑥ and ⑦, are coupled through a common intermediate.

The outcome of these coupled reactions, both reversible under cellular conditions, is that the energy released on oxidation of an aldehyde to a carboxylate group is conserved by the coupled formation of ATP from ADP and P_i . The formation of ATP by phosphoryl group transfer from a substrate such as 1,3-bisphosphoglycerate is referred to as a **substrate-level phosphorylation**, to distinguish this mechanism from **respiration-linked phosphorylation**. Substrate-level phosphorylations involve soluble enzymes and chemical intermediates (1,3-bisphosphoglycerate in this case). Respiration-linked phosphorylations, on the other hand, involve membrane-bound enzymes and transmembrane gradients of protons (Chapter 19).

⑧ Conversion of 3-Phosphoglycerate to 2-Phosphoglycerate

The enzyme **phosphoglycerate mutase** catalyzes a reversible shift of the phosphoryl group between C-2 and C-3 of glycerate; Mg^{2+} is essential for this reaction:



The reaction occurs in two steps (Fig. 14–8). A phosphoryl group initially attached to a His residue of the mutase is transferred to the hydroxyl group at C-2 of 3-phosphoglycerate, forming 2,3-bisphosphoglycerate (2,3-BPG). The phosphoryl group at C-3 of 2,3-BPG is then transferred to the same His residue, producing 2-phosphoglycerate and regenerating the phosphorylated enzyme. Phosphoglycerate mutase is initially phosphorylated by phosphoryl transfer from 2,3-BPG, which is required in small quantities to initiate the catalytic cycle and is continuously regenerated by that cycle. Although in most cells 2,3-BPG is present in only trace amounts, it is a major component (~5 mM) of erythrocytes, where it regulates the affinity of hemoglobin for

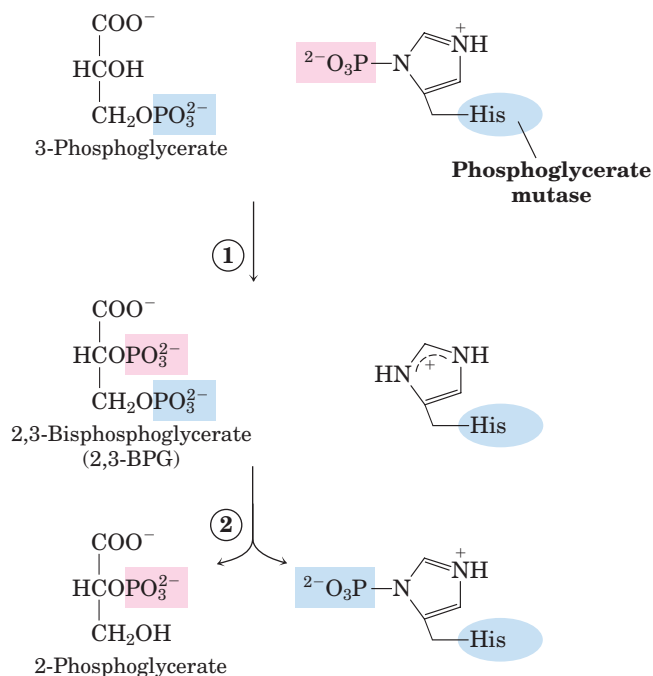
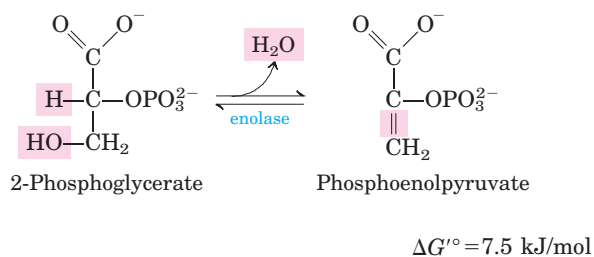


FIGURE 14-8 The phosphoglycerate mutase reaction. The enzyme is initially phosphorylated on a His residue. ① The phosphoenzyme transfers its phosphoryl group to 3-phosphoglycerate, forming 2,3-BPG. ② The phosphoryl group at C-3 of 2,3-BPG is transferred to the same His residue on the enzyme, producing 2-phosphoglycerate and regenerating the phosphoenzyme.

oxygen (see Fig. 5-17; note that in the context of hemoglobin regulation, 2,3-bisphosphoglycerate is usually abbreviated as simply BPG).

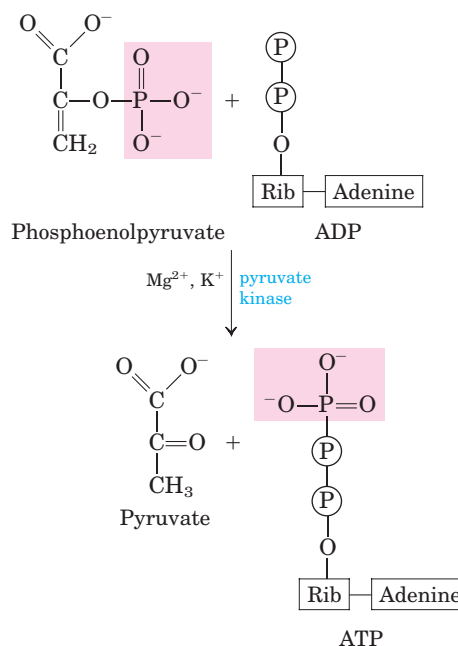
⑨ **Dehydration of 2-Phosphoglycerate to Phosphoenolpyruvate** In the second glycolytic reaction that generates a compound with high phosphoryl group transfer potential, **enolase** promotes reversible removal of a molecule of water from 2-phosphoglycerate to yield **phosphoenolpyruvate (PEP)**:



The mechanism of the enolase reaction is presented in Figure 6-23. Despite the relatively small standard free-energy change of this reaction, there is a very large difference in the standard free energy of hydrolysis of

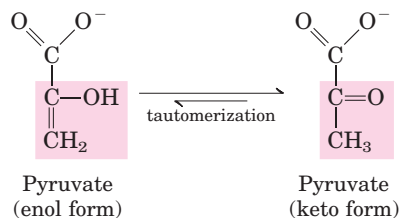
the phosphoryl groups of the reactant and product: -17.6 kJ/mol for 2-phosphoglycerate (a low-energy phosphate ester) and -61.9 kJ/mol for phosphoenolpyruvate (a compound with a very high standard free energy of hydrolysis) (see Fig. 13-3, Table 13-6). Although 2-phosphoglycerate and phosphoenolpyruvate contain nearly the same *total* amount of energy, the loss of the water molecule from 2-phosphoglycerate causes a redistribution of energy within the molecule, greatly increasing the standard free energy of hydrolysis of the phosphoryl group.

⑩ **Transfer of the Phosphoryl Group from Phosphoenolpyruvate to ADP** The last step in glycolysis is the transfer of the phosphoryl group from phosphoenolpyruvate to ADP, catalyzed by **pyruvate kinase**, which requires K^+ and either Mg^{2+} or Mn^{2+} :



$$\Delta G'^{\circ} = -31.4 \text{ kJ/mol}$$

In this substrate-level phosphorylation, the product **pyruvate** first appears in its enol form, then tautomerizes rapidly and nonenzymatically to its keto form, which predominates at pH 7:

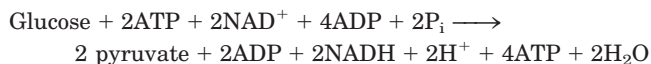


The overall reaction has a large, negative standard free-energy change, due in large part to the spontaneous conversion of the enol form of pyruvate to the keto form (see Fig. 13-3). The $\Delta G'^{\circ}$ of phosphoenolpyruvate

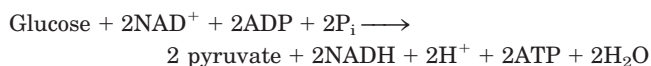
hydrolysis is -61.9 kJ/mol; about half of this energy is conserved in the formation of the phosphoanhydride bond of ATP ($\Delta G'^{\circ} = -30.5$ kJ/mol), and the rest (-31.4 kJ/mol) constitutes a large driving force pushing the reaction toward ATP synthesis. The pyruvate kinase reaction is essentially irreversible under intracellular conditions and is an important site of regulation, as described in Chapter 15.

The Overall Balance Sheet Shows a Net Gain of ATP

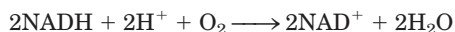
We can now construct a balance sheet for glycolysis to account for (1) the fate of the carbon skeleton of glucose, (2) the input of P_i and ADP and the output of ATP, and (3) the pathway of electrons in the oxidation-reduction reactions. The left-hand side of the following equation shows all the inputs of ATP, NAD^+ , ADP, and P_i (consult Fig. 14-2), and the right-hand side shows all the outputs (keep in mind that each molecule of glucose yields two molecules of pyruvate):



Canceling out common terms on both sides of the equation gives the overall equation for glycolysis under aerobic conditions:



The two molecules of NADH formed by glycolysis in the cytosol are, under aerobic conditions, reoxidized to NAD^+ by transfer of their electrons to the electron-transfer chain, which in eukaryotic cells is located in the mitochondria. The electron-transfer chain passes these electrons to their ultimate destination, O_2 :



Electron transfer from NADH to O_2 in mitochondria provides the energy for synthesis of ATP by respiration-linked phosphorylation (Chapter 19).

In the overall glycolytic process, one molecule of glucose is converted to two molecules of pyruvate (the pathway of carbon). Two molecules of ADP and two of P_i are converted to two molecules of ATP (the pathway of phosphoryl groups). Four electrons, as two hydride ions, are transferred from two molecules of glyceraldehyde 3-phosphate to two of NAD^+ (the pathway of electrons).

Glycolysis Is under Tight Regulation

During his studies on the fermentation of glucose by yeast, Louis Pasteur discovered that both the rate and the total amount of glucose consumption were many times greater under anaerobic than aerobic conditions. Later studies of muscle showed the same large differ-

ence in the rates of anaerobic and aerobic glycolysis. The biochemical basis of this “Pasteur effect” is now clear. The ATP yield from glycolysis under anaerobic conditions (2 ATP per molecule of glucose) is much smaller than that from the complete oxidation of glucose to CO_2 under aerobic conditions (30 or 32 ATP per glucose; see Table 19-5). About 15 times as much glucose must therefore be consumed anaerobically as aerobically to yield the same amount of ATP.

The flux of glucose through the glycolytic pathway is regulated to maintain nearly constant ATP levels (as well as adequate supplies of glycolytic intermediates that serve biosynthetic roles). The required adjustment in the rate of glycolysis is achieved by a complex interplay among ATP consumption, NADH regeneration, and allosteric regulation of several glycolytic enzymes—including hexokinase, PFK-1, and pyruvate kinase—and by second-to-second fluctuations in the concentration of key metabolites that reflect the cellular balance between ATP production and consumption. On a slightly longer time scale, glycolysis is regulated by the hormones glucagon, epinephrine, and insulin, and by changes in the expression of the genes for several glycolytic enzymes. We return to a more detailed discussion of the regulation of glycolysis in Chapter 15.

Cancerous Tissue Has Deranged Glucose Catabolism



Glucose uptake and glycolysis proceed about ten times faster in most solid tumors than in non-cancerous tissues. Tumor cells commonly experience hypoxia (limited oxygen supply), because they initially lack an extensive capillary network to supply the tumor with oxygen. As a result, cancer cells more than 100 to 200 μm from the nearest capillaries depend on anaerobic glycolysis for much of their ATP production. They take up more glucose than normal cells, converting it to pyruvate and then to lactate as they recycle NADH. The high glycolytic rate may also result in part from smaller numbers of mitochondria in tumor cells; less ATP made by respiration-linked phosphorylation in mitochondria means more ATP is needed from glycolysis. In addition, some tumor cells overproduce several glycolytic enzymes, including an isozyme of hexokinase that associates with the cytosolic face of the mitochondrial inner membrane and is insensitive to feedback inhibition by glucose 6-phosphate. This enzyme may monopolize the ATP produced in mitochondria, using it to convert glucose to glucose 6-phosphate and committing the cell to continued glycolysis. The hypoxia-inducible transcription factor (HIF-1) is a protein that acts at the level of mRNA synthesis to stimulate the synthesis of at least eight of the glycolytic enzymes. This gives the tumor cell the capacity to survive anaerobic conditions until the supply of blood vessels has caught up with tumor growth.

The German biochemist Otto Warburg was the first to show, as early as 1928, that tumors have a higher rate of glucose metabolism than other tissues. With his associates, Warburg purified and crystallized seven of the enzymes of glycolysis. In these studies he developed and used an experimental tool that revolutionized biochemical studies of oxidative metabolism: the Warburg manometer, which measured directly the consumption of oxygen by monitoring changes in gas volume, and therefore allowed quantitative measurement of any enzyme with oxidase activity.

Warburg, considered by many the preeminent biochemist of the first half of the twentieth century, made seminal contributions to many other areas of biochemistry, including respiration, photosynthesis, and the enzymology of intermediary metabolism. Trained in carbohydrate chemistry in the laboratory of the great Emil Fischer (who won the Nobel Prize in Chemistry in 1902), Warburg himself won the Nobel Prize in Physiology or Medicine in 1931. A number of Warburg's students and colleagues also were awarded Nobel Prizes:

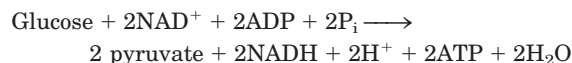


Otto Warburg,
1883–1970

Otto Meyerhof in 1922, Hans Krebs and Fritz Lipmann in 1953, and Hugo Theorell in 1955. Meyerhof's laboratory provided training for Lipmann, and for several other Nobel Prize winners: Severo Ochoa (1959), Andre Lwoff (1965), and George Wald (1967). ■

SUMMARY 14.1 Glycolysis

- Glycolysis is a near-universal pathway by which a glucose molecule is oxidized to two molecules of pyruvate, with energy conserved as ATP and NADH.
- All ten glycolytic enzymes are in the cytosol, and all ten intermediates are phosphorylated compounds of three or six carbons.
- In the preparatory phase of glycolysis, ATP is invested to convert glucose to fructose 1,6-bisphosphate. The bond between C-3 and C-4 is then broken to yield two molecules of triose phosphate.
- In the payoff phase, each of the two molecules of glyceraldehyde 3-phosphate derived from glucose undergoes oxidation at C-1; the energy of this oxidation reaction is conserved in the formation of one NADH and two ATP per triose phosphate oxidized. The net equation for the overall process is



- Glycolysis is tightly regulated in coordination with other energy-yielding pathways to assure a steady supply of ATP. Hexokinase, PFK-1, and pyruvate kinase are all subject to allosteric regulation that controls the flow of carbon through the pathway and maintains constant levels of metabolic intermediates.

14.2 Feeder Pathways for Glycolysis

Many carbohydrates besides glucose meet their catabolic fate in glycolysis, after being transformed into one of the glycolytic intermediates. The most significant are the storage polysaccharides glycogen and starch; the disaccharides maltose, lactose, trehalose, and sucrose; and the monosaccharides fructose, mannose, and galactose (Fig. 14–9).

Glycogen and Starch Are Degraded by Phosphorolysis

Glycogen in animal tissues and in microorganisms (and starch in plants) can be mobilized for use within the same cell by a phosphorolytic reaction catalyzed by **glycogen phosphorylase** (**starch phosphorylase** in plants). These enzymes catalyze an attack by P_i on the ($\alpha 1 \rightarrow 4$) glycosidic linkage that joins the last two glucose residues at a nonreducing end, generating glucose 1-phosphate and a polymer one glucose unit shorter (Fig. 14–10). *Phosphorolysis* preserves some of the energy of the glycosidic bond in the phosphate ester glucose 1-phosphate. Glycogen phosphorylase (or starch phosphorylase) acts repetitively until it approaches an ($\alpha 1 \rightarrow 6$) branch point (see Fig. 7–15), where its action stops. A **debranching enzyme** removes the branches. The mechanisms and control of glycogen degradation are described in detail in Chapter 15.

Glucose 1-phosphate produced by glycogen phosphorylase is converted to glucose 6-phosphate by **phosphoglucomutase**, which catalyzes the reversible reaction



The glucose 6-phosphate thus formed can enter glycolysis or another pathway such as the pentose phosphate pathway, described in Section 14.5. Phosphoglucomutase employs essentially the same mechanism as phosphoglycerate mutase (p. 531). The general name **mutase** is given to enzymes that catalyze the transfer of a functional group from one position to another in the same molecule. Mutases are a subclass of **isomerases**, enzymes that interconvert stereoisomers or structural or positional isomers (see Table 6–3).

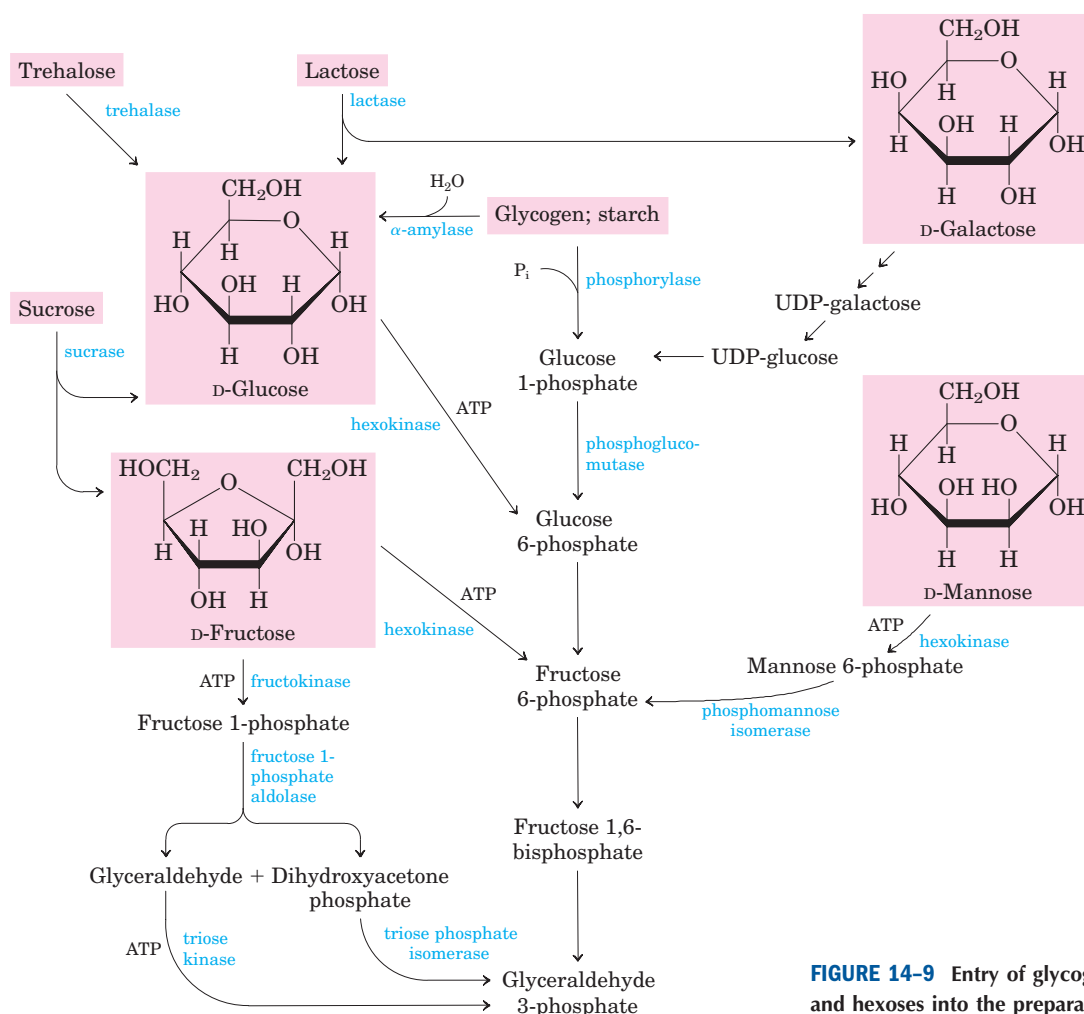
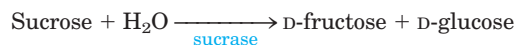
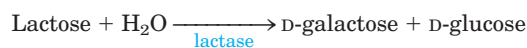


FIGURE 14-9 Entry of glycogen, starch, disaccharides, and hexoses into the preparatory stage of glycolysis.

Dietary Polysaccharides and Disaccharides Undergo Hydrolysis to Monosaccharides

For most humans, starch is the major source of carbohydrates in the diet. Digestion begins in the mouth, where salivary α -amylase (Fig. 14-9) hydrolyzes the internal glycosidic linkages of starch, producing short polysaccharide fragments or oligosaccharides. (Note that in this *hydrolysis* reaction, water, not P_i , is the attacking species.) In the stomach, salivary α -amylase is inactivated by the low pH, but a second form of α -amylase, secreted by the pancreas into the small intestine, continues the breakdown process. Pancreatic α -amylase yields mainly maltose and maltotriose (the di- and trisaccharides of $\alpha(1\rightarrow4)$ glucose) and oligosaccharides called limit dextrins, fragments of amylopectin containing $\alpha(1\rightarrow6)$ branch points. Maltose and dextrins are degraded by enzymes of the intestinal brush border (the fingerlike microvilli of intestinal epithelial cells, which greatly increase the area of the intestinal surface). Dietary glycogen has essentially the same structure as starch, and its digestion proceeds by the same pathway.

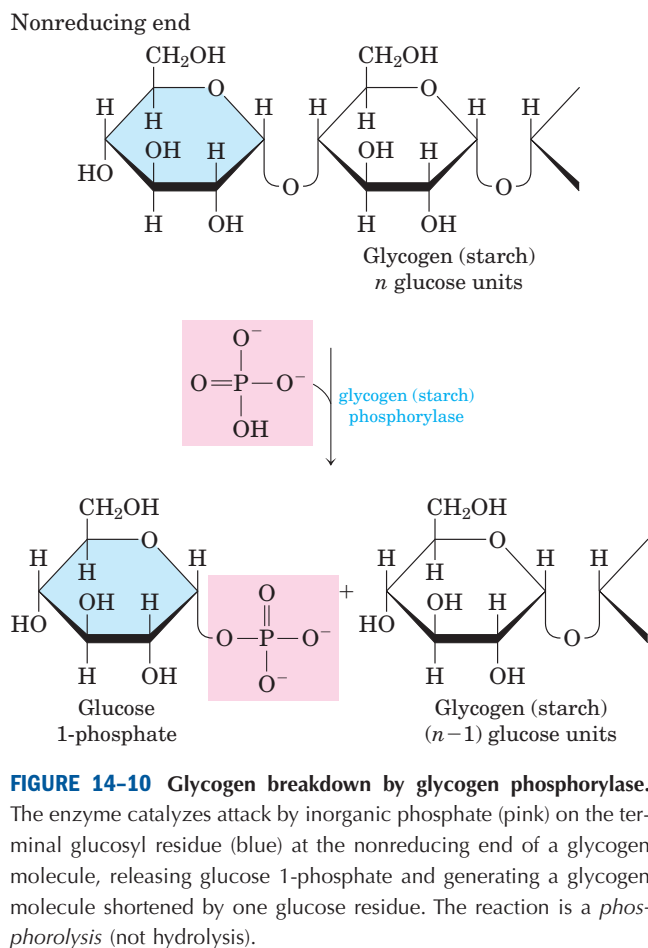
Disaccharides must be hydrolyzed to monosaccharides before entering cells. Intestinal disaccharides and dextrins are hydrolyzed by enzymes attached to the outer surface of the intestinal epithelial cells:



The monosaccharides so formed are actively transported into the epithelial cells (see Fig. 11-44), then passed into the blood to be carried to various tissues, where they are phosphorylated and funneled into the glycolytic sequence.



Lactose intolerance, common among adults of most human populations except those originating



in Northern Europe and some parts of Africa, is due to the disappearance after childhood of most or all of the lactase activity of the intestinal cells. Lactose cannot be completely digested and absorbed in the small intestine and passes into the large intestine, where bacteria convert it to toxic products that cause abdominal cramps and diarrhea. The problem is further complicated because undigested lactose and its metabolites increase the osmolarity of the intestinal contents, favoring the retention of water in the intestine. In most parts of the world where lactose intolerance is prevalent, milk is not used as a food by adults, although milk products predigested with lactase are commercially available in some countries. In certain human disorders, several or all of the intestinal disaccharidases are missing. In these cases, the digestive disturbances triggered by dietary disaccharides can sometimes be minimized by a controlled diet. ■

Other Monosaccharides Enter the Glycolytic Pathway at Several Points

In most organisms, hexoses other than glucose can undergo glycolysis after conversion to a phosphorylated

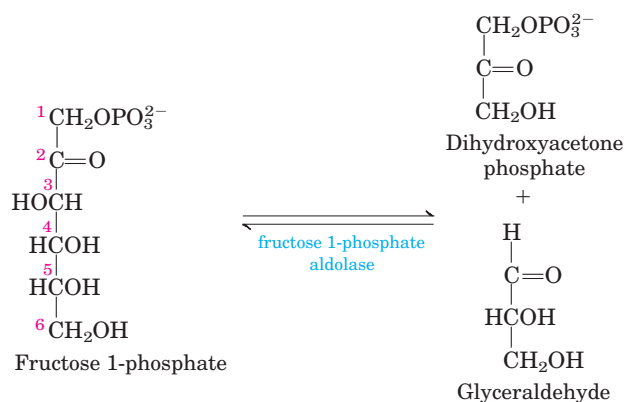
derivative. D-Fructose, present in free form in many fruits and formed by hydrolysis of sucrose in the small intestine of vertebrates, is phosphorylated by hexokinase:



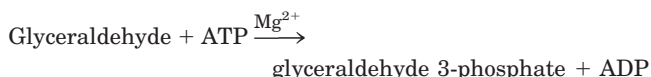
This is a major pathway of fructose entry into glycolysis in the muscles and kidney. In the liver, however, fructose enters by a different pathway. The liver enzyme **fructokinase** catalyzes the phosphorylation of fructose at C-1 rather than C-6:




The fructose 1-phosphate is then cleaved to glyceraldehyde and dihydroxyacetone phosphate by **fructose 1-phosphate aldolase**:



Dihydroxyacetone phosphate is converted to glyceraldehyde 3-phosphate by the glycolytic enzyme triose phosphate isomerase. Glyceraldehyde is phosphorylated by ATP and **triose kinase** to glyceraldehyde 3-phosphate:



Thus both products of fructose 1-phosphate hydrolysis enter the glycolytic pathway as glyceraldehyde 3-phosphate.

 D-Galactose, a product of hydrolysis of the disaccharide lactose (milk sugar), passes in the blood from the intestine to the liver, where it is first phosphorylated at C-1, at the expense of ATP, by the enzyme **galactokinase**:



The galactose 1-phosphate is then converted to its epimer at C-4, glucose 1-phosphate, by a set of reactions in which **uridine diphosphate** (UDP) functions as a coenzyme-like carrier of hexose groups (Fig. 14-11). The epimerization involves first the oxidation of the C-4 —OH group to a ketone, then reduction of the ketone to an —OH, with inversion of the configuration at C-4. NAD is the cofactor for both the oxidation and the reduction.

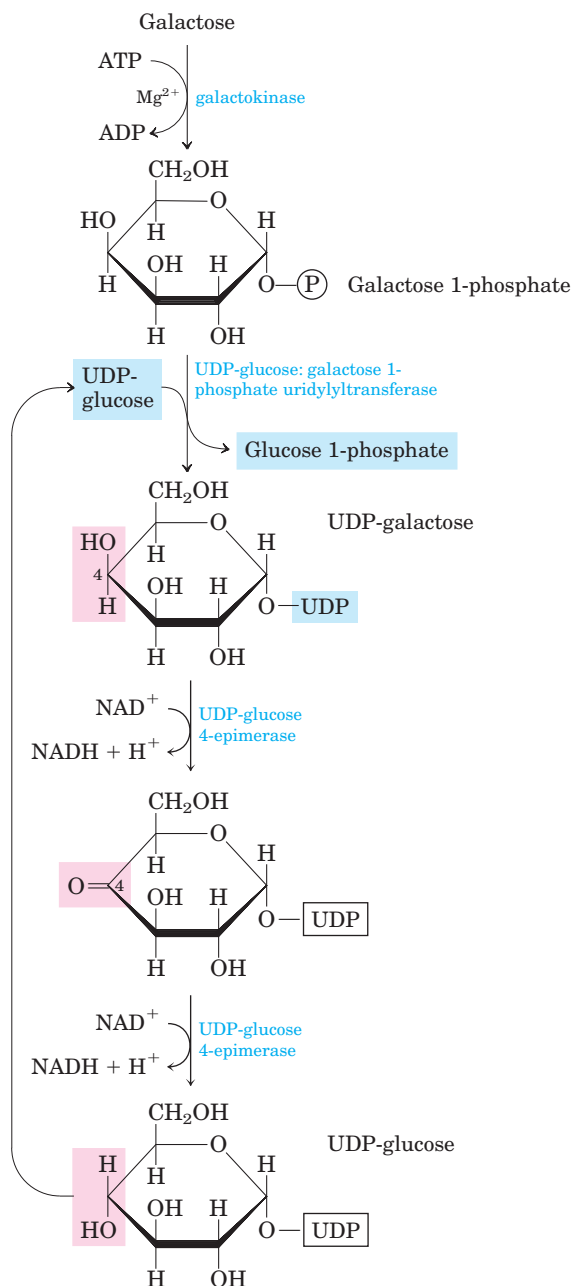
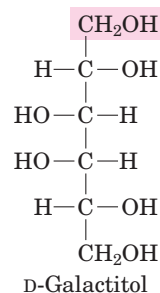


FIGURE 14-11 Conversion of galactose to glucose 1-phosphate. The conversion proceeds through a sugar-nucleotide derivative, UDP-galactose, which is formed when galactose 1-phosphate displaces glucose 1-phosphate from UDP-glucose. UDP-galactose is then converted by UDP-glucose 4-epimerase to UDP-glucose, in a reaction that involves oxidation of C-4 (pink) by NAD^+ , then reduction of C-4 by $NADH$; the result is inversion of the configuration at C-4. The UDP-glucose is recycled through another round of the same reaction. The net effect of this cycle is the conversion of galactose 1-phosphate to glucose 1-phosphate; there is no net production or consumption of UDP-galactose or UDP-glucose.

Defects in any of the three enzymes in this pathway cause **galactosemia** in humans. In galactokinase-deficiency galactosemia, high galactose concentrations are found in blood and urine. Infants develop cataracts, caused by deposition of the galactose metabolite galactitol in the lens.



The symptoms in this disorder are relatively mild, and strict limitation of galactose in the diet greatly diminishes their severity.

Transferase-deficiency galactosemia is more serious; it is characterized by poor growth in children, speech abnormality, mental deficiency, and liver damage that may be fatal, even when galactose is withheld from the diet. Epimerase-deficiency galactosemia leads to similar symptoms, but is less severe when dietary galactose is carefully controlled. ■

D-Mannose, released in the digestion of various polysaccharides and glycoproteins of foods, can be phosphorylated at C-6 by hexokinase:



Mannose 6-phosphate is isomerized by **phosphomannose isomerase** to yield fructose 6-phosphate, an intermediate of glycolysis.

SUMMARY 14.2 Feeder Pathways for Glycolysis

- Glycogen and starch, polymeric storage forms of glucose, enter glycolysis in a two-step process. Phosphorolytic cleavage of a glucose residue from an end of the polymer, forming glucose 1-phosphate, is catalyzed by glycogen phosphorylase or starch phosphorylase. Phosphoglucomutase then converts the glucose 1-phosphate to glucose 6-phosphate, which can enter glycolysis.
- Ingested polysaccharides and disaccharides are converted to monosaccharides by intestinal hydrolytic enzymes, and the monosaccharides then enter intestinal cells and are transported to the liver or other tissues.
- A variety of D-hexoses, including fructose, galactose, and mannose, can be funneled into glycolysis. Each is phosphorylated and converted to either glucose 6-phosphate or fructose 6-phosphate.
- Conversion of galactose 1-phosphate to glucose 1-phosphate involves two nucleotide derivatives: UDP-galactose and UDP-glucose. Genetic defects in any of the three enzymes that catalyze conversion of galactose to glucose 1-phosphate result in galactosemias of varying severity.

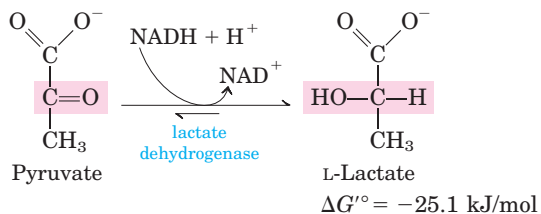
14.3 Fates of Pyruvate under Anaerobic Conditions: Fermentation

Pyruvate occupies an important junction in carbohydrate catabolism (Fig. 14–3). Under aerobic conditions pyruvate is oxidized to acetate, which enters the citric acid cycle and is oxidized to CO_2 and H_2O , and NADH formed by the dehydrogenation of glyceraldehyde 3-phosphate is ultimately reoxidized to NAD^+ by passage of its electrons to O_2 in mitochondrial respiration. However, under hypoxic conditions, as in very active skeletal muscle, in submerged plant tissues, or in lactic acid bacteria, NADH generated by glycolysis cannot be reoxidized by O_2 . Failure to regenerate NAD^+ would leave the cell with no electron acceptor for the oxidation of glyceraldehyde 3-phosphate, and the energy-yielding reactions of glycolysis would stop. NAD^+ must therefore be regenerated in some other way.

The earliest cells lived in an atmosphere almost devoid of oxygen and had to develop strategies for deriving energy from fuel molecules under anaerobic conditions. Most modern organisms have retained the ability to constantly regenerate NAD^+ during anaerobic glycolysis by transferring electrons from NADH to form a reduced end product such as lactate or ethanol.

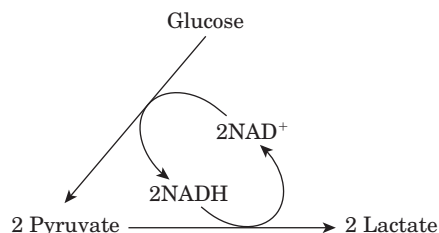
Pyruvate Is the Terminal Electron Acceptor in Lactic Acid Fermentation

When animal tissues cannot be supplied with sufficient oxygen to support aerobic oxidation of the pyruvate and NADH produced in glycolysis, NAD^+ is regenerated from NADH by the reduction of pyruvate to **lactate**. As mentioned earlier, some tissues and cell types (such as erythrocytes, which have no mitochondria and thus cannot oxidize pyruvate to CO_2) produce lactate from glucose even under aerobic conditions. The reduction of pyruvate is catalyzed by **lactate dehydrogenase**, which forms the L isomer of lactate at pH 7:



The overall equilibrium of this reaction strongly favors lactate formation, as shown by the large negative standard free-energy change.

In glycolysis, dehydrogenation of the two molecules of glyceraldehyde 3-phosphate derived from each molecule of glucose converts two molecules of NAD^+ to two of NADH. Because the reduction of two molecules of pyruvate to two of lactate regenerates two molecules of NAD^+ , there is no net change in NAD^+ or NADH:

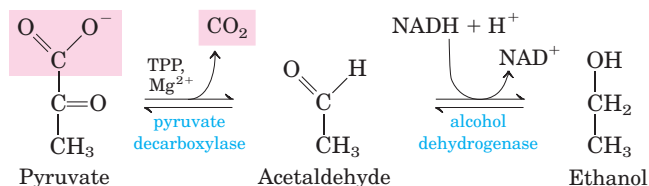


The lactate formed by active skeletal muscles (or by erythrocytes) can be recycled; it is carried in the blood to the liver, where it is converted to glucose during the recovery from strenuous muscular activity. When lactate is produced in large quantities during vigorous muscle contraction (during a sprint, for example), the acidification that results from ionization of lactic acid in muscle and blood limits the period of vigorous activity. The best-conditioned athletes can sprint at top speed for no more than a minute (Box 14–1).

Although conversion of glucose to lactate includes two oxidation-reduction steps, there is no net change in the oxidation state of carbon; in glucose ($\text{C}_6\text{H}_{12}\text{O}_6$) and lactic acid ($\text{C}_3\text{H}_6\text{O}_3$), the H:C ratio is the same. Nevertheless, some of the energy of the glucose molecule has been extracted by its conversion to lactate—enough to give a net yield of two molecules of ATP for every glucose molecule consumed. **Fermentation** is the general term for such processes, which extract energy (as ATP) but do not consume oxygen or change the concentrations of NAD^+ or NADH. Fermentations are carried out by a wide range of organisms, many of which occupy anaerobic niches, and they yield a variety of end products, some of which find commercial uses.

Ethanol Is the Reduced Product in Ethanol Fermentation

Yeast and other microorganisms ferment glucose to ethanol and CO_2 , rather than to lactate. Glucose is converted to pyruvate by glycolysis, and the pyruvate is converted to ethanol and CO_2 in a two-step process:



In the first step, pyruvate is decarboxylated in an irreversible reaction catalyzed by **pyruvate decarboxylase**. This reaction is a simple decarboxylation and does not involve the net oxidation of pyruvate. Pyruvate decarboxylase requires Mg^{2+} and has a tightly bound coenzyme, thiamine pyrophosphate, discussed below. In the second step, acetaldehyde is reduced to ethanol through the action of **alcohol dehydrogenase**, with

BOX 14-1 THE WORLD OF BIOCHEMISTRY**Athletes, Alligators, and Coelacanths: Glycolysis at Limiting Concentrations of Oxygen**

Most vertebrates are essentially aerobic organisms; they convert glucose to pyruvate by glycolysis, then use molecular oxygen to oxidize the pyruvate completely to CO_2 and H_2O . Anaerobic catabolism of glucose to lactate occurs during short bursts of extreme muscular activity, for example in a 100 m sprint, during which oxygen cannot be carried to the muscles fast enough to oxidize pyruvate. Instead, the muscles use their stored glucose (glycogen) as fuel to generate ATP by fermentation, with lactate as the end product. In a sprint, lactate in the blood builds up to high concentrations. It is slowly converted back to glucose by gluconeogenesis in the liver in the subsequent rest or recovery period, during which oxygen is consumed at a gradually diminishing rate until the breathing rate returns to normal. The excess oxygen consumed in the recovery period represents a repayment of the oxygen debt. This is the amount of oxygen required to supply ATP for gluconeogenesis during recovery respiration, in order to regenerate the glycogen “borrowed” from liver and muscle to carry out intense muscular activity in the sprint. The cycle of reactions that includes glucose conversion to lactate in muscle and lactate conversion to glucose in liver is called the Cori cycle, for Carl and Gerty Cori, whose studies in the 1930s and 1940s clarified the pathway and its role (see Box 15-1).

The circulatory systems of most small vertebrates can carry oxygen to their muscles fast enough to avoid having to use muscle glycogen anaerobically. For example, migrating birds often fly great distances at high speeds without rest and without incurring an oxygen debt. Many running animals of moderate size also maintain an essentially aerobic metabolism in their skeletal muscle. However, the circulatory systems of larger animals, including humans, cannot completely sustain aerobic metabolism in skeletal muscles over long periods of intense muscular activity. These animals generally are slow-moving under normal circumstances and engage in intense muscular activity only in the gravest emergencies, because such bursts of activity require long recovery periods to repay the oxygen debt.

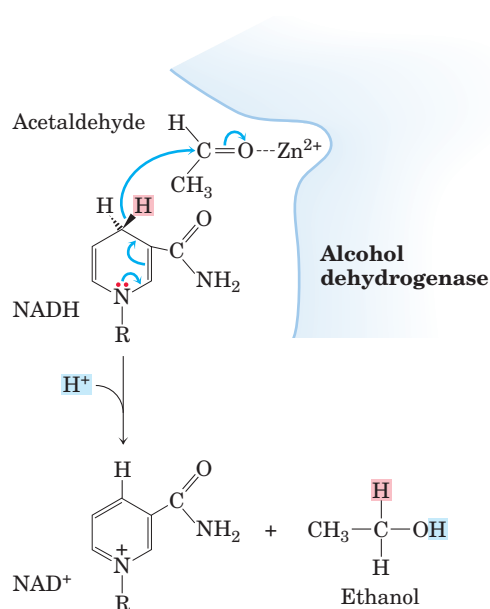
Alligators and crocodiles, for example, are normally sluggish animals. Yet when provoked they are capable of lightning-fast charges and dangerous lashings of their powerful tails. Such intense bursts of activity are short and must be followed by long periods of recovery. The fast emergency movements require

lactic acid fermentation to generate ATP in skeletal muscles. The stores of muscle glycogen are rapidly expended in intense muscular activity, and lactate reaches very high concentrations in muscles and extracellular fluid. Whereas a trained athlete can recover from a 100 m sprint in 30 min or less, an alligator may require many hours of rest and extra oxygen consumption to clear the excess lactate from its blood and regenerate muscle glycogen after a burst of activity.

Other large animals, such as the elephant and rhinoceros, have similar metabolic characteristics, as do diving mammals such as whales and seals. Dinosaurs and other huge, now-extinct animals probably had to depend on lactic acid fermentation to supply energy for muscular activity, followed by very long recovery periods during which they were vulnerable to attack by smaller predators better able to use oxygen and thus better adapted to continuous, sustained muscular activity.

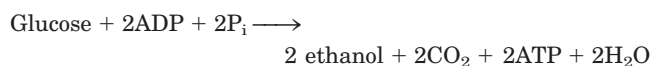
Deep-sea explorations have revealed many species of marine life at great ocean depths, where the oxygen concentration is near zero. For example, the primitive coelacanth, a large fish recovered from depths of 4,000 m or more off the coast of South Africa, has an essentially anaerobic metabolism in virtually all its tissues. It converts carbohydrates to lactate and other products, most of which must be excreted. Some marine vertebrates ferment glucose to ethanol and CO_2 in order to generate ATP.





MECHANISM FIGURE 14-12 The alcohol dehydrogenase reaction. A Zn^{2+} at the active site polarizes the carbonyl oxygen of acetaldehyde, allowing transfer of a hydride ion (red) from the reduced cofactor NADH. The reduced intermediate acquires a proton from the medium (blue) to form ethanol. **Alcohol Dehydrogenase Mechanism**

the reducing power furnished by NADH derived from the dehydrogenation of glyceraldehyde 3-phosphate. This reaction is a well-studied case of hydride transfer from NADH (Fig. 14-12). Ethanol and CO_2 are thus the end products of ethanol fermentation, and the overall equation is



As in lactic acid fermentation, there is no net change in the ratio of hydrogen to carbon atoms when glucose (H:C ratio = $12/6 = 2$) is fermented to two ethanol and

two CO_2 (combined H:C ratio = $12/6 = 2$). In all fermentations, the H:C ratio of the reactants and products remains the same.

Pyruvate decarboxylase is present in brewer's and baker's yeast and in all other organisms that ferment glucose to ethanol, including some plants. The CO_2 produced by pyruvate decarboxylation in brewer's yeast is responsible for the characteristic carbonation of champagne. The ancient art of brewing beer involves a number of enzymatic processes in addition to the reactions of ethanol fermentation (Box 14-2). In baking, CO_2 released by pyruvate decarboxylase when yeast is mixed with a fermentable sugar causes dough to rise. The enzyme is absent in vertebrate tissues and in other organisms that carry out lactic acid fermentation.

Alcohol dehydrogenase is present in many organisms that metabolize ethanol, including humans. In human liver it catalyzes the oxidation of ethanol, either ingested or produced by intestinal microorganisms, with the concomitant reduction of NAD^+ to NADH.

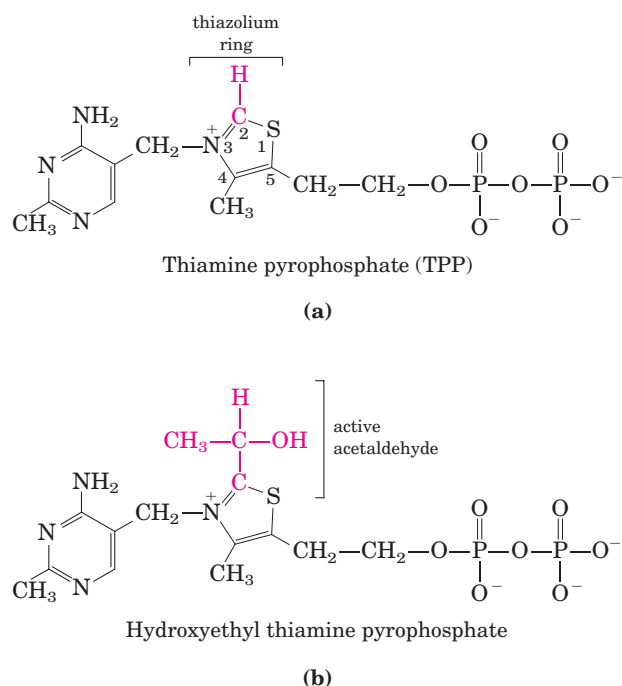
Thiamine Pyrophosphate Carries “Active Acetaldehyde” Groups

The pyruvate decarboxylase reaction provides our first encounter with **thiamine pyrophosphate (TPP)** (Fig. 14-13), a coenzyme derived from vitamin B_1 . Lack of vitamin B_1 in the human diet leads to the condition known as beriberi, characterized by an accumulation of body fluids (swelling), pain, paralysis, and ultimately death.

Thiamine pyrophosphate plays an important role in the cleavage of bonds adjacent to a carbonyl group, such as the decarboxylation of α -keto acids, and in chemical rearrangements in which an activated acetaldehyde group is transferred from one carbon atom to another (Table 14-1). The functional part of TPP, the thiazolium ring, has a relatively acidic proton at C-2. Loss of this

TABLE 14-1 Some TPP-Dependent Reactions

Enzyme	Pathway(s)	Bond cleaved	Bond formed
Pyruvate decarboxylase	Ethanol fermentation		
Pyruvate dehydrogenase α -Ketoglutarate dehydrogenase	Synthesis of acetyl-CoA Citric acid cycle		
Transketolase	Carbon-assimilation reactions Pentose phosphate pathway		

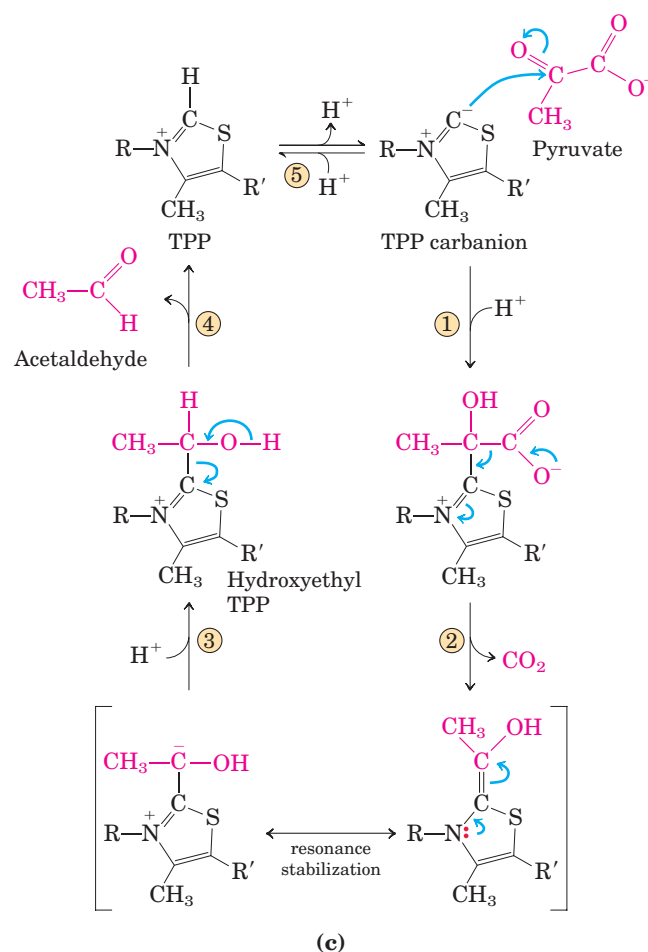


MECHANISM FIGURE 14-13 Thiamine pyrophosphate (TPP) and its role in pyruvate decarboxylation. (a) TPP is the coenzyme form of vitamin B₁ (thiamine). The reactive carbon atom in the thiazolium ring of TPP is shown in red. In the reaction catalyzed by pyruvate decarboxylase, two of the three carbons of pyruvate are carried transiently on TPP in the form of a hydroxyethyl, or “active acetaldehyde,” group (b), which is subsequently released as acetaldehyde. (c) After cleavage of a carbon–carbon bond, one product often has a free electron pair, or carbanion, which because of its strong tendency to form a new bond is generally unstable. The thiazolium ring of TPP stabilizes carbanion intermediates by providing an electrophilic (electron-deficient) structure into which the carbanion electrons can be delocalized by resonance. Structures with this property, often called “electron sinks,” play a role in many biochemical reactions. This principle is illustrated here for the reaction catalyzed by pyruvate decarboxylase. ① The TPP carbanion acts as a nucleophile, attacking the carbonyl group of pyruvate. ② Decarboxylation produces a carbanion that is stabilized by the thiazolium ring. ③ Protonation to form hydroxyethyl TPP is followed by ④ release of acetaldehyde. ⑤ A proton dissociates to regenerate the carbanion. **Thiamine Pyrophosphate Mechanism**

proton produces a carbanion that is the active species in TPP-dependent reactions (Fig. 14–13). The carbanion readily adds to carbonyl groups, and the thiazolium ring is thereby positioned to act as an “electron sink” that greatly facilitates reactions such as the decarboxylation catalyzed by pyruvate decarboxylase.

Fermentations Yield a Variety of Common Foods and Industrial Chemicals

Our progenitors learned millennia ago to use fermentation in the production and preservation of foods. Certain microorganisms present in raw food products fer-



ment the carbohydrates and yield metabolic products that give the foods their characteristic forms, textures, and tastes. Yogurt, already known in Biblical times, is produced when the bacterium *Lactobacillus bulgaricus* ferments the carbohydrate in milk, producing lactic acid; the resulting drop in pH causes the milk proteins to precipitate, producing the thick texture and sour taste of unsweetened yogurt. Another bacterium, *Propionibacterium freudenreichii*, ferments milk to produce propionic acid and CO₂; the propionic acid precipitates milk proteins, and bubbles of CO₂ cause the holes characteristic of Swiss cheese. Many other food products are the result of fermentations: pickles, sauerkraut, sausage, soy sauce, and a variety of national favorites, such as kimchi (Korea), tempoyak (Indonesia), kefir (Russia), dahi (India), and pozol (Mexico). The drop in pH associated with fermentation also helps to preserve foods, because most of the microorganisms that cause food spoilage cannot grow at low pH. In agriculture, plant byproducts such as corn stalks are preserved for use as animal feed by packing them into a large container (a silo) with limited access to air; microbial fermentation produces acids that lower the pH. The silage that results from this fermentation

BOX 14-2 THE WORLD OF BIOCHEMISTRY

Brewing Beer

Brewers prepare beer by ethanol fermentation of the carbohydrates in cereal grains (seeds) such as barley, carried out by yeast glycolytic enzymes. The carbohydrates, largely polysaccharides, must first be degraded to disaccharides and monosaccharides. In a process called malting, the barley seeds are allowed to germinate until they form the hydrolytic enzymes required to break down their polysaccharides, at which point germination is stopped by controlled heating. The product is malt, which contains enzymes that catalyze the hydrolysis of the β linkages of cellulose and other cell wall polysaccharides of the barley husks, and enzymes such as α -amylase and maltase.

The brewer next prepares the wort, the nutrient medium required for fermentation by yeast cells. The malt is mixed with water and then mashed or crushed. This allows the enzymes formed in the malting process to act on the cereal polysaccharides to form maltose, glucose, and other simple sugars, which are soluble in the aqueous medium. The remaining cell matter is then separated, and the liquid wort is boiled with hops to give flavor. The wort is cooled and then aerated.

Now the yeast cells are added. In the aerobic wort the yeast grows and reproduces very rapidly, using energy obtained from available sugars. No ethanol forms during this stage, because the yeast, amply supplied with oxygen, oxidizes the pyruvate formed by glycolysis to CO_2 and H_2O via the citric acid cycle. When all the dissolved oxygen in the vat of wort has been consumed, the yeast cells switch to anaerobic metabolism, and from this point they ferment the sugars into ethanol and CO_2 . The fermentation process is controlled in part by the concentration of the ethanol formed, by the pH, and by the amount of remaining sugar. After fermentation has been stopped, the cells are removed and the “raw” beer is ready for final processing.

In the final steps of brewing, the amount of foam or head on the beer, which results from dissolved proteins, is adjusted. Normally this is controlled by proteolytic enzymes that arise in the malting process. If these enzymes act on the proteins too long, the beer will have very little head and will be flat; if they do not act long enough, the beer will not be clear when it is cold. Sometimes proteolytic enzymes from other sources are added to control the head.

process can be kept as animal feed for long periods without spoilage.

In 1910 Chaim Weizmann (later to become the first president of Israel) discovered that the bacterium *Clostridium acetobutyricum* ferments starch to butanol and acetone. This discovery opened the field of industrial fermentations, in which some readily available material rich in carbohydrate (corn starch or molasses, for example) is supplied to a pure culture of a specific microorganism, which ferments it into a product of greater value. The methanol used to make “gasohol” is produced by microbial fermentation, as are formic, acetic, propionic, butyric, and succinic acids, and glycerol, ethanol, isopropanol, butanol, and butanediol. These fermentations are generally carried out in huge closed vats in which temperature and access to air are adjusted to favor the multiplication of the desired microorganism and to exclude contaminating organisms (Fig. 14-14). The beauty of industrial fermentations is that complicated, multistep chemical transformations are carried out in high yields and with few side products by chemical factories that reproduce themselves—microbial cells. For some industrial fermentations, technology has been developed to immobilize the cells in an inert support, to pass the starting material continuously through the bed of immobilized cells, and to collect the desired product in the effluent—an engineer’s dream!



FIGURE 14-14 Industrial-scale fermentation. Microorganisms are cultured in a sterilizable vessel containing thousands of liters of growth medium—an inexpensive source of both carbon and energy—under carefully controlled conditions, including low oxygen concentration and constant temperature. After centrifugal separation of the cells from the growth medium, the valuable products of the fermentation are recovered from the cells or from the supernatant fluid.

SUMMARY 14.3 Fates of Pyruvate under Anaerobic Conditions: Fermentation

- The NADH formed in glycolysis must be recycled to regenerate NAD^+ , which is required as an electron acceptor in the first step of the payoff phase. Under aerobic conditions, electrons pass from NADH to O_2 in mitochondrial respiration.
- Under anaerobic or hypoxic conditions, many organisms regenerate NAD^+ by transferring electrons from NADH to pyruvate, forming lactate. Other organisms, such as yeast, regenerate NAD^+ by reducing pyruvate to ethanol and CO_2 . In these anaerobic processes (fermentations), there is no *net* oxidation or reduction of the carbons of glucose.
- A variety of microorganisms can ferment sugar in fresh foods, resulting in changes in pH, taste, and texture, and preserving food from spoilage. Fermentations are used in industry to produce a wide variety of commercially valuable organic compounds from inexpensive starting materials.

14.4 Gluconeogenesis

The central role of glucose in metabolism arose early in evolution, and this sugar remains the nearly universal fuel and building block in modern organisms, from microbes to humans. In mammals, some tissues depend almost completely on glucose for their metabolic energy. For the human brain and nervous system, as well as the erythrocytes, testes, renal medulla, and embryonic tissues, glucose from the blood is the sole or major fuel source. The brain alone requires about 120 g of glucose each day—more than half of all the glucose stored as glycogen in muscle and liver. However, the supply of glucose from these stores is not always sufficient; between meals and during longer fasts, or after vigorous exercise, glycogen is depleted. For these times, organisms need a method for synthesizing glucose from noncarbohydrate precursors. This is accomplished by a pathway called **gluconeogenesis** (“formation of new sugar”), which converts pyruvate and related three- and four-carbon compounds to glucose.

Gluconeogenesis occurs in all animals, plants, fungi, and microorganisms. The reactions are essentially the same in all tissues and all species. The important precursors of glucose in animals are three-carbon compounds such as lactate, pyruvate, and glycerol, as well as certain amino acids (Fig. 14–15). In mammals, gluconeogenesis takes place mainly in the liver, and to a lesser extent in renal cortex. The glucose produced passes into the blood to supply other tissues. After vigorous exercise, lactate produced by anaerobic glycoly-

sis in skeletal muscle returns to the liver and is converted to glucose, which moves back to muscle and is converted to glycogen—a circuit called the Cori cycle (Box 14–1; see also Fig. 23–18). In plant seedlings, stored fats and proteins are converted, via paths that include gluconeogenesis, to the disaccharide sucrose for transport throughout the developing plant. Glucose and its derivatives are precursors for the synthesis of plant cell walls, nucleotides and coenzymes, and a variety of other essential metabolites. In many microorganisms, gluconeogenesis starts from simple organic compounds of two or three carbons, such as acetate, lactate, and propionate, in their growth medium.

Although the reactions of gluconeogenesis are the same in all organisms, the metabolic context and the regulation of the pathway differ from one species to another and from tissue to tissue. In this section we focus on gluconeogenesis as it occurs in the mammalian liver. In Chapter 20 we show how photosynthetic organisms use this pathway to convert the primary products of photosynthesis into glucose, to be stored as sucrose or starch.

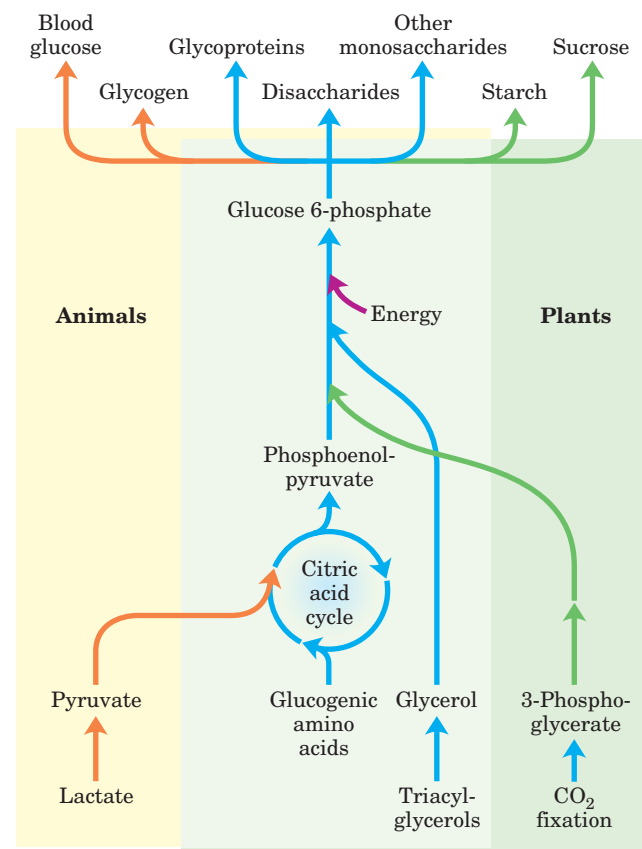
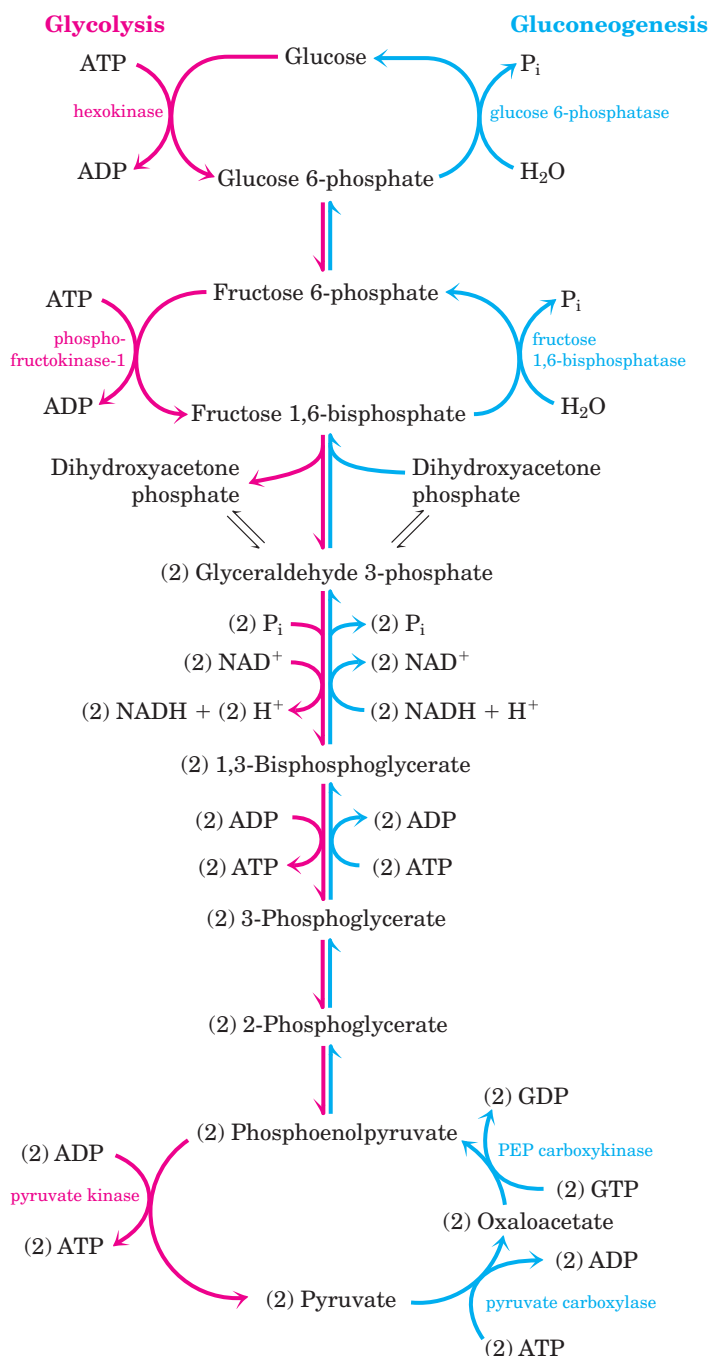


FIGURE 14–15 Carbohydrate synthesis from simple precursors. The pathway from phosphoenolpyruvate to glucose 6-phosphate is common to the biosynthetic conversion of many different precursors of carbohydrates in animals and plants. Plants and photosynthetic bacteria are uniquely able to convert CO_2 to carbohydrates.



Gluconeogenesis and glycolysis are not identical pathways running in opposite directions, although they do share several steps (Fig. 14–16); seven of the ten enzymatic reactions of gluconeogenesis are the reverse of glycolytic reactions. However, three reactions of glycolysis are essentially irreversible *in vivo* and cannot be used in gluconeogenesis: the conversion of glucose to glucose 6-phosphate by hexokinase, the phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate by phosphofructokinase-1, and the conversion of phosphoenolpyruvate to pyruvate by pyruvate kinase (Fig. 14–16). In cells, these three reactions are charac-

FIGURE 14–16 Opposing pathways of glycolysis and gluconeogenesis in rat liver. The reactions of glycolysis are shown on the left side in blue; the opposing pathway of gluconeogenesis is shown on the right in red. The major sites of regulation of gluconeogenesis shown here are discussed later in this chapter, and in detail in Chapter 15. Figure 14–19 illustrates an alternative route for oxaloacetate produced in mitochondria.

terized by a large negative free-energy change, ΔG , whereas other glycolytic reactions have a ΔG near 0 (Table 14–2). In gluconeogenesis, the three irreversible steps are bypassed by a separate set of enzymes, catalyzing reactions that are sufficiently exergonic to be effectively irreversible in the direction of glucose synthesis. Thus, both glycolysis and gluconeogenesis are irreversible processes in cells. In animals, both pathways occur largely in the cytosol, necessitating their reciprocal and coordinated regulation. Separate regulation of the two pathways is brought about through controls exerted on the enzymatic steps unique to each.

We begin by considering the three bypass reactions of gluconeogenesis. (Keep in mind that “bypass” refers throughout to the bypass of irreversible glycolytic reactions.)

Conversion of Pyruvate to Phosphoenolpyruvate Requires Two Exergonic Reactions

The first of the bypass reactions in gluconeogenesis is the conversion of pyruvate to phosphoenolpyruvate (PEP). This reaction cannot occur by reversal of the pyruvate kinase reaction of glycolysis (p. 532), which has a large, negative standard free-energy change and is irreversible under the conditions prevailing in intact cells (Table 14–2, step 10). Instead, the phosphorylation of pyruvate is achieved by a roundabout sequence of reactions that in eukaryotes requires enzymes in both the cytosol and mitochondria. As we shall see, the pathway shown in Figure 14–16 and described in detail here is one of two routes from pyruvate to PEP; it is the predominant path when pyruvate or alanine is the glucogenic precursor. A second pathway, described later, predominates when lactate is the glucogenic precursor.

Pyruvate is first transported from the cytosol into mitochondria or is generated from alanine within mitochondria by transamination, in which the α -amino group is removed from alanine (leaving pyruvate) and added to an α -keto carboxylic acid (transamination reactions are discussed in detail in Chapter 18). Then **pyruvate carboxylase**, a mitochondrial enzyme that requires the coenzyme **biotin**, converts the pyruvate to oxaloacetate (Fig. 14–17):

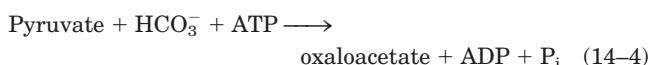


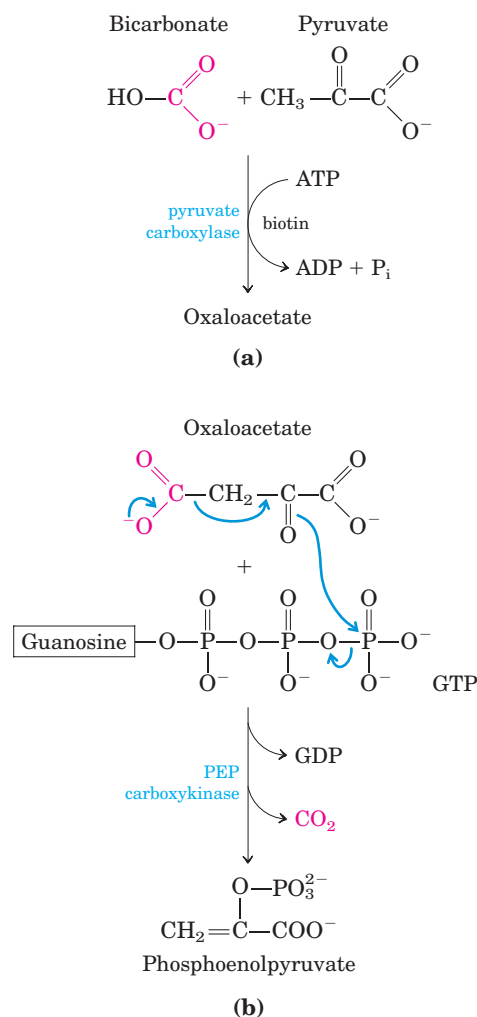
TABLE 14-2 Free-Energy Changes of Glycolytic Reactions in Erythrocytes

Glycolytic reaction step	$\Delta G'^{\circ}$ (kJ/mol)	ΔG (kJ/mol)
① Glucose + ATP \longrightarrow glucose 6-phosphate + ADP	-16.7	-33.4
② Glucose 6-phosphate \rightleftharpoons fructose 6-phosphate	1.7	0 to 25
③ Fructose 6-phosphate + ATP \longrightarrow fructose 1,6-bisphosphate + ADP	-14.2	-22.2
④ Fructose 1,6-bisphosphate \rightleftharpoons dihydroxyacetone phosphate + glyceraldehyde 3-phosphate	23.8	0 to -6
⑤ Dihydroxyacetone phosphate \rightleftharpoons glyceraldehyde 3-phosphate	7.5	0 to 4
⑥ Glyceraldehyde 3-phosphate + P_i + NAD^+ \rightleftharpoons 1,3-bisphosphoglycerate + $NADH$ + H^+	6.3	-2 to 2
⑦ 1,3-Bisphosphoglycerate + ADP \rightleftharpoons 3-phosphoglycerate + ATP	-18.8	0 to 2
⑧ 3-Phosphoglycerate \rightleftharpoons 2-phosphoglycerate	4.4	0 to 0.8
⑨ 2-Phosphoglycerate \rightleftharpoons phosphoenolpyruvate + H_2O	7.5	0 to 3.3
⑩ Phosphoenolpyruvate + ADP \longrightarrow pyruvate + ATP	-31.4	-16.7

Note: $\Delta G'^{\circ}$ is the standard free-energy change, as defined in Chapter 13 (p. 491). ΔG is the free-energy change calculated from the actual concentrations of glycolytic intermediates present under physiological conditions in erythrocytes, at pH 7. The glycolytic reactions bypassed in gluconeogenesis are shown in red. Biochemical equations are not necessarily balanced for H or charge (p. 506).

The reaction involves biotin as a carrier of activated HCO_3^- (Fig. 14-18). The reaction mechanism is shown in Figure 16-16. Pyruvate carboxylase is the first regulatory enzyme in the gluconeogenic pathway, requiring acetyl-CoA as a positive effector. (Acetyl-CoA is produced by fatty acid oxidation (Chapter 17), and its accumulation signals the availability of fatty acids as fuel.) As we shall see in Chapter 16 (see Fig. 16-15), the pyruvate carboxylase reaction can replenish intermediates in another central metabolic pathway, the citric acid cycle.

Because the mitochondrial membrane has no transporter for oxaloacetate, before export to the cytosol the oxaloacetate formed from pyruvate must be reduced to malate by mitochondrial **malate dehydrogenase**, at the expense of NADH:

**FIGURE 14-17** Synthesis of phosphoenolpyruvate from pyruvate.

(a) In mitochondria, pyruvate is converted to oxaloacetate in a biotin-requiring reaction catalyzed by pyruvate carboxylase. **(b)** In the cytosol, oxaloacetate is converted to phosphoenolpyruvate by PEP carboxykinase. The CO_2 incorporated in the pyruvate carboxylase reaction is lost here as CO_2 . The decarboxylation leads to a rearrangement of electrons that facilitates attack of the carbonyl oxygen of the pyruvate moiety on the γ phosphate of GTP.

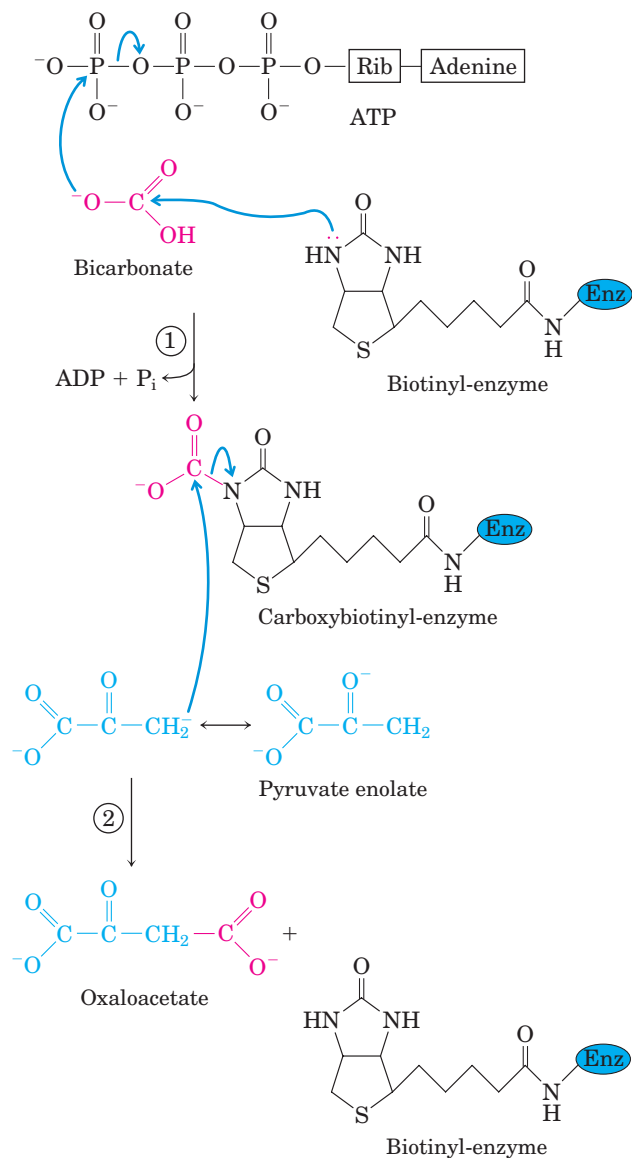


FIGURE 14-18 Role of biotin in the pyruvate carboxylase reaction.

The cofactor biotin is covalently attached to the enzyme through an amide linkage to the ϵ -amino group of a Lys residue, forming a biotinyl-enzyme. The reaction occurs in two phases, which occur at two different sites in the enzyme. At catalytic site 1, bicarbonate ion is converted to CO_2 at the expense of ATP. Then CO_2 reacts with biotin, forming carboxybiotinyl-enzyme. The long arm composed of biotin and the side chain of the Lys to which it is attached then carry the CO_2 of carboxybiotinyl-enzyme to catalytic site 2 on the enzyme surface, where CO_2 is released and reacts with the pyruvate, forming oxaloacetate and regenerating the biotinyl-enzyme. The general role of flexible arms in carrying reaction intermediates between enzyme active sites is described in Figure 16-17, and the mechanistic details of the pyruvate carboxylase reaction are shown in Figure 16-16. Similar mechanisms occur in other biotin-dependent carboxylation reactions, such as those catalyzed by propionyl-CoA carboxylase (see Fig. 17-11) and acetyl-CoA carboxylase (see Fig. 21-1).

The standard free-energy change for this reaction is quite high, but under physiological conditions (including a very low concentration of oxaloacetate) $\Delta G \approx 0$ and the reaction is readily reversible. Mitochondrial malate dehydrogenase functions in both gluconeogenesis and the citric acid cycle, but the overall flow of metabolites in the two processes is in opposite directions.

Malate leaves the mitochondrion through a specific transporter in the inner mitochondrial membrane (see Fig. 19-27), and in the cytosol it is reoxidized to oxaloacetate, with the production of cytosolic NADH:

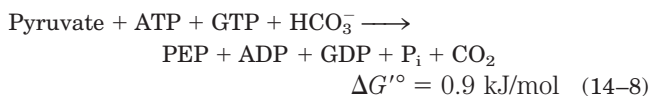


The oxaloacetate is then converted to PEP by **phosphoenolpyruvate carboxykinase** (Fig. 14-17). This Mg^{2+} -dependent reaction requires GTP as the phosphoryl group donor :



The reaction is reversible under intracellular conditions; the formation of one high-energy phosphate compound (PEP) is balanced by the hydrolysis of another (GTP).

The overall equation for this set of bypass reactions, the sum of Equations 14-4 through 14-7, is



Two high-energy phosphate equivalents (one from ATP and one from GTP), each yielding about 50 kJ/mol under cellular conditions, must be expended to phosphorylate one molecule of pyruvate to PEP. In contrast, when PEP is converted to pyruvate during glycolysis, only one ATP is generated from ADP. Although the standard free-energy change ($\Delta G'^{\circ}$) of the two-step path from pyruvate to PEP is 0.9 kJ/mol, the actual free-energy change (ΔG), calculated from measured cellular concentrations of intermediates, is very strongly negative (-25 kJ/mol); this results from the ready consumption of PEP in other reactions such that its concentration remains relatively low. The reaction is thus effectively irreversible in the cell.

Note that the CO_2 added to pyruvate in the pyruvate carboxylase step is the same molecule that is lost in the PEP carboxykinase reaction (Fig. 14-17). This carboxylation-decarboxylation sequence represents a way of “activating” pyruvate, in that the decarboxylation of oxaloacetate facilitates PEP formation. In Chapter 21 we shall see how a similar carboxylation-decarboxylation sequence is used to activate acetyl-CoA for fatty acid biosynthesis (see Fig. 21-1).

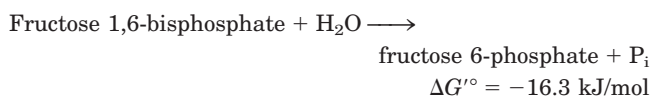
There is a logic to the route of these reactions through the mitochondrion. The $[\text{NADH}]/[\text{NAD}^+]$ ratio in the cytosol is 8×10^{-4} , about 10^5 times lower than in mitochondria. Because cytosolic NADH is consumed in gluconeogenesis (in the conversion of 1,3-bisphos-

phoglycerate to glyceraldehyde 3-phosphate; Fig. 14–16), glucose biosynthesis cannot proceed unless NADH is available. The transport of malate from the mitochondrion to the cytosol and its reconversion there to oxaloacetate effectively moves reducing equivalents to the cytosol, where they are scarce. This path from pyruvate to PEP therefore provides an important balance between NADH produced and consumed in the cytosol during gluconeogenesis.

A second pyruvate \rightarrow PEP bypass predominates when lactate is the glucogenic precursor (Fig. 14–19). This pathway makes use of lactate produced by glycolysis in erythrocytes or anaerobic muscle, for example, and it is particularly important in large vertebrates after vigorous exercise (Box 14–1). The conversion of lactate to pyruvate in the cytosol of hepatocytes yields NADH, and the export of reducing equivalents (as malate) from mitochondria is therefore unnecessary. After the pyruvate produced by the lactate dehydrogenase reaction is transported into the mitochondrion, it is converted to oxaloacetate by pyruvate carboxylase, as described above. This oxaloacetate, however, is converted directly to PEP by a mitochondrial isozyme of PEP carboxykinase, and the PEP is transported out of the mitochondrion to continue on the gluconeogenic path. The mitochondrial and cytosolic isozymes of PEP carboxykinase are encoded by separate genes in the nuclear chromosomes, providing another example of two distinct enzymes catalyzing the same reaction but having different cellular locations or metabolic roles (recall the isozymes of hexokinase).

Conversion of Fructose 1,6-Bisphosphate to Fructose 6-Phosphate Is the Second Bypass

The second glycolytic reaction that cannot participate in gluconeogenesis is the phosphorylation of fructose 6-phosphate by PFK-1 (Table 14–2, step ③). Because this reaction is highly exergonic and therefore irreversible in intact cells, the generation of fructose 6-phosphate from fructose 1,6-bisphosphate (Fig. 14–16) is catalyzed by a different enzyme, Mg^{2+} -dependent **fructose 1,6-bisphosphatase (FBPase-1)**, which promotes the essentially irreversible *hydrolysis* of the C-1 phosphate (*not* phosphoryl group transfer to ADP):



Conversion of Glucose 6-Phosphate to Glucose Is the Third Bypass

The third bypass is the final reaction of gluconeogenesis, the dephosphorylation of glucose 6-phosphate to yield glucose (Fig. 14–16). Reversal of the hexokinase reaction (p. 526) would require phosphoryl group trans-

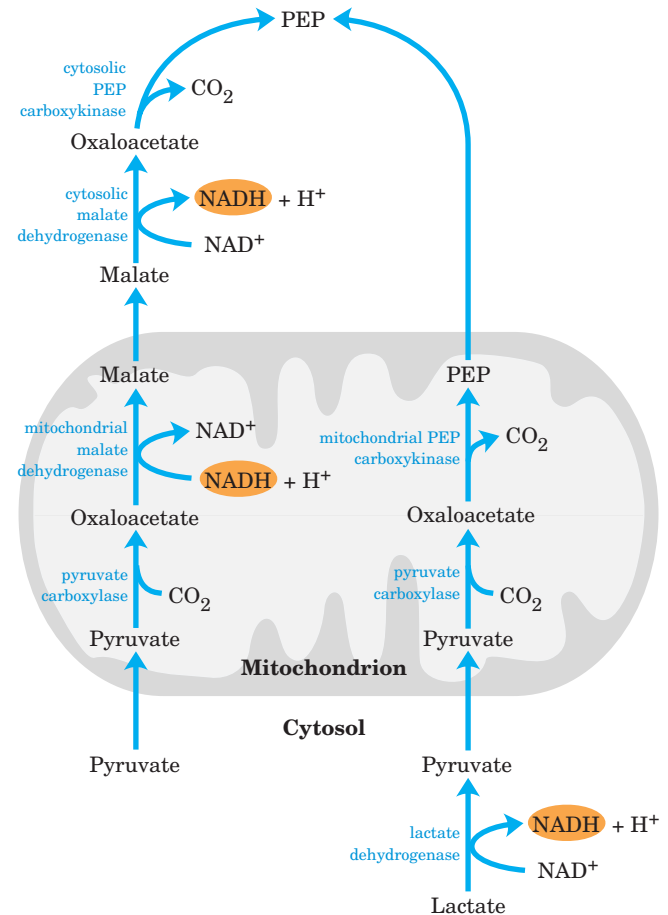
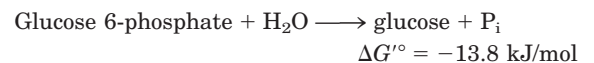


FIGURE 14–19 Alternative paths from pyruvate to phosphoenolpyruvate. The path that predominates depends on the glucogenic precursor (lactate or pyruvate). The path on the right predominates when lactate is the precursor, because cytosolic NADH is generated in the lactate dehydrogenase reaction and does not have to be shuttled out of the mitochondrion (see text). The relative importance of the two pathways depends on the availability of lactate and the cytosolic requirements for NADH by gluconeogenesis.

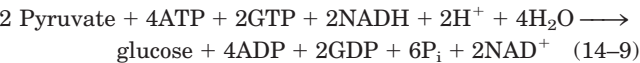
fer from glucose 6-phosphate to ADP, forming ATP, an energetically unfavorable reaction (Table 14–2, step ①). The reaction catalyzed by **glucose 6-phosphatase** does not require synthesis of ATP; it is a simple hydrolysis of a phosphate ester:



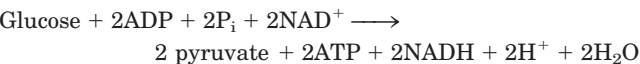
This Mg^{2+} -activated enzyme is found on the luminal side of the endoplasmic reticulum of hepatocytes and renal cells (see Fig. 15–6). Muscle and brain tissue do not contain this enzyme and so cannot carry out gluconeogenesis. Glucose produced by gluconeogenesis in the liver or kidney or ingested in the diet is delivered to brain and muscle through the bloodstream.

Gluconeogenesis Is Energetically Expensive, but Essential

The sum of the biosynthetic reactions leading from pyruvate to free blood glucose (Table 14–3) is



For each molecule of glucose formed from pyruvate, six high-energy phosphate groups are required, four from ATP and two from GTP. In addition, two molecules of NADH are required for the reduction of two molecules of 1,3-bisphosphoglycerate. Clearly, Equation 14–9 is not simply the reverse of the equation for conversion of glucose to pyruvate by glycolysis, which requires only two molecules of ATP:



The synthesis of glucose from pyruvate is a relatively expensive process. Much of this high energy cost is necessary to ensure the irreversibility of gluconeogenesis. Under intracellular conditions, the overall free-energy change of glycolysis is at least –63 kJ/mol. Under the same conditions the overall ΔG of gluconeogenesis is –16 kJ/mol. Thus both glycolysis and gluconeogenesis are essentially irreversible processes in cells.

Citric Acid Cycle Intermediates and Many Amino Acids Are Glucogenic

The biosynthetic pathway to glucose described above allows the net synthesis of glucose not only from pyruvate but also from the four-, five-, and six-carbon intermediates of the citric acid cycle (Chapter 16). Citrate,

isocitrate, α-ketoglutarate, succinyl-CoA, succinate, fumarate, and malate—all are citric acid cycle intermediates that can undergo oxidation to oxaloacetate (see Fig. 16–7). Some or all of the carbon atoms of most amino acids derived from proteins are ultimately catabolized to pyruvate or to intermediates of the citric acid cycle. Such amino acids can therefore undergo net conversion to glucose and are said to be **glucogenic** (Table 14–4). Alanine and glutamine, the principal molecules that transport amino groups from extrahepatic tissues to the liver (see Fig. 18–9), are particularly important glucogenic amino acids in mammals. After removal of their amino groups in liver mitochondria, the carbon skeletons remaining (pyruvate and α-ketoglutarate, respectively) are readily funneled into gluconeogenesis.

In contrast, no net conversion of fatty acids to glucose occurs in mammals. As we shall see in Chapter 17, the catabolism of most fatty acids yields only acetyl-CoA. Mammals cannot use acetyl-CoA as a precursor of glucose, because the pyruvate dehydrogenase reaction is irreversible and cells have no other pathway to convert acetyl-CoA to pyruvate. Plants, yeast, and many bacteria do have a pathway (the glyoxylate cycle; see Fig. 16–20) for converting acetyl-CoA to oxaloacetate, so these organisms can use fatty acids as the starting material for gluconeogenesis. This is especially important during the germination of seedlings, before photosynthesis can serve as a source of glucose.

Glycolysis and Gluconeogenesis Are Regulated Reciprocally

If glycolysis (the conversion of glucose to pyruvate) and gluconeogenesis (the conversion of pyruvate to glucose) were allowed to proceed simultaneously at high rates,

TABLE 14–3 Sequential Reactions in Gluconeogenesis Starting from Pyruvate		
Pyruvate + HCO ₃ [–] + ATP → oxaloacetate + ADP + P _i		×2
Oxaloacetate + GTP ⇌ phosphoenolpyruvate + CO ₂ + GDP		×2
Phosphoenolpyruvate + H ₂ O ⇌ 2-phosphoglycerate		×2
2-Phosphoglycerate ⇌ 3-phosphoglycerate		×2
3-Phosphoglycerate + ATP ⇌ 1,3-bisphosphoglycerate + ADP		×2
1,3-Bisphosphoglycerate + NADH + H ⁺ ⇌ glyceraldehyde 3-phosphate + NAD ⁺ + P _i		×2
Glyceraldehyde 3-phosphate ⇌ dihydroxyacetone phosphate		
Glyceraldehyde 3-phosphate + dihydroxyacetone phosphate ⇌ fructose 1,6-bisphosphate		
Fructose 1,6-bisphosphate → fructose 6-phosphate + P _i		
Fructose 6-phosphate ⇌ glucose 6-phosphate		
Glucose 6-phosphate + H ₂ O → glucose + P _i		
Sum: 2 Pyruvate + 4ATP + 2GTP + 2NADH + 2H ⁺ + 4H ₂ O → glucose + 4ADP + 2GDP + 6P _i + 2NAD ⁺		

Note: The bypass reactions are in red; all other reactions are reversible steps of glycolysis. The figures at the right indicate that the reaction is to be counted twice, because two three-carbon precursors are required to make a molecule of glucose. The reactions required to replace the cytosolic NADH consumed in the glyceraldehyde 3-phosphate dehydrogenase reaction (the conversion of lactate to pyruvate in the cytosol or the transport of reducing equivalents from mitochondria to the cytosol in the form of malate) are not considered in this summary. Biochemical equations are not necessarily balanced for H and charge (p. 506).

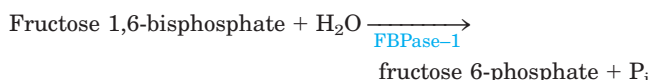
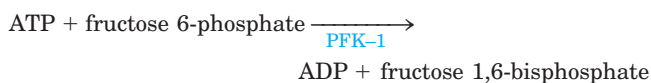
TABLE 14-4 Glucogenic Amino Acids, Grouped by Site of Entry

Pyruvate	Succinyl-CoA
Alanine	Isoleucine*
Cysteine	Methionine
Glycine	Threonine
Serine	Valine
Threonine	Fumarate
Tryptophan*	Phenylalanine*
α-Ketoglutarate	Tyrosine*
Arginine	Oxaloacetate
Glutamate	Asparagine
Glutamine	Aspartate
Histidine	
Proline	

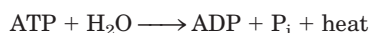
Note: All these amino acids are precursors of blood glucose or liver glycogen, because they can be converted to pyruvate or citric acid cycle intermediates. Of the 20 common amino acids, only leucine and lysine are unable to furnish carbon for net glucose synthesis.

*These amino acids are also ketogenic (see Fig. 18-21).

the result would be the consumption of ATP and the production of heat. For example, PFK-1 and FBPase-1 catalyze opposing reactions:



The sum of these two reactions is



These two enzymatic reactions, and a number of others in the two pathways, are regulated allosterically and by covalent modification (phosphorylation). In Chapter 15 we take up the mechanisms of this regulation in detail. For now, suffice it to say that the pathways are regulated so that when the flux of glucose through glycolysis goes up, the flux of pyruvate toward glucose goes down, and vice versa.

SUMMARY 14.4 Gluconeogenesis

- Gluconeogenesis is a ubiquitous multistep process in which pyruvate or a related three-carbon compound (lactate, alanine) is converted to glucose. Seven of the steps in gluconeogenesis are catalyzed by the same enzymes used in glycolysis; these are the reversible reactions.
- Three irreversible steps in the glycolytic pathway are bypassed by reactions catalyzed by gluconeogenic enzymes: (1) conversion of

pyruvate to PEP via oxaloacetate, catalyzed by pyruvate carboxylase and PEP carboxykinase; (2) dephosphorylation of fructose 1,6-bisphosphate by FBPase-1; and (3) dephosphorylation of glucose 6-phosphate by glucose 6-phosphatase.

- Formation of one molecule of glucose from pyruvate requires 4 ATP, 2 GTP, and 2 NADH; it is expensive.
- In mammals, gluconeogenesis in the liver and kidney provides glucose for use by the brain, muscles, and erythrocytes.
- Pyruvate carboxylase is stimulated by acetyl-CoA, increasing the rate of gluconeogenesis when the cell already has adequate supplies of other substrates (fatty acids) for energy production.
- Animals cannot convert acetyl-CoA derived from fatty acids into glucose; plants and microorganisms can.
- Glycolysis and gluconeogenesis are reciprocally regulated to prevent wasteful operation of both pathways at the same time.

14.5 Pentose Phosphate Pathway of Glucose Oxidation



In most animal tissues, the major catabolic fate of glucose 6-phosphate is glycolytic breakdown to pyruvate, much of which is then oxidized via the citric acid cycle, ultimately leading to the formation of ATP. Glucose 6-phosphate does have other catabolic fates, however, which lead to specialized products needed by the cell. Of particular importance in some tissues is the oxidation of glucose 6-phosphate to pentose phosphates by the **pentose phosphate pathway** (also called the **phosphogluconate pathway** or the **hexose monophosphate pathway**; Fig. 14-20). In this oxidative pathway, NADP^+ is the electron acceptor, yielding NADPH. Rapidly dividing cells, such as those of bone marrow, skin, and intestinal mucosa, use the pentoses to make RNA, DNA, and such coenzymes as ATP, NADH, FADH_2 , and coenzyme A.

In other tissues, the essential product of the pentose phosphate pathway is not the pentoses but the electron donor NADPH, needed for reductive biosynthesis or to counter the damaging effects of oxygen radicals. Tissues that carry out extensive fatty acid synthesis (liver, adipose, lactating mammary gland) or very active synthesis of cholesterol and steroid hormones (liver, adrenal gland, gonads) require the NADPH provided by the pathway. Erythrocytes and the cells of the lens and cornea are directly exposed to oxygen and thus to the damaging free radicals generated by oxygen.

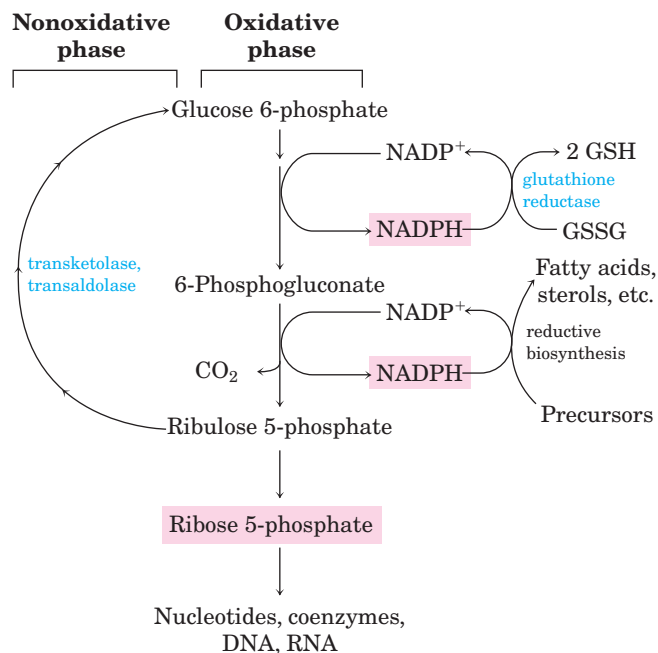


FIGURE 14-20 General scheme of the pentose phosphate pathway.

NADPH formed in the oxidative phase is used to reduce glutathione, GSSG (see Box 14-3) and to support reductive biosynthesis. The other product of the oxidative phase is ribose 5-phosphate, which serves as precursor for nucleotides, coenzymes, and nucleic acids. In cells that are not using ribose 5-phosphate for biosynthesis, the nonoxidative phase recycles six molecules of the pentose into five molecules of the hexose glucose 6-phosphate, allowing continued production of NADPH and converting glucose 6-phosphate (in six cycles) to CO₂.

By maintaining a reducing atmosphere (a high ratio of NADPH to NADP⁺ and a high ratio of reduced to oxidized glutathione), they can prevent or undo oxidative damage to proteins, lipids, and other sensitive molecules. In erythrocytes, the NADPH produced by the pentose phosphate pathway is so important in preventing oxidative damage that a genetic defect in glucose 6-phosphate dehydrogenase, the first enzyme of the pathway, can have serious medical consequences (Box 14-3). ■

The Oxidative Phase Produces Pentose Phosphates and NADPH

The first reaction of the pentose phosphate pathway (Fig. 14-21) is the oxidation of glucose 6-phosphate by **glucose 6-phosphate dehydrogenase (G6PD)** to form 6-phosphoglucono- δ -lactone, an intramolecular ester. NADP⁺ is the electron acceptor, and the overall equilibrium lies far in the direction of NADPH formation. The lactone is hydrolyzed to the free acid 6-phosphogluconate by a specific **lactonase**, then 6-phosphogluconate undergoes oxidation and decarboxylation by **6-phosphogluconate dehydrogenase** to form the ketopentose ribulose 5-phosphate. This reaction generates

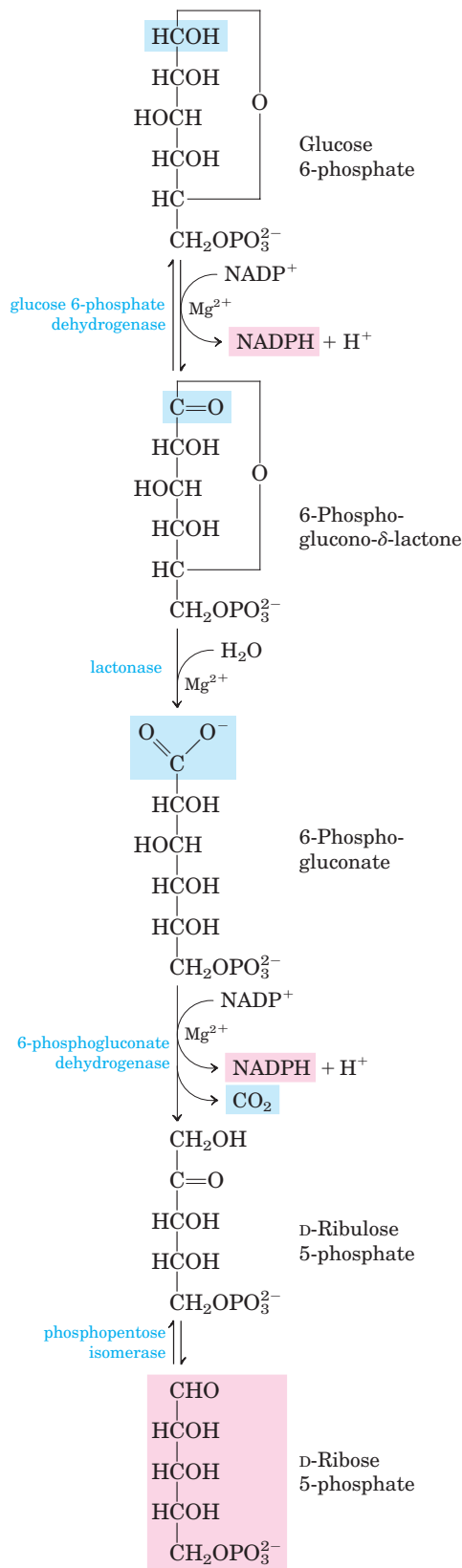


FIGURE 14-21 Oxidative reactions of the pentose phosphate pathway. The end products are ribose 5-phosphate, CO₂, and NADPH.



BOX 14-3 BIOCHEMISTRY IN MEDICINE

Why Pythagoras Wouldn't Eat Falafel: Glucose 6-Phosphate Dehydrogenase Deficiency

Fava beans, an ingredient of falafel, have been an important food source in the Mediterranean and Middle East since antiquity. The Greek philosopher and mathematician Pythagoras prohibited his followers from dining on fava beans, perhaps because they make many people sick with a condition called favism, which can be fatal. In favism, erythrocytes begin to lyse 24 to 48 hours after ingestion of the beans, releasing free hemoglobin into the blood. Jaundice and sometimes kidney failure can result. Similar symptoms can occur with ingestion of the antimalarial drug primaquine or of sulfa antibiotics or following exposure to certain herbicides. These symptoms have a genetic basis: glucose 6-phosphate dehydrogenase (G6PD) deficiency, which affects about 400 million people. Most G6PD-deficient individuals are asymptomatic; only the combination of G6PD deficiency and certain environmental factors produces the clinical manifestations.

G6PD catalyzes the first step in the pentose phosphate pathway (see Fig. 14-21), which produces NADPH. This reductant, essential in many biosynthetic pathways, also protects cells from oxidative damage by hydrogen peroxide (H_2O_2) and superoxide free radicals, highly reactive oxidants generated as metabolic byproducts and through the actions of drugs such as primaquine and natural products such as divicine—the toxic ingredient of fava beans. During normal detoxification, H_2O_2 is converted to H_2O by reduced glutathione and glutathione peroxidase, and the oxidized glutathione is converted back to the reduced form by glutathione reductase and NADPH (Fig. 1). H_2O_2 is also broken down to H_2O and O_2 by catalase, which also requires NADPH. In G6PD-deficient individuals, the NADPH production is diminished and detoxification of H_2O_2 is inhibited. Cellular damage results: lipid peroxidation leading to breakdown of erythrocyte membranes and oxidation of proteins and DNA.

The geographic distribution of G6PD deficiency is instructive. Frequencies as high as 25% occur in tropical Africa, parts of the Middle East, and Southeast Asia, areas where malaria is most prevalent. In addition to such epidemiological observations, in vitro studies show that growth of one malaria parasite, *Plasmodium falciparum*, is inhibited in G6PD-deficient erythrocytes. The parasite is very sensitive to oxidative damage and is killed by a level of oxidative stress that is tolerable to a G6PD-deficient human host. Because the advantage of resistance to malaria balances the disadvantage of lowered resistance to oxidative damage, natural selection sustains the G6PD-deficient genotype in human populations where malaria is prevalent. Only under overwhelming oxidative stress, caused by drugs, herbicides, or divicine, does G6PD deficiency cause serious medical problems.

An antimalarial drug such as primaquine is believed to act by causing oxidative stress to the parasite. It is ironic that antimalarial drugs can cause illness through the same biochemical mechanism that provides resistance to malaria. Divicine also acts as an antimalarial drug, and ingestion of fava beans may protect against malaria. By refusing to eat falafel, many Pythagoreans with normal G6PD activity may have unwittingly increased their risk of malaria!

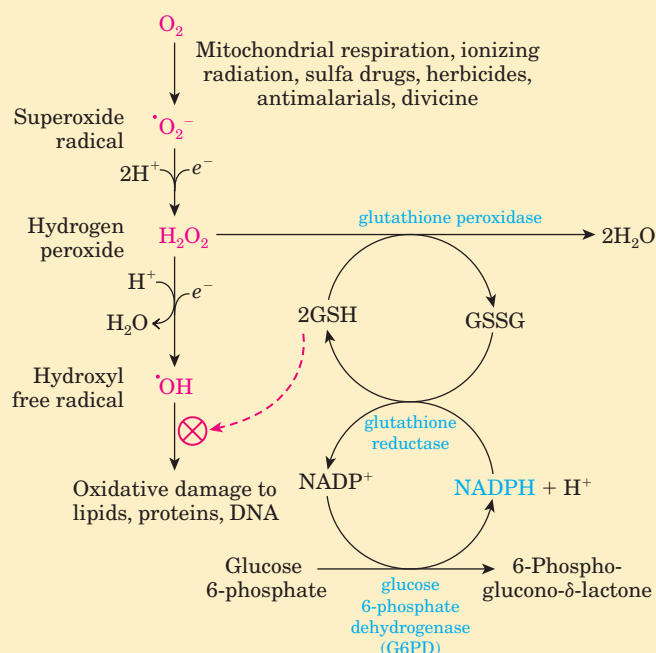
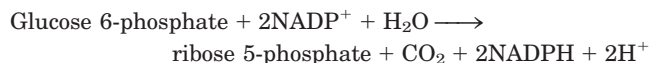


FIGURE 1 Role of NADPH and glutathione in protecting cells against highly reactive oxygen derivatives. Reduced glutathione (GSH) protects the cell by destroying hydrogen peroxide and hydroxyl free radicals. Regeneration of GSH from its oxidized form (GSSG) requires the NADPH produced in the glucose 6-phosphate dehydrogenase reaction.

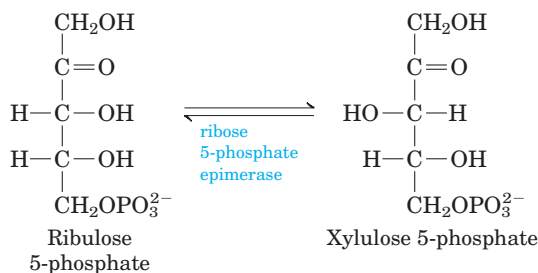
a second molecule of NADPH. **Phosphopentose isomerase** converts ribulose 5-phosphate to its aldose isomer, ribose 5-phosphate. In some tissues, the pentose phosphate pathway ends at this point, and its overall equation is



The net result is the production of NADPH, a reductant for biosynthetic reactions, and ribose 5-phosphate, a precursor for nucleotide synthesis.

The Nonoxidative Phase Recycles Pentose Phosphates to Glucose 6-Phosphate

In tissues that require primarily NADPH, the pentose phosphates produced in the oxidative phase of the pathway are recycled into glucose 6-phosphate. In this non-oxidative phase, ribulose 5-phosphate is first epimerized to xylulose 5-phosphate:



Then, in a series of rearrangements of the carbon skeletons (Fig. 14–22), six five-carbon sugar phosphates are

converted to five six-carbon sugar phosphates, completing the cycle and allowing continued oxidation of glucose 6-phosphate with production of NADPH. Continued recycling leads ultimately to the conversion of glucose 6-phosphate to six CO_2 . Two enzymes unique to the pentose phosphate pathway act in these interconversions of sugars: transketolase and transaldolase. **Transketolase** catalyzes the transfer of a two-carbon fragment from a ketose donor to an aldose acceptor (Fig. 14–23a). In its first appearance in the pentose phosphate pathway, transketolase transfers C-1 and C-2 of xylulose 5-phosphate to ribose 5-phosphate, forming the seven-carbon product sedoheptulose 7-phosphate (Fig. 14–23b). The remaining three-carbon fragment from xylulose is glyceraldehyde 3-phosphate.

Next, **transaldolase** catalyzes a reaction similar to the aldolase reaction of glycolysis: a three-carbon fragment is removed from sedoheptulose 7-phosphate and condensed with glyceraldehyde 3-phosphate, forming fructose 6-phosphate and the tetrose erythrose 4-phosphate (Fig. 14–24). Now transketolase acts again, forming fructose 6-phosphate and glyceraldehyde 3-phosphate from erythrose 4-phosphate and xylulose 5-phosphate (Fig. 14–25). Two molecules of glyceraldehyde 3-phosphate formed by two iterations of these reactions can be converted to a molecule of fructose 1,6-bisphosphate as in gluconeogenesis (Fig. 14–16), and finally FBPase-1 and phosphohexose isomerase convert fructose 1,6-bisphosphate to glucose 6-phosphate. The cycle is complete: six pentose phosphates have been converted to five hexose phosphates (Fig. 14–22b).

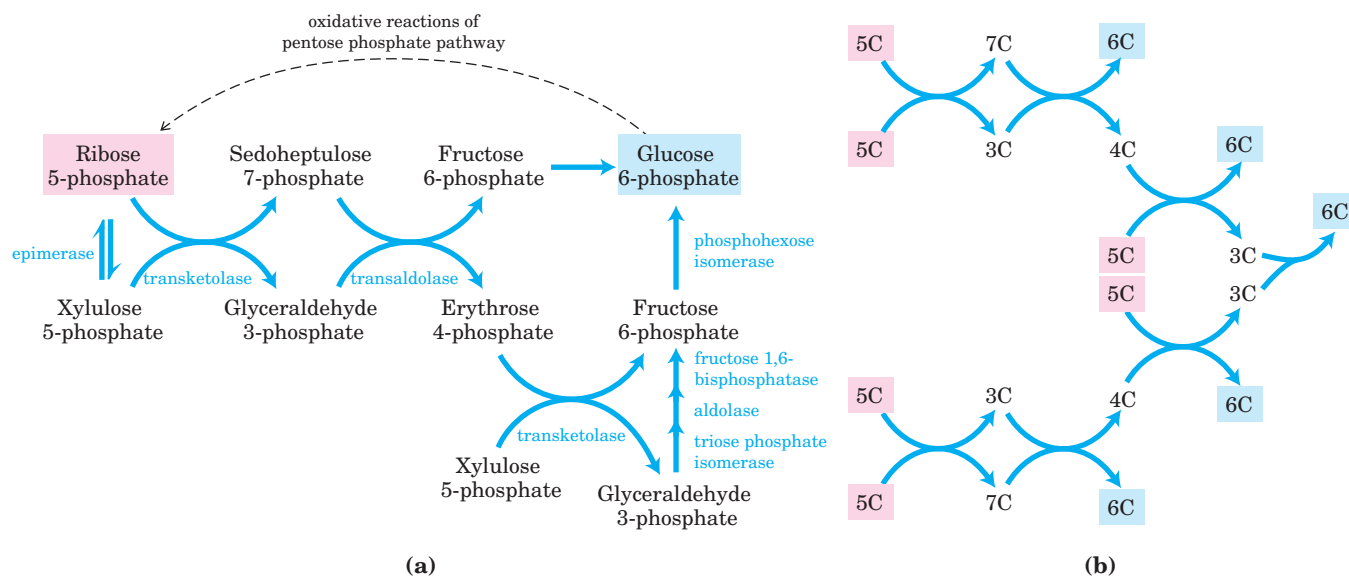
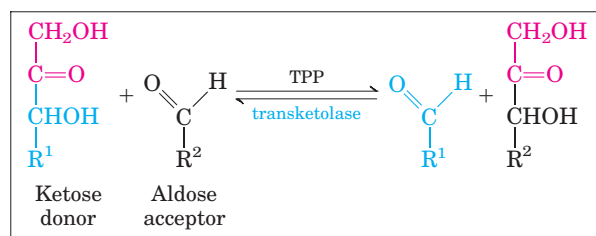
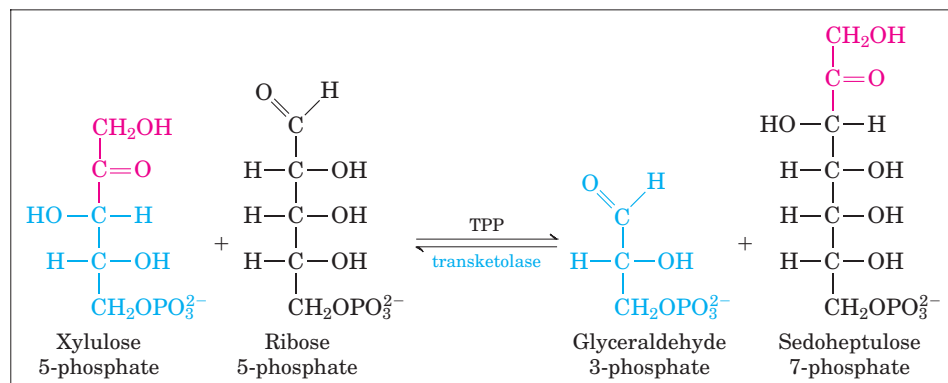


FIGURE 14–22 Nonoxidative reactions of the pentose phosphate pathway. (a) These reactions convert pentose phosphates to hexose phosphates, allowing the oxidative reactions (see Fig. 14–21) to continue. The enzymes transketolase and transaldolase are specific to this pathway; the other enzymes also serve in the glycolytic or gluconeogenic pathways. (b) A schematic diagram showing the pathway

from six pentoses (5C) to five hexoses (6C). Note that this involves two sets of the interconversions shown in (a). Every reaction shown here is reversible; unidirectional arrows are used only to make clear the direction of the reactions during continuous oxidation of glucose 6-phosphate. In the light-independent reactions of photosynthesis, the direction of these reactions is reversed (see Fig. 20–10).



(a)



(b)

FIGURE 14-23 The first reaction catalyzed by transketolase. (a) The general reaction catalyzed by transketolase is the transfer of a two-carbon group, carried temporarily on enzyme-bound TPP, from a ketose donor to an aldose acceptor. (b) Conversion of two pentose phosphates to a triose phosphate and a seven-carbon sugar phosphate, sedoheptulose 7-phosphate.

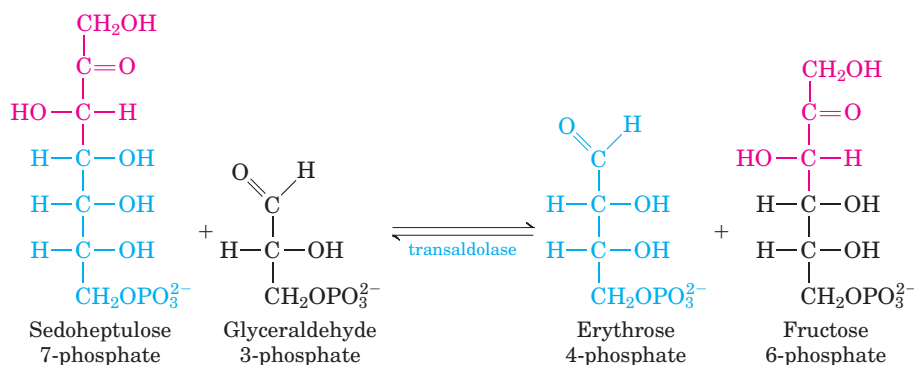


FIGURE 14-24 The reaction catalyzed by transaldolase.

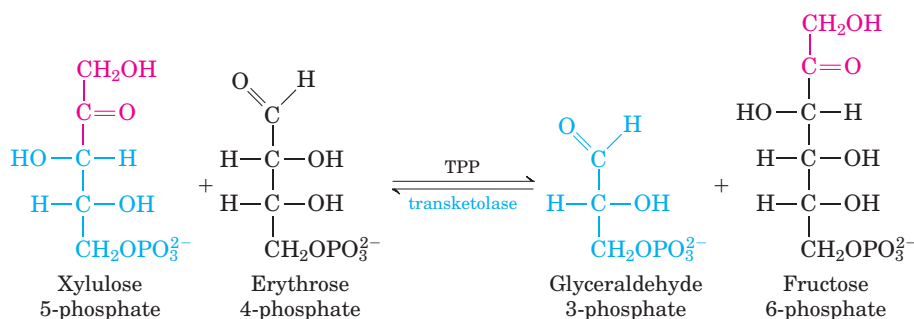
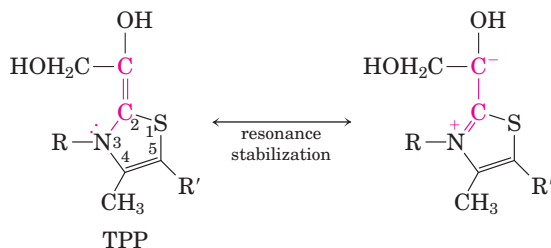
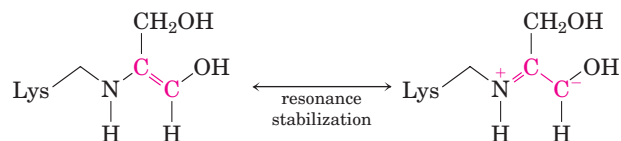


FIGURE 14-25 The second reaction catalyzed by transketolase.

Transketolase requires the cofactor thiamine pyrophosphate (TPP), which stabilizes a two-carbon carbanion in this reaction (Fig. 14-26a), just as it does in the pyruvate decarboxylase reaction (Fig. 14-13). Transaldolase uses a Lys side chain to form a Schiff base with the carbonyl group of its substrate, a ketose,

thereby stabilizing a carbanion (Fig. 14-26b) that is central to the reaction mechanism.

The process described in Figure 14-21 is known as the **oxidative pentose phosphate pathway**. The first two steps are oxidations with large, negative standard free-energy changes and are essentially irreversible in

(a) Transketolase**(b) Transaldolase**

Protonated Schiff base

FIGURE 14-26 Carbanion intermediates stabilized by covalent interactions with transketolase and transaldolase. **(a)** The ring of TPP stabilizes the two-carbon carbanion carried by transketolase; see Fig. 14-13 for the chemistry of TPP action. **(b)** In the transaldolase reaction, the protonated Schiff base formed between the ϵ -amino group of a Lys side chain and the substrate stabilizes a three-carbon carbanion.

the cell. The reactions of the nonoxidative part of the pentose phosphate pathway (Fig. 14-22) are readily reversible and thus also provide a means of converting hexose phosphates to pentose phosphates. As we shall see in Chapter 20, a process that converts hexose phosphates to pentose phosphates is crucial to the photosynthetic assimilation of CO_2 by plants. That pathway, the **reductive pentose phosphate pathway**, is essentially the reversal of the reactions shown in Figure 14-22 and employs many of the same enzymes.

All the enzymes in the pentose phosphate pathway are located in the cytosol, like those of glycolysis and most of those of gluconeogenesis. In fact, these three pathways are connected through several shared intermediates and enzymes. The glyceraldehyde 3-phosphate formed by the action of transketolase is readily converted to dihydroxyacetone phosphate by the glycolytic enzyme triose phosphate isomerase, and these two trioses can be joined by the aldolase as in gluconeogenesis, forming fructose 1,6-bisphosphate. Alternatively, the triose phosphates can be oxidized to pyruvate by the glycolytic reactions. The fate of the trioses is determined by the cell's relative needs for pentose phosphates, NADPH, and ATP.

Wernicke-Korsakoff Syndrome Is Exacerbated by a Defect in Transketolase



In humans with Wernicke-Korsakoff syndrome, a mutation in the gene for transketolase results in

an enzyme having an affinity for its coenzyme TPP that is one-tenth that of the normal enzyme. Although moderate deficiencies in the vitamin thiamine have little effect on individuals with an unmutated transketolase gene, in those with the altered gene, thiamine deficiency drops the level of TPP below that needed to saturate the enzyme. The lowering of transketolase activity slows the whole pentose phosphate pathway, and the result is the Wernicke-Korsakoff syndrome: severe memory loss, mental confusion, and partial paralysis. The syndrome is more common among alcoholics than in the general population; chronic alcohol consumption interferes with the intestinal absorption of some vitamins, including thiamine. ■

Glucose 6-Phosphate Is Partitioned between Glycolysis and the Pentose Phosphate Pathway

Whether glucose 6-phosphate enters glycolysis or the pentose phosphate pathway depends on the current needs of the cell and on the concentration of NADP^+ in the cytosol. Without this electron acceptor, the first reaction of the pentose phosphate pathway (catalyzed by G6PD) cannot proceed. When a cell is rapidly converting NADPH to NADP^+ in biosynthetic reductions, the level of NADP^+ rises, allosterically stimulating G6PD and thereby increasing the flux of glucose 6-phosphate through the pentose phosphate pathway (Fig. 14-27). When the demand for NADPH slows, the level of NADP^+ drops, the pentose phosphate pathway slows, and glucose 6-phosphate is instead used to fuel glycolysis.

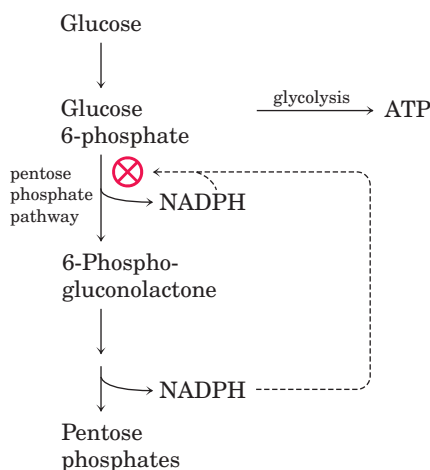


FIGURE 14-27 Role of NADPH in regulating the partitioning of glucose 6-phosphate between glycolysis and the pentose phosphate pathway. When NADPH is forming faster than it is being used for biosynthesis and glutathione reduction (see Fig. 14-20), $[\text{NADPH}]$ rises and inhibits the first enzyme in the pentose phosphate pathway. As a result, more glucose 6-phosphate is available for glycolysis.

SUMMARY 14.5 Pentose Phosphate Pathway of Glucose Oxidation

- The *oxidative* pentose phosphate pathway (phosphogluconate pathway, or hexose monophosphate pathway) brings about oxidation and decarboxylation at C-1 of glucose 6-phosphate, reducing NADP^+ to NADPH and producing pentose phosphates.
- NADPH provides reducing power for biosynthetic reactions, and ribose 5-phosphate is a precursor for nucleotide and nucleic acid synthesis. Rapidly growing tissues and tissues carrying out active biosynthesis of fatty acids, cholesterol, or steroid hormones send more glucose 6-phosphate through the pentose phosphate pathway than do tissues with less demand for pentose phosphates and reducing power.
- The first phase of the pentose phosphate pathway consists of two oxidations that convert glucose 6-phosphate to ribulose 5-phosphate and reduce NADP^+ to NADPH. The second phase comprises nonoxidative steps that convert pentose phosphates to glucose 6-phosphate, which begins the cycle again.
- In the second phase, transaldolase (with TPP as cofactor) and transketolase catalyze the interconversion of three-, four-, five-, six-, and seven-carbon sugars, with the reversible conversion of six pentose phosphates to five hexose phosphates. In the carbon-assimilating reactions of photosynthesis, the same enzymes catalyze the reverse process, called the *reductive* pentose phosphate pathway: conversion of five hexose phosphates to six pentose phosphates.
- A genetic defect in transketolase that lowers its affinity for TPP exacerbates the Wernicke-Korsakoff syndrome.
- Entry of glucose 6-phosphate either into glycolysis or into the pentose phosphate pathway is largely determined by the relative concentrations of NADP^+ and NADPH.

Key Terms

Terms in bold are defined in the glossary.

glycolysis 522	acyl phosphate 530	mutases 534	biotin 544
fermentation 522	substrate-level phosphorylation 531	isomerases 534	pentose phosphate pathway 549
lactic acid fermentation	respiration-linked phosphorylation 531	lactose intolerance	phosphogluconate pathway 549
hypoxia 523	phosphoenolpyruvate (PEP) 532	galactosemia 537	hexose monophosphate pathway 549
ethanol (alcohol) fermentation 523		thiamine pyrophosphate (TPP) 540	
isozymes 526		gluconeogenesis 543	

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Problems

1. Equation for the Preparatory Phase of Glycolysis

Write balanced biochemical equations for all the reactions in the catabolism of glucose to two molecules of glyceraldehyde 3-phosphate (the preparatory phase of glycolysis), including the standard free-energy change for each reaction. Then write the overall or net equation for the preparatory phase of glycolysis, with the net standard free-energy change.

2. The Payoff Phase of Glycolysis in Skeletal Muscle

In working skeletal muscle under anaerobic conditions, glyceraldehyde 3-phosphate is converted to pyruvate (the payoff phase of glycolysis), and the pyruvate is reduced to lactate. Write balanced biochemical equations for all the reactions in this process, with the standard free-energy change for each reaction. Then write the overall or net equation for the payoff phase of glycolysis (with lactate as the end product), including the net standard free-energy change.

3. Pathway of Atoms in Fermentation A “pulse-chase” experiment using ^{14}C -labeled carbon sources is carried out on a yeast extract maintained under strictly anaerobic conditions to produce ethanol. The experiment consists of incubating a small amount of ^{14}C -labeled substrate (the pulse) with the yeast extract just long enough for each intermediate in the fermentation pathway to become labeled. The label is then “chased” through the pathway by the addition of excess unlabeled glucose. The chase effectively prevents any further entry of labeled glucose into the pathway.

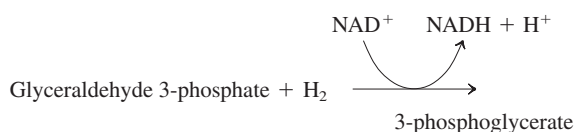
(a) If $[1\text{-}^{14}\text{C}]\text{glucose}$ (glucose labeled at C-1 with ^{14}C) is used as a substrate, what is the location of ^{14}C in the product ethanol? Explain.

(b) Where would ^{14}C have to be located in the starting glucose to ensure that all the ^{14}C activity is liberated as $^{14}\text{CO}_2$ during fermentation to ethanol? Explain.

4. Fermentation to Produce Soy Sauce Soy sauce is prepared by fermenting a salted mixture of soybeans and wheat with several microorganisms, including yeast, over a period of 8 to 12 months. The resulting sauce (after solids are removed) is rich in lactate and ethanol. How are these two compounds produced? To prevent the soy sauce from having a strong vinegar taste (vinegar is dilute acetic acid), oxygen must be kept out of the fermentation tank. Why?

5. Equivalence of Triose Phosphates ^{14}C -Labeled glyceraldehyde 3-phosphate was added to a yeast extract. After a short time, fructose 1,6-bisphosphate labeled with ^{14}C at C-3 and C-4 was isolated. What was the location of the ^{14}C label in the starting glyceraldehyde 3-phosphate? Where did the second ^{14}C label in fructose 1,6-bisphosphate come from? Explain.

6. Glycolysis Shortcut Suppose you discovered a mutant yeast whose glycolytic pathway was shorter because of the presence of a new enzyme catalyzing the reaction:



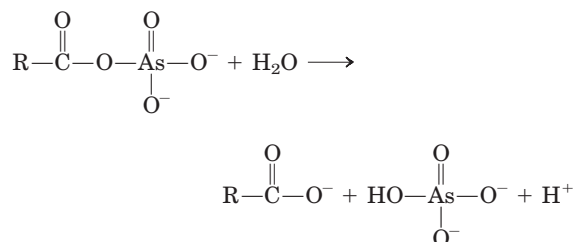
Would shortening the glycolytic pathway in this way benefit the cell? Explain.

7. Role of Lactate Dehydrogenase During strenuous activity, the demand for ATP in muscle tissue is vastly increased. In rabbit leg muscle or turkey flight muscle, the ATP is produced almost exclusively by lactic acid fermentation. ATP is formed in the payoff phase of glycolysis by two reactions, promoted by phosphoglycerate kinase and pyruvate kinase. Suppose skeletal muscle were devoid of lactate dehydrogenase. Could it carry out strenuous physical activity; that is, could it generate ATP at a high rate by glycolysis? Explain.

8. Efficiency of ATP Production in Muscle The transformation of glucose to lactate in myocytes releases only about 7% of the free energy released when glucose is completely oxidized to CO_2 and H_2O . Does this mean that anaerobic glycolysis in muscle is a wasteful use of glucose? Explain.

9. Free-Energy Change for Triose Phosphate Oxidation The oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate, catalyzed by glyceraldehyde 3-phosphate dehydrogenase, proceeds with an unfavorable equilibrium constant ($K'_{\text{eq}} = 0.08$; $\Delta G'^{\circ} = 6.3 \text{ kJ/mol}$), yet the flow through this point in the glycolytic pathway proceeds smoothly. How does the cell overcome the unfavorable equilibrium?

10. Arsenate Poisoning Arsenate is structurally and chemically similar to inorganic phosphate (P_i), and many enzymes that require phosphate will also use arsenate. Organic compounds of arsenate are less stable than analogous phosphate compounds, however. For example, acyl *arsenates* decompose rapidly by hydrolysis:



On the other hand, acyl *phosphates*, such as 1,3-bisphosphoglycerate, are more stable and undergo further enzyme-catalyzed transformation in cells.

(a) Predict the effect on the net reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase if phosphate were replaced by arsenate.

(b) What would be the consequence to an organism if arsenate were substituted for phosphate? Arsenate is very toxic to most organisms. Explain why.

11. Requirement for Phosphate in Ethanol Fermentation In 1906 Harden and Young, in a series of classic studies on the fermentation of glucose to ethanol and CO_2 by extracts of brewer's yeast, made the following observations. (1) Inorganic phosphate was essential to fermentation; when the supply of phosphate was exhausted, fermentation ceased before all the glucose was used. (2) During fermentation under these conditions, ethanol, CO_2 , and a hexose bisphosphate

accumulated. (3) When arsenate was substituted for phosphate, no hexose biphosphate accumulated, but the fermentation proceeded until all the glucose was converted to ethanol and CO_2 .

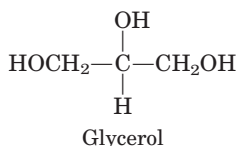
(a) Why did fermentation cease when the supply of phosphate was exhausted?

(b) Why did ethanol and CO_2 accumulate? Was the conversion of pyruvate to ethanol and CO_2 essential? Why? Identify the hexose biphosphate that accumulated. Why did it accumulate?

(c) Why did the substitution of arsenate for phosphate prevent the accumulation of the hexose biphosphate yet allow fermentation to ethanol and CO_2 to go to completion? (See Problem 10.)

12. Role of the Vitamin Niacin Adults engaged in strenuous physical activity require an intake of about 160 g of carbohydrate daily but only about 20 mg of niacin for optimal nutrition. Given the role of niacin in glycolysis, how do you explain the observation?

13. Metabolism of Glycerol Glycerol obtained from the breakdown of fat is metabolized by conversion to dihydroxyacetone phosphate, a glycolytic intermediate, in two enzyme-catalyzed reactions. Propose a reaction sequence for glycerol metabolism. On which known enzyme-catalyzed reactions is your proposal based? Write the net equation for the conversion of glycerol to pyruvate according to your scheme.

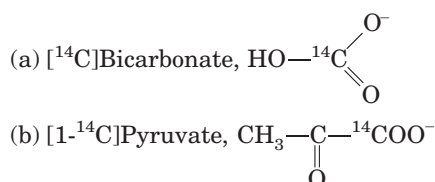


14. Severity of Clinical Symptoms Due to Enzyme Deficiency

The clinical symptoms of two forms of galactosemia—deficiency of galactokinase or of UDP-glucose:galactose 1-phosphate uridylyltransferase—show radically different severity. Although both types produce gastric discomfort after milk ingestion, deficiency of the transferase also leads to liver, kidney, spleen, and brain dysfunction and eventual death. What products accumulate in the blood and tissues with each type of enzyme deficiency? Estimate the relative toxicities of these products from the above information.

15. Muscle Wasting in Starvation One consequence of starvation is a reduction in muscle mass. What happens to the muscle proteins?

16. Pathway of Atoms in Gluconeogenesis A liver extract capable of carrying out all the normal metabolic reactions of the liver is briefly incubated in separate experiments with the following ^{14}C -labeled precursors:



Trace the pathway of each precursor through gluconeogenesis. Indicate the location of ^{14}C in all intermediates and in the product, glucose.

17. Pathway of CO_2 in Gluconeogenesis In the first bypass step of gluconeogenesis, the conversion of pyruvate to phosphoenolpyruvate, pyruvate is carboxylated by pyruvate carboxylase to oxaloacetate, which is subsequently decarboxylated by PEP carboxykinase to yield phosphoenolpyruvate. The observation that the addition of CO_2 is directly followed by the loss of CO_2 suggests that ^{14}C of $^{14}\text{CO}_2$ would not be incorporated into PEP, glucose, or any intermediates in gluconeogenesis. However, when a rat liver preparation synthesizes glucose in the presence of $^{14}\text{CO}_2$, ^{14}C slowly appears in PEP and eventually at C-3 and C-4 of glucose. How does the ^{14}C label get into PEP and glucose? (Hint: During gluconeogenesis in the presence of $^{14}\text{CO}_2$, several of the four-carbon citric acid cycle intermediates also become labeled.)

18. Energy Cost of a Cycle of Glycolysis and Gluconeogenesis What is the cost (in ATP equivalents) of transforming glucose to pyruvate via glycolysis and back again to glucose via gluconeogenesis?

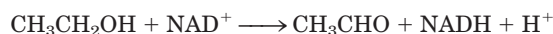
19. Glucogenic Substrates A common procedure for determining the effectiveness of compounds as precursors of glucose in mammals is to starve the animal until the liver glycogen stores are depleted and then administer the compound in question. A substrate that leads to a *net* increase in liver glycogen is termed glucogenic, because it must first be converted to glucose 6-phosphate. Show by means of known enzymatic reactions which of the following substances are glucogenic:

- (a) Succinate, $^-\text{OOC}-\text{CH}_2-\text{CH}_2-\text{COO}^-$
- (b) Glycerol, $\begin{array}{c} \text{OH} \quad \text{OH} \quad \text{OH} \\ | \quad | \quad | \\ \text{CH}_2 - \text{C} - \text{CH}_2 \\ | \\ \text{H} \end{array}$
- (c) Acetyl-CoA, $\begin{array}{c} \text{O} \\ || \\ \text{CH}_3 - \text{C} - \text{S-CoA} \end{array}$
- (d) Pyruvate, $\begin{array}{c} \text{O} \\ || \\ \text{CH}_3 - \text{C} - \text{COO}^- \end{array}$
- (e) Butyrate, $\text{CH}_3-\text{CH}_2-\text{CH}_2-\text{COO}^-$



20. Ethanol Affects Blood Glucose Levels

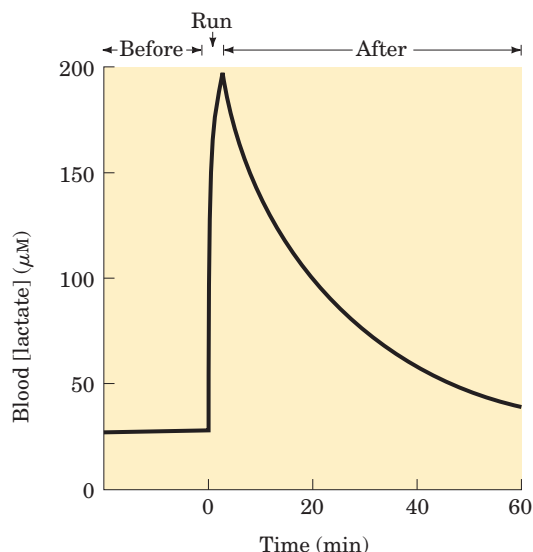
The consumption of alcohol (ethanol), especially after periods of strenuous activity or after not eating for several hours, results in a deficiency of glucose in the blood, a condition known as hypoglycemia. The first step in the metabolism of ethanol by the liver is oxidation to acetaldehyde, catalyzed by liver alcohol dehydrogenase:



Explain how this reaction inhibits the transformation of lactate to pyruvate. Why does this lead to hypoglycemia?

21. Blood Lactate Levels during Vigorous Exercise

The concentrations of lactate in blood plasma before, during, and after a 400 m sprint are shown in the graph.



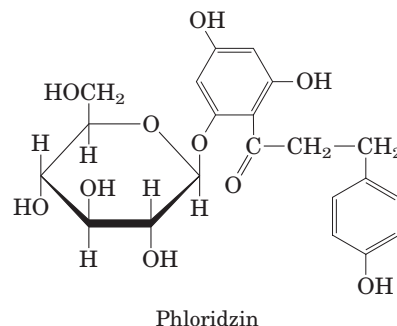
- What causes the rapid rise in lactate concentration?
- What causes the decline in lactate concentration after completion of the sprint? Why does the decline occur more slowly than the increase?
- Why is the concentration of lactate not zero during the resting state?

22. Relationship between Fructose 1,6-Bisphosphatase and Blood Lactate Levels

A congenital defect in the liver enzyme fructose 1,6-bisphosphatase results in abnormally high levels of lactate in the blood plasma. Explain.

23. Effect of Phloridzin on Carbohydrate Metabolism

Phloridzin, a toxic glycoside from the bark of the pear tree, blocks the normal reabsorption of glucose from the kidney tubule, thus causing blood glucose to be almost completely excreted in the urine. In an experiment, rats fed phloridzin and sodium succinate excreted about 0.5 mol of glucose (made by gluconeogenesis) for every 1 mol of sodium succinate ingested. How is the succinate transformed to glucose? Explain the stoichiometry.

**24. Excess O₂ Uptake during Gluconeogenesis**

Lactate absorbed by the liver is converted to glucose, with the input of 6 mol of ATP for every mole of glucose produced. The extent of this process in a rat liver preparation can be monitored by administering [¹⁴C]lactate and measuring the amount of [¹⁴C]glucose produced. Because the stoichiometry of O₂ consumption and ATP production is known (about 5 ATP per O₂), we can predict the extra O₂ consumption above the normal rate when a given amount of lactate is administered. However, when the extra O₂ used in the synthesis of glucose from lactate is actually measured, it is always higher than predicted by known stoichiometric relationships. Suggest a possible explanation for this observation.