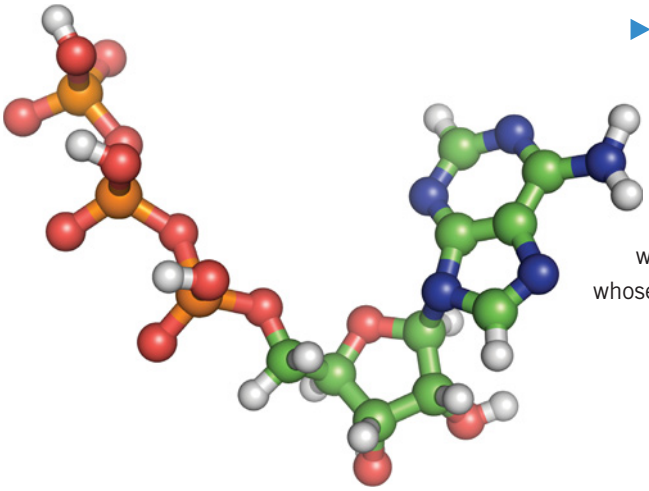
chapter 12

METABOLISM AND BIOENERGETICS



▸▸ WHAT'S so special about ATP?

ATP is a relatively abundant nucleotide, serving as a building block for RNA and, in its deoxy form, for DNA. Yet ATP is also known as the cell's energy currency, and we can speak of the energetic cost of a metabolic process in terms of the ATP that a cell must spend. In this chapter we'll see that ATP is not some kind of magic coin with a special chemical structure. Rather, it is an ordinary nucleotide whose *reactions* play a vital part in the metabolism of all cells.

THIS CHAPTER IN CONTEXT

Part 1 Foundations

Part 2 Molecular Structure and Function

Part 3 Metabolism

12 Metabolism and Bioenergetics

Part 4 Genetic Information

Do You Remember?

- **Living organisms obey the laws of thermodynamics (Section 1-3).**
- **Amino acids are linked by peptide bonds to form a polypeptide (Section 4-1).**
- **Allosteric regulators can inhibit or activate enzymes (Section 7-3).**
- **Lipids are predominantly hydrophobic molecules that can be esterified but cannot form polymers (Section 8-1).**
- **Monosaccharides can be linked by glycosidic bonds in various arrangements (Section 11-2).**

Some organisms, known as **chemoautotrophs** (from the Greek *trophe,* "nourishment"), obtain virtually all their metabolic building materials and free energy from the simple inorganic compounds CO_2, N_2, H_2, and S_2. **Photoautotrophs,** such as the familiar green plants, need little more than CO_2, H_2O, a source of nitrogen, and sunlight. In contrast, **heterotrophs,** a group that includes animals, directly or indirectly obtain all their building materials and free energy from organic compounds produced by chemo- or photoautotrophs. Despite their different trophic strategies, all organisms have remarkably similar cellular structures, make the same types of biomolecules, and use similar enzymes to build and break down those molecules.

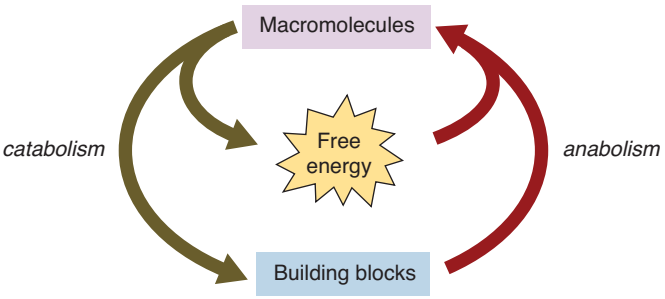


Figure 12-1 Catabolism and anabolism. Catabolic (degradative) reactions yield free energy and small molecules that can be used for anabolic (synthetic) reactions. Metabolism is the sum of all catabolic and anabolic processes.

Cells break down or **catabolize** large molecules to release free energy and small molecules. The cells then use the free energy and small molecules to rebuild larger molecules, a process called **anabolism** (Fig. 12-1). The set of all catabolic and anabolic activities constitutes an organism's **metabolism.** A catalog of all the metabolic reactions undertaken by plants, animals, and bacteria is far beyond the scope of this book. Instead, we will examine a few common metabolic processes, focusing primarily on mammalian systems. In the next few chapters, we will examine some catabolic processes that release free energy and some anabolic processes that consume free energy. But first we will introduce a few of the major molecular players in metabolism, including their precursors and degradation products, and further explore the meaning of free energy in biological systems.

12-1 Food and Fuel

KEY CONCEPTS
- The macromolecules in food are hydrolyzed, and the monomeric products are absorbed by the intestine.
- Cells store fatty acids, glucose, and amino acids in the form of polymers.
- Metabolic fuels can be mobilized by breaking down glycogen, triacylglycerols, and proteins.

As heterotrophs, mammals rely on food produced by other organisms. After food is digested and absorbed, it becomes a source of metabolic energy and materials to support the animal's growth and other activities. The human diet includes the four types of biological molecules introduced in Section 1-2 and described in more detail in subsequent chapters. These molecules are often present as macromolecular polymers, namely proteins, nucleic acids, polysaccharides, and triacylglycerols (technically, fats are not polymers since the monomeric units are not linked to each other but to glycerol). *Digestion reduces the polymers to their monomeric components: amino acids, nucleotides, monosaccharides, and fatty acids.* The breakdown of nucleotides does not yield significant amounts of metabolic free energy, so we will devote more attention to the catabolism of other types of biomolecules.

Cells take up the products of digestion

Digestion takes place extracellularly in the mouth, stomach, and small intestine and is catalyzed by hydrolytic enzymes (Fig. 12-2). For example, salivary amylase begins to break down starch, which consists of linear polymers of glucose residues (amylose) and branched polymers (amylopectin; Section 11-2). Gastric and pancreatic proteases (including trypsin, chymotrypsin, and elastase) degrade proteins to small peptides and amino acids. Lipases synthesized by the pancreas and secreted into the small intestine catalyze the release of fatty acids from triacylglycerols. Water-insoluble lipids do not freely mix with the other digested molecules but instead form micelles (Fig. 2-10).

The products of digestion are absorbed by the cells lining the intestine. Monosaccharides enter the cells via active transporters such as the Na^+-glucose system diagrammed in Figure 9-18. Similar symport systems bring amino acids and di- and tripeptides into the cells. Some highly hydrophobic lipids diffuse through the cell membrane; others require transporters. Inside the cell, the triacylglycerol digestion

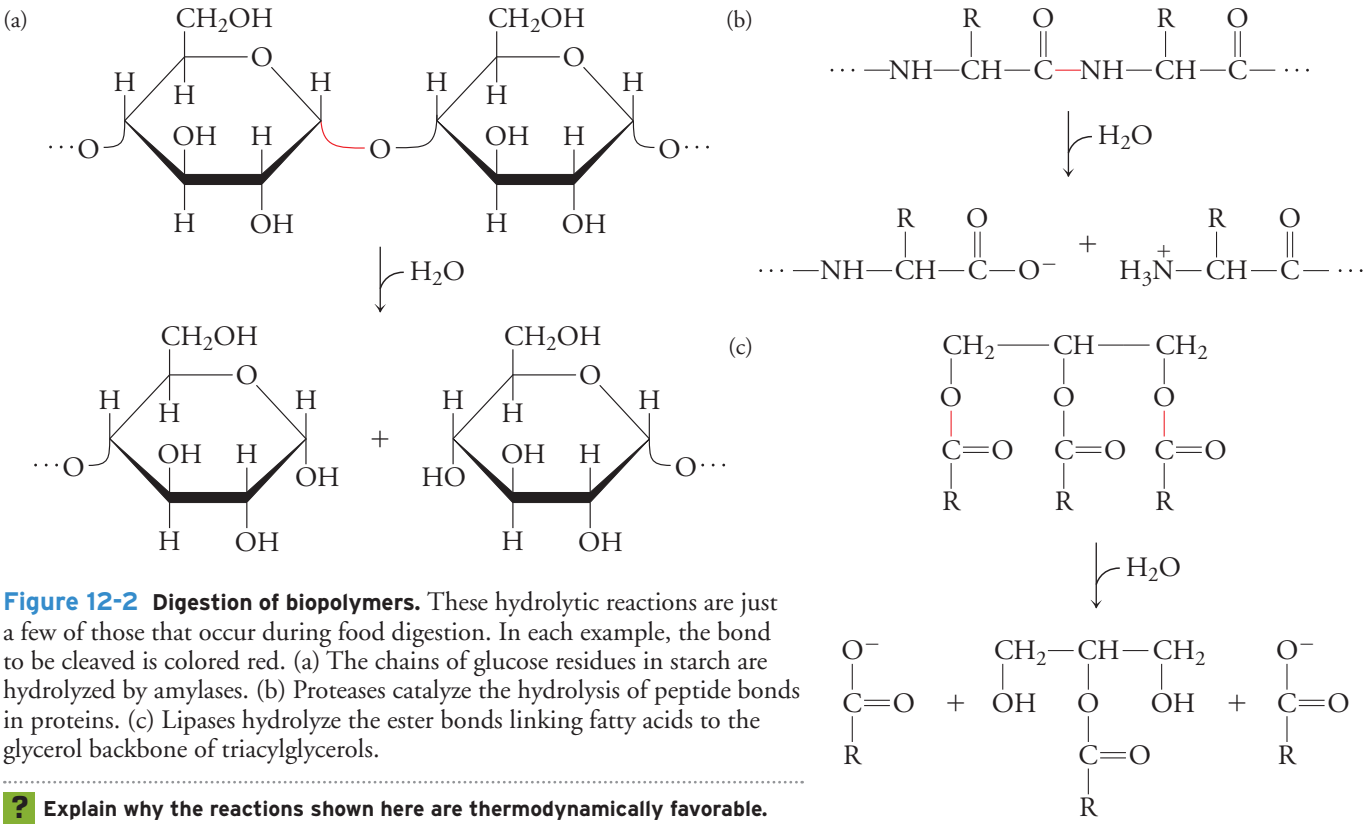
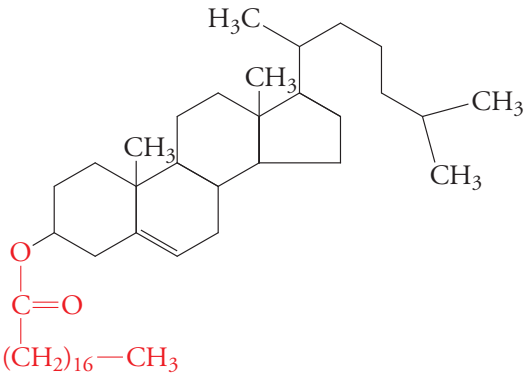


Figure 12-2 **Digestion of biopolymers.** These hydrolytic reactions are just a few of those that occur during food digestion. In each example, the bond to be cleaved is colored red. (a) The chains of glucose residues in starch are hydrolyzed by amylases. (b) Proteases catalyze the hydrolysis of peptide bonds in proteins. (c) Lipases hydrolyze the ester bonds linking fatty acids to the glycerol backbone of triacylglycerols.

? **Explain why the reactions shown here are thermodynamically favorable.**

products re-form triacylglycerols, and some fatty acids are linked to cholesterol to form cholesteryl esters, for example,



Cholesteryl stearate

Triacylglycerols and cholesteryl esters are packaged, together with specific proteins, to form **lipoproteins.** These particles, known specifically as chylomicrons, are released into the lymphatic circulation before entering the bloodstream for delivery to tissues.

Water-soluble substances such as amino acids and monosaccharides leave the intestinal cells and enter the portal vein, which drains the intestine and other visceral organs and leads directly to the liver. *The liver therefore receives the bulk of a meal's nutrients and catabolizes them, stores them, or releases them back into the bloodstream.* The liver also takes up chylomicrons and repackages the lipids with different proteins to form other lipoproteins, which circulate throughout the body, carrying cholesterol, triacylglycerols, and other lipids (lipoproteins are discussed in greater detail in Chapter 17). The allocation of resources following a meal varies with the individual's needs at that time and with the type of nutrients consumed. Fortunately, the body does this efficiently, regardless of what food was eaten (Box 12-A).

BOX 12-A BIOCHEMISTRY NOTE

Dietary Guidelines

Nutritionists have yet to come up with the ideal diet; the best they can do is identify the body's overall needs and roughly outline dietary requirements. For example, scientists have compiled lists of recommended daily intakes for various substances in terms of grams of the substance or the proportion of total energy intake contributed by that substance:

Distribution of Macronutrients for Adults

Carbohydrate	45–65%
Fat	20–35%
Protein	10–35%

However, few foods are composed of pure substances, so more practical guidelines focus on types of foods, with units that are more familiar to most consumers, such as ounces or cups. One source of information is the U.S. Department of Agriculture, which has published the following guidelines:

Food Group Choices

	Moderately active female, age 21–25	Moderately active male, age 21–25
Total calories	2200	2800
Fruits	2 cups	2.5 cups
Grains	7 oz	10 oz
Milk products	3 cups	3 cups
Meat, beans, nuts	6 oz	7 oz
Oils	6 tsp	8 tsp
Vegetables	3 cups	3.5 cups

[From www.cnpp.usda.gov/]

Even these recommendations are somewhat clumsy, since most individuals do not determine the volume or mass of what they place on their plates. Nutrition educators strive to translate some formal quantities into yet more familiar units: A cup of rice or a medium apple is about the size of a baseball, and three ounces of meat is about the size of a deck of cards.

An additional drawback of dietary guidelines formulated like those above is that in the United States, recommendations are based on a traditional Western diet that includes meat and dairy products. Vegetarians (who do not consume meat), vegans (who avoid consuming any animal products), and those who do not drink milk must be more diligent in assessing whether the foods they consume meet the basic requirements for carbohydrates, proteins, and so on.

Finally, a serious challenge for anyone interested in tracking their nutrient consumption is that many foods are processed; that is, raw ingredients are combined, sometimes in unknown proportions, to generate a product that can be sold as a convenience item (think: instant soup). Such foods are typically accompanied by a nutrition facts label that lists, among other things, the serving size; calories per serving; and the quantities of carbohydrates, fats, and proteins (in grams) and their percentage of the recommended daily value.

The availability of different types of dietary guidelines, along with a plethora of advice (which may or may not be grounded in the scientific method), suggests that there is significant leeway regarding what humans can or should consume. Indeed, consideration of how eating patterns have varied across centuries and across continents indicates that the human body must be remarkably versatile in converting a variety of raw materials into the molecular building blocks and metabolic energy required to sustain life.

Question: How would the recommended intake of protein vary from infancy to old age? Should intakes be adjusted according to body mass?

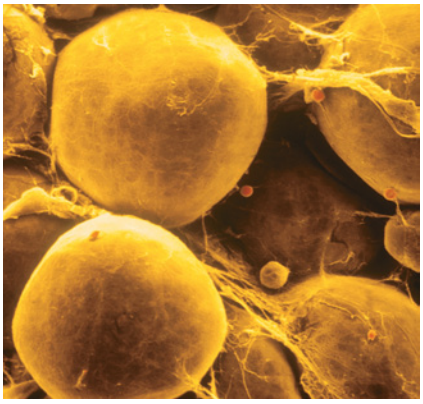


Figure 12-3 Adipocytes. These cells, which make up adipose tissue, contain a small amount of cytoplasm surrounding a large globule of triacylglycerols (fat). [© CNRI/ Phototake.]

Monomers are stored as polymers

Immediately following a meal, the circulating concentrations of monomeric compounds are relatively high. All cells can take up these materials to some extent to fulfill their immediate needs, but *some tissues are specialized for the long-term storage of nutrients.* For example, fatty acids are used to build triacylglycerols, many of which travel in the form of lipoproteins to adipose tissue. Here, adipocytes take up the triacylglycerols and store them as intracellular fat globules. Because the mass of lipid is hydrophobic and does not interfere with activities in the aqueous cytoplasm, the fat globule can be enormous, occupying most of the volume of the adipocyte **(Fig. 12-3)**.

Virtually all cells can take up monosaccharides and immediately catabolize them to produce free energy. Some tissues, primarily liver and muscle (which makes up a significant portion of the human body), use monosaccharides to synthesize glycogen, the storage polymer of glucose. Glycogen is a highly branched polymer with a compact shape. Several glycogen molecules may clump together to form granules that are visible by electron microscopy **(Fig. 12-4)**. Glycogen's branched structure means that a single molecule can be expanded quickly, by adding glucose residues to its many branches, and degraded quickly, by simultaneously removing glucose from the ends of many branches. Glucose that does not become part of glycogen can be catabolized to two-carbon acetyl units and converted into fatty acids for storage as triacylglycerols.

Amino acids can be used to build polypeptides. A protein is not a dedicated storage molecule for amino acids, as glycogen is for glucose and triacylglycerols are for fatty acids, so excess amino acids cannot be saved for later. However, in certain cases, such as during starvation, proteins are catabolized to supply the body's energy needs. If the intake of amino acids exceeds the body's immediate protein-building needs, the excess amino acids can be broken down and converted to carbohydrate (which can be stored as glycogen) or converted to acetyl units (which can then be converted to fat).

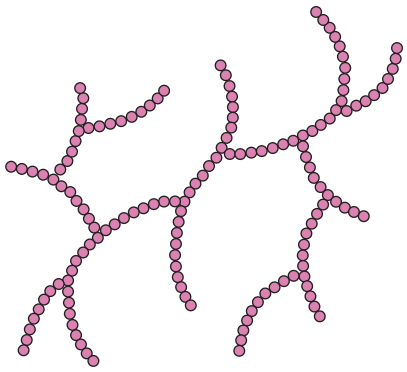
Amino acids and glucose are both required to synthesize nucleotides. Asp, Gln, and Gly supply some of the carbon and nitrogen atoms used to build the purine and pyrimidine bases (Section 18-3). The ribose-5-phosphate component of nucleotides is derived from glucose by a pathway that converts the six-carbon sugar to a five-carbon sugar (Section 13-4). In sum, the allocation of resources within a cell depends on the type of tissue and its need to build cellular structures, provide free energy, or stockpile resources in anticipation of future needs.

Fuels are mobilized as needed

Amino acids, monosaccharides, and fatty acids are known as ***metabolic fuels*** *because they can be broken down by processes that make free energy available for the cell's activities.* After a meal, free glucose and amino acids are catabolized to release their free energy. When these fuel supplies are exhausted, the body **mobilizes** its stored resources; that is, it converts its polysaccharide and triacylglycerol storage molecules (and sometimes proteins) to their respective monomeric units. Most of the body's tissues prefer to use glucose as their primary metabolic fuel, and the central nervous system can run on almost nothing else. In response to this demand, the liver mobilizes glucose by breaking down glycogen.

In general, depolymerization reactions are hydrolytic, but in the case of glycogen, the molecule that breaks the bonds between glucose residues is not water but phosphate. Thus, the degradation of glycogen is called **phosphorolysis.** This reaction is catalyzed by glycogen phosphorylase, which releases residues from the ends of branches in the glycogen polymer.

(a)



(b)

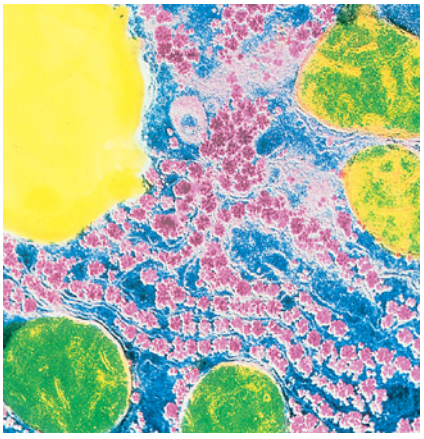
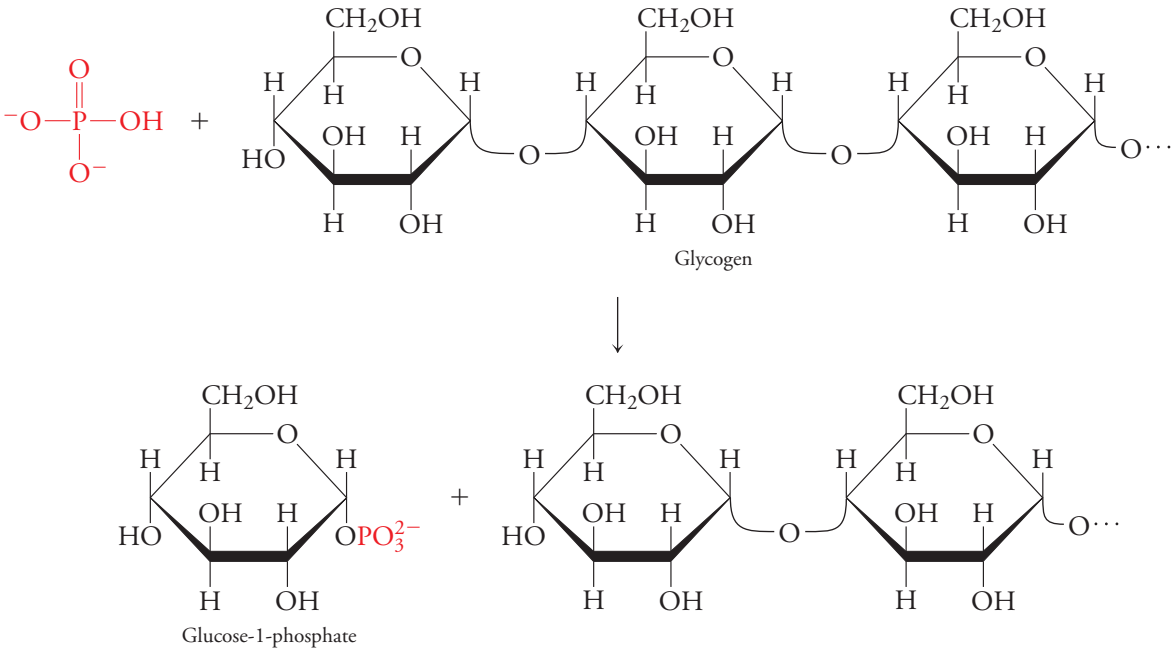


Figure 12-4 Glycogen structure. (a) Schematic diagram of a glycogen molecule. Each circle represents a glucose monomer, and branches occur every 8 to 14 residues. (b) Electron micrograph of a liver cell showing glycogen granules (colored pink). Mitochondria are green, and a fat globule is yellow. [© CNRI/Science Photo Library/Photo Researchers.]



The phosphate group of glucose-1-phosphate is removed before glucose is released from the liver into the circulation. Other tissues absorb glucose from the blood. In the disease **diabetes mellitus,** this does not occur, and the concentration of circulating glucose may become elevated.

Only when the supply of glucose runs low does adipose tissue mobilize its fat stores. A lipase hydrolyzes triacylglycerols so that fatty acids can be released into the bloodstream. These free fatty acids are not water-soluble and therefore bind to circulating proteins. Except for the heart, which uses fatty acids as its primary fuel, the body does not have a budget for burning fatty acids. In general, as long as dietary carbohydrates and amino acids can meet the body's energy needs, stored fat will not be mobilized, even if the diet includes almost no fat. This feature of mammalian fuel metabolism is a source of misery for many dieters!

Amino acids are not mobilized to generate energy except during a fast, when glycogen stores are depleted (in this situation, the liver can also convert some amino acids into glucose). However, cellular proteins are continuously degraded and rebuilt with the changing demand for particular enzymes, transporters, cytoskeletal elements, and so on. There are two major mechanisms for degrading unneeded proteins. In the first, the **lysosome,** an organelle containing proteases and other hydrolytic enzymes, breaks down proteins that are enclosed in a membranous vesicle. Membrane proteins and extracellular proteins taken up by endocytosis are degraded by this pathway, but intracellular proteins that become enclosed in vesicles can also be broken down by lysosomal enzymes.

A second pathway for degrading intracellular proteins requires a barrel-shaped structure known as a **proteasome.** The 700-kD core of this multiprotein complex encloses an inner chamber with multiple active sites that carry out peptide bond hydrolysis (Fig. 12-5). A protein can enter the proteasome only after it has been covalently tagged with a small protein called ubiquitin. This 76-residue protein is ubiquitous (hence its name) and highly conserved in eukaryotes (Fig. 12-6). Ubiquitin is attached to a protein by the action of a set of enzymes that link the C-terminus of ubiquitin to a Lys side chain. Additional ubiquitin molecules are then added to the first, each one linked via its C-terminus to a Lys side chain of the preceding ubiquitin. A chain of at least four ubiquitins is required to mark a protein for destruction by a proteasome.

The structural features that allow a protein to be ubiquitinated are not completely understood, but the system is sophisticated enough to allow unneeded or defective proteins to be destroyed while sparing essential proteins. A cap at the end of the proteasome barrel (not shown in Fig. 12-5) regulates the entry of ubiquitinated proteins into the inner chamber. The free energy of ATP drives conformational

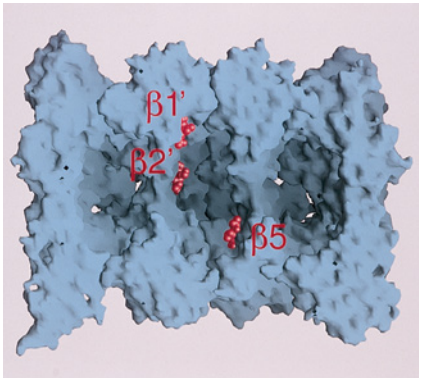


Figure 12-5 Structure of the yeast proteasome core. This cutaway view shows the inner chamber, where proteolysis occurs. Additional protein complexes (not shown) assist the entry of proteins into the proteasome. The red structures mark the locations of three protease active sites. [Courtesy Robert Huber, Max-Planck-Institut fur Biochemie, Germany.]

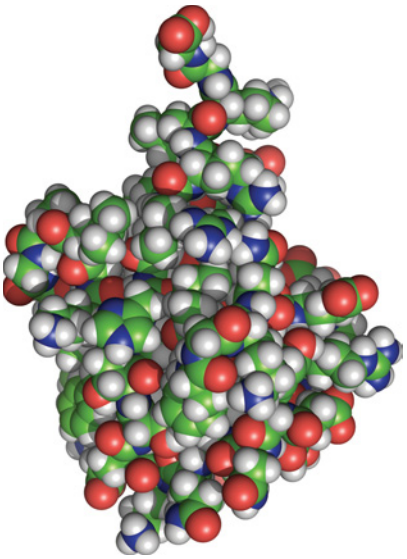


Figure 12-6 Ubiquitin. Several copies of this 76-residue protein are linked to Lys residues in proteins that are to be degraded by a proteasome. Atoms are color-coded: C green, O red, N blue, and H white. [Structure (pdb 1UBQ) determined by S. Vijay-Kumar, C. E. Bugg, and W. J. Cook.]

? Draw the linkage between a protein's C-terminus and a ubiquitin Lys residue.

changes that apparently help the condemned protein to unfold so that it can be more easily hydrolyzed. The ubiquitin molecules are not degraded; instead they are detached and reused. The three protease active sites inside the proteasome cleave the unfolded polypeptide substrate, releasing peptides of about eight residues that can diffuse out of the proteasome (Fig. 12-7). These peptides are further broken down by cytosolic peptidases so that the amino acids can be catabolized or recycled.

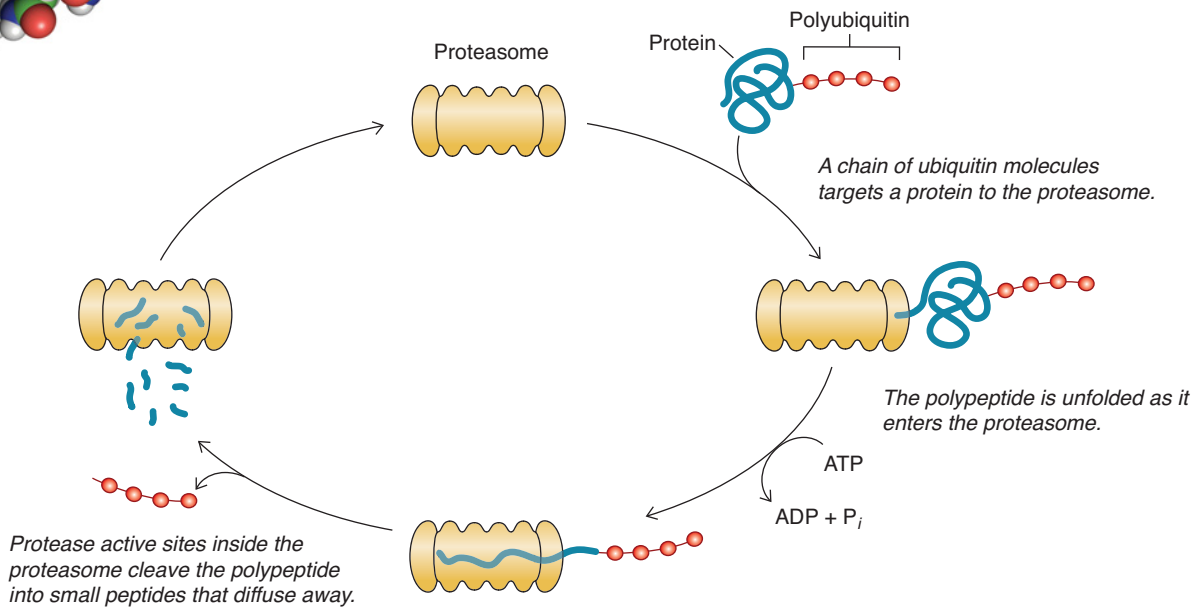


Figure 12-7 Protein degradation by the proteasome.

CONCEPT REVIEW

- Review the steps by which nutrients from food molecules reach the body's tissues.
- What are metabolic fuels and how are they stored?
- How are metabolic fuels mobilized?
- Describe the pathways for intracellular protein degradation.

12-2 Metabolic Pathways

KEY CONCEPTS

- A few metabolites appear in several metabolic pathways.
- Coenzymes such as NAD^+ and ubiquinone collect electrons from compounds that become oxidized.
- Metabolic pathways in cells are connected and are regulated.
- Many vitamins, substances that humans cannot synthesize, are components of coenzymes.

The interconversion of a biopolymer and its monomeric units is usually accomplished in just one or a few enzyme-catalyzed steps. In contrast, many steps are required to break down the monomeric compounds or build them up from smaller precursors. These series of reactions are known as **metabolic pathways.** A metabolic pathway can be considered from many viewpoints: as a series of intermediates or **metabolites,** as a set of enzymes that catalyze the reactions by which metabolites are interconverted, as an energy-producing or energy-requiring phenomenon, or as a dynamic process whose activity can be turned up or down. As we explore metabolic pathways in the coming chapters, we will take on each of these issues.

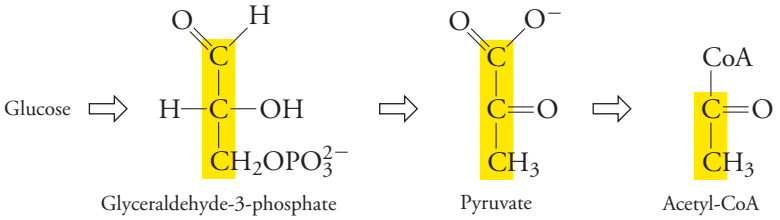


Figure 12-8 **Some intermediates resulting from glucose catabolism.**

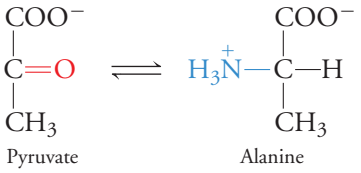
? Compare the oxidation states of the carbons in glyceraldehyde-3-phosphate and in pyruvate.

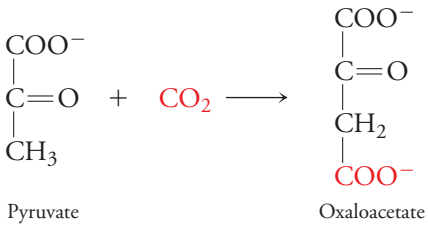
Some major metabolic pathways share a few common intermediates

One of the challenges of studying metabolism is dealing with the large number of reactions that occur in a cell—involving thousands of different intermediates. However, *a handful of metabolites appear as precursors or products in the pathways that lead to or from virtually all other types of biomolecules.* These intermediates are worth examining at this point, since they will reappear several times in the coming chapters.

In **glycolysis,** the pathway that degrades the monosaccharide glucose, the six-carbon sugar is phosphorylated and split in half, yielding two molecules of glyceraldehyde-3-phosphate **(Fig. 12-8)**. This compound is then converted in several more steps to another three-carbon molecule, pyruvate. The decarboxylation of pyruvate (removal of a carbon atom as CO_2) yields acetyl-CoA, in which a two-carbon acetyl group is linked to the carrier molecule coenzyme A (CoA).

Glyceraldehyde-3-phosphate, pyruvate, and acetyl-CoA are key players in other metabolic pathways. For example, glyceraldehyde-3-phosphate is the metabolic precursor of the three-carbon glycerol backbone of triacylglycerols. In plants, it is also the entry point for the carbon "fixed" by photosynthesis; in this case, two molecules of glyceraldehyde-3-phosphate combine to form a six-carbon monosaccharide. Pyruvate can undergo a reversible amino-group transfer reaction to yield alanine (at right). This makes pyruvate both a precursor of an amino acid and the degradation product of one. Pyruvate can also be carboxylated to yield oxaloacetate, a four-carbon precursor of several other amino acids:





Fatty acids are built by the sequential addition of two-carbon units derived from acetyl-CoA; fatty acid breakdown yields acetyl-CoA. These relationships are summarized in **Figure 12-9**. If not used to synthesize other compounds, two-carbon

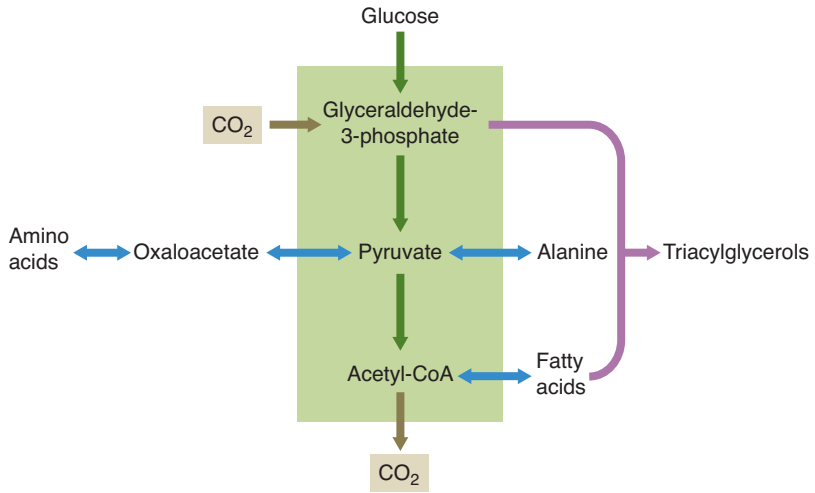


Figure 12-9 **Some of the metabolic roles of the common intermediates.**

? Without looking at the text, draw the structures of glyceraldehyde-3-phosphate, pyruvate, and oxaloacetate.

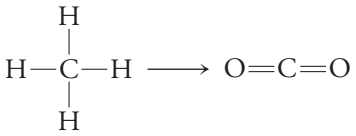
intermediates can be broken down to CO_2 by the **citric acid cycle,** a metabolic pathway essential for the catabolism of all metabolic fuels.

Many metabolic pathways include oxidation–reduction reactions

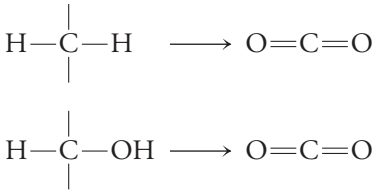
In general, *the catabolism of amino acids, monosaccharides, and fatty acids is a process of oxidizing carbon atoms, and the synthesis of these compounds involves carbon reduction.* Recall from Section 1-3 that **oxidation** is defined as the loss of electrons and **reduction** is the gain of electrons. Oxidation–reduction, or **redox,** reactions occur in pairs so that as one compound becomes more oxidized (gives up electrons or loosens its hold on them), another compound becomes reduced (receives the electrons or tightens its grip on them).

For the metabolic reactions that we are concerned with, the oxidation of carbon atoms frequently appears as the replacement of C—H bonds (in which the C and H atoms share the bonding electrons equally) with C—O bonds (in which the more electronegative O atom "pulls" the electrons away from the carbon atom). Carbon has given up some of its electrons, even though the electrons are still participating in a covalent bond.

The transformation of methane to carbon dioxide represents the conversion of carbon from its most reduced state to its most oxidized state:



Similarly, oxidation occurs during the catabolism of a fatty acid, when saturated methylene (—CH_2—) groups are converted to CO_2 and when the carbons of a carbohydrate (represented as CH_2O) are converted to CO_2:



The reverse of either of these processes—converting the carbons of CO_2 to the carbons of fatty acids or carbohydrates—is a reduction process (this is what occurs during photosynthesis, for example). In reduction processes, the carbon atoms regain electrons as C—O bonds are replaced by C—H bonds.

Turning CO_2 into carbohydrate (CH_2O) requires the input of free energy (think: sunlight). Therefore, *the reduced carbons of the carbohydrate represent a form of stored free energy.* This energy is recovered when cells break the carbohydrate back down to CO_2. Of course, such a metabolic conversion does not happen all at once but takes place through many enzyme-catalyzed steps.

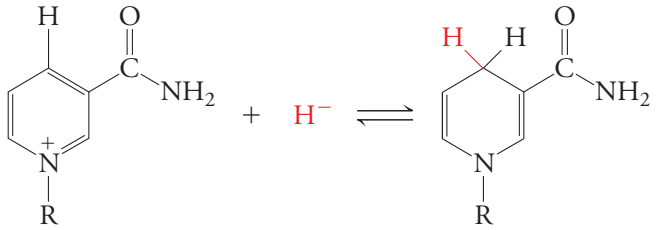
In following metabolic pathways that include oxidation–reduction reactions, we can examine the redox state of the carbon atoms, and we can also trace the path of the electrons that are transferred during the oxidation–reduction reaction. In some cases, this is straightforward, as when an oxidized metal ion such as iron gains an electron (represented as e^-) to become reduced.

$$Fe^{3+} + e^- \rightarrow Fe^{2+}$$

But in some cases, an electron travels along with a proton as an H atom, or a pair of electrons travels with a proton as a hydride ion (H^-).

When a metabolic fuel molecule is oxidized, its electrons may be transferred to a compound such as nicotinamide adenine dinucleotide (NAD^+) or nicotinamide adenine dinucleotide phosphate ($NADP^+$). The structure of these nucleotides is shown in Figure 3-3b. NAD^+ and $NADP^+$ are called **cofactors** or **coenzymes,**

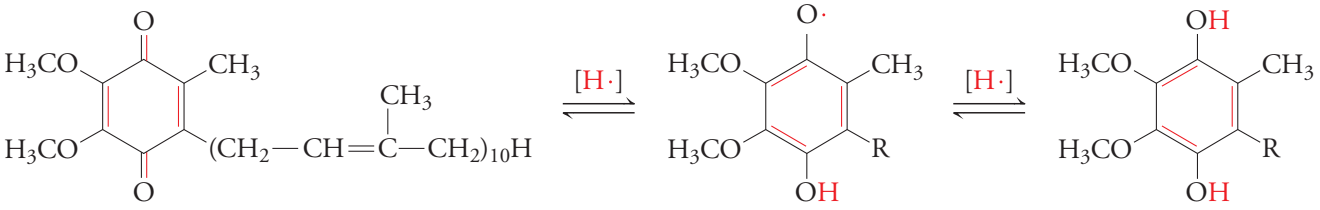
organic compounds that allow an enzyme to carry out a particular chemical reaction (Section 6-2). The redox-active portion of NAD^+ and $NADP^+$ is the nicotinamide group, which accepts a hydride ion to form NADH or NADPH.



$NAD(P)^+$ (*oxidized*) NAD(P)H (*reduced*)

This reaction is reversible, so the reduced cofactors can become oxidized by giving up a hydride ion. In general, NAD^+ participates in catabolic reactions and $NADP^+$ in anabolic reactions. Because these electron carriers are soluble in aqueous solution, they can travel throughout the cell, shuttling electrons from reduced compounds to oxidized compounds.

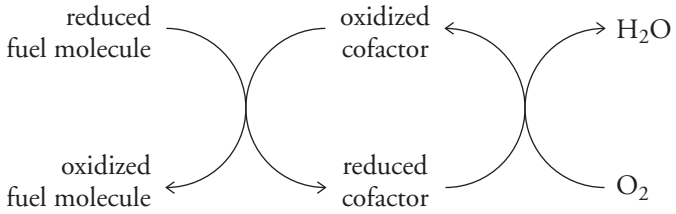
Many cellular oxidation–reduction reactions take place at membrane surfaces, for example, in the inner membranes of mitochondria and chloroplasts in eukaryotes and in the plasma membrane of prokaryotes. In these cases, a membrane-associated enzyme may transfer electrons from a substrate to a lipid-soluble electron carrier such as ubiquinone (coenzyme Q, abbreviated Q; see Section 8-1). Ubiquinone's hydrophobic tail, containing 10 five-carbon isoprenoid units in mammals, allows it to diffuse within the membrane. *Ubiquinone can take up one or two electrons* (in contrast to NAD^+, which is strictly a two-electron carrier). A one-electron reduction of ubiquinone (addition of an H atom) produces a semiquinone, a stable free radical (shown as QH·). A two-electron reduction (two H atoms) yields ubiquinol (QH_2):



Ubiquinone (Q) Ubisemiquinone (QH·) Ubiquinol (QH_2)

The reduced ubiquinol can then diffuse through the membrane to donate its electrons in another oxidation–reduction reaction.

Catabolic pathways, such as the citric acid cycle, generate considerable amounts of reduced cofactors. Some of them are reoxidized in anabolic reactions. The rest are reoxidized by a process that is accompanied by the synthesis of ATP from ADP and P_i. In mammals, the reoxidation of NADH and QH_2 and the concomitant production of ATP require the reduction of O_2 to H_2O. This pathway is known as **oxidative phosphorylation.**



In effect, NAD^+ and ubiquinone collect electrons (and hence free energy) from reduced fuel molecules. When the electrons are ultimately transferred to O_2, this free energy is harvested in the form of ATP.

Metabolic pathways are complex

So far we have sketched the outlines of mammalian fuel metabolism, in which macromolecules are stored and mobilized so that their monomeric units can be broken down into smaller intermediates. These intermediates can be further degraded (oxidized) and their electrons collected by cofactors. We have also briefly mentioned anabolic (synthetic) reactions in which the common two- and three-carbon intermediates give rise to larger compounds. At this point, we can present this information in schematic form in order to highlight some important features of metabolism **(Fig. 12-10)**.

1. *Metabolic pathways are all connected.* In a cell, a metabolic pathway does not operate in isolation; its substrates are the products of other pathways, and vice versa. For example, the NADH and QH_2 generated by the citric acid cycle are the starting materials for oxidative phosphorylation.
2. *Pathway activity is regulated.* Cells do not synthesize polymers when monomers are in short supply. Conversely, they do not catabolize fuels when the need for ATP is low. The **flux,** or flow of intermediates, through a metabolic pathway is regulated in various ways according to substrate availability and the cell's need for the pathway's products. The activity of one or more enzymes in a pathway may be controlled by allosteric effectors (Sections 5-1 and 7-3). These changes in turn may reflect extracellular signals that activate intracellular kinases, phosphatases, and second messengers (Section 10-1). Regulation of pathways is especially critical when the simultaneous operation of two opposing processes, such as fatty acid synthesis and degradation, would be wasteful.
3. *Not every cell carries out every pathway.* Figure 12-10 is a composite of a number of metabolic processes, and a given cell or organism may undertake only a subset of these. Mammals do not perform photosynthesis, and only the liver and kidney can synthesize glucose from noncarbohydrate precursors.
4. *Each cell has a unique metabolic repertoire.* In addition to the pathways outlined in Figure 12-10, which are centered on fuel metabolism, cells carry out a plethora of biosynthetic reactions that are not explicitly shown. Such pathways contribute to the unique metabolic capabilities of different cells and organisms (Box 12-B).
5. *Organisms may be metabolically interdependent.* Photosynthetic plants and the heterotrophs that consume them are an obvious example of metabolic complementarity, but there are numerous other examples, especially in the microbial world. Certain organisms that release methane as a waste product live in close proximity to methanotrophic species (which consume CH_4 as a fuel); neither organism can survive without the other. Humans also exhibit interspecific

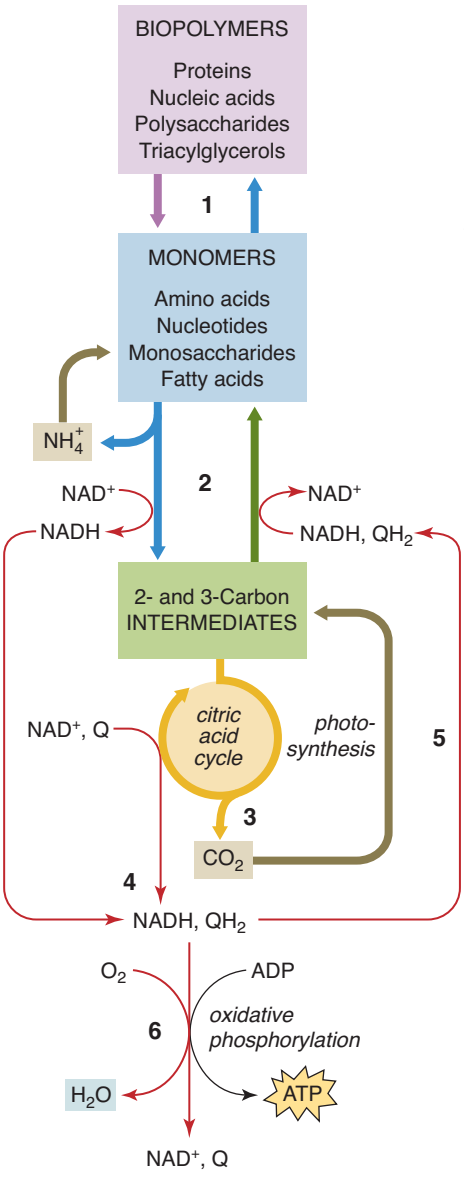


Figure 12-10 Outline of metabolism. In this composite diagram, downward arrows represent catabolic processes, and upward arrows represent anabolic processes. Red arrows indicate some major oxidation–reduction reactions. The major metabolic processes are highlighted: (1) Biological polymers (proteins, nucleic acids, polysaccharides, and triacylglycerols) are built from and are degraded to monomers (amino acids, nucleotides, monosaccharides, and fatty acids). (2) The monomers are broken down into two- and three-carbon intermediates such as glyceraldehyde-3-phosphate, pyruvate, and acetyl-CoA, which are also the precursors of many other biological compounds. (3) The complete degradation of biological molecules yields inorganic compounds such as NH_4^+, CO_2, and H_2O. These substances are returned to the pool of intermediates by processes such as photosynthesis. (4) Electron carriers (NAD^+ and ubiquinone) accept the electrons released by metabolic fuels (amino acids, monosaccharides, and fatty acids) as they are degraded and then completely oxidized by the citric acid cycle. (5) The reduced cofactors (NADH and QH_2) are required for many biosynthetic reactions. (6) The reoxidation of reduced cofactors drives the production of ATP from ADP + P_i (oxidative phosphorylation).

? Is the citric acid cycle a process of carbon oxidation or reduction? Is photosynthesis a process of carbon oxidation or reduction?

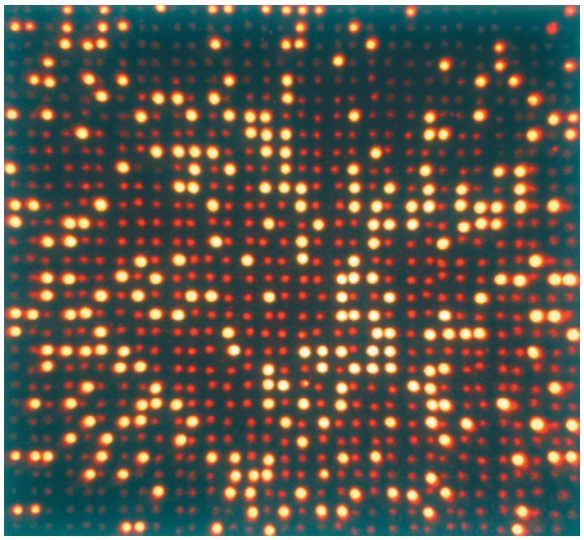
cooperativity: Thousands of different microbial species, amounting to some 100 trillion cells, can live in or on the human body. Collectively, these organisms express millions of different genes and carry out a correspondingly rich set of metabolic activities.

BOX 12-B BIOCHEMISTRY NOTE

The Transcriptome, the Proteome, and the Metabolome

Modern biologists have developed research tools that use the power of computers to collect enormous data sets and analyze them. Such endeavors provide great insights but also have limitations. As we saw in Section 3-3, genomics, the study of an organism's complete set of genes, yields a glimpse of that organism's overall metabolic repertoire. But what the organism, or a single cell, is actually doing at a particular moment depends in part on which genes are active.

A cell's population of mRNA molecules represents genes that are turned on, or transcribed. The study of these mRNAs is known as **transcriptomics.** Identifying and quantifying all the mRNA transcripts (the **transcriptome**) from a single cell type can be done by assembling short strands of DNA with known sequences on a solid support, then allowing them to hybridize, or form double-stranded structures, with fluorescent-labeled mRNAs from a cell preparation. The strength of fluorescence indicates how much mRNA binds to a particular complementary DNA sequence. The collection of DNA sequences is called a **microarray** or **DNA chip** because thousands of sequences fit in a few square centimeters. The microarray may represent an entire genome or just a few selected genes. Each bright spot in the DNA chip shown here represents a DNA sequence to which a fluorescent mRNA molecule has bound.



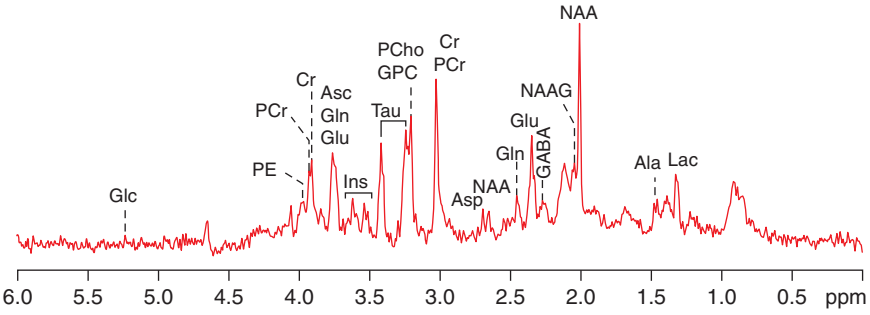
[Voker Steger/Science Photo Library/Photo Researchers.]

Biologists use DNA chips to identify genes whose expression changes under certain conditions or at different developmental stages.

Unfortunately, the correlation between the amount of a particular mRNA and the amount of its protein product is not perfect; some mRNAs are rapidly degraded, whereas others are translated many times, yielding large quantities of the corresponding protein. Hence, a more reliable way to assess gene expression is through **proteomics**—by examining a cell's **proteome,** the complete set of proteins that are synthesized by the cell at a particular point in its life cycle. However, this approach is limited by the technical problems of detecting minute quantities of thousands of different proteins. Nucleic acids can be amplified by the polymerase chain reaction (see Section 3-4), but there is no comparable procedure for amplifying proteins.

(*continued on the next page*)

Where genomics, transcriptomics, and proteomics fall short, **metabolomics** steps in, attempting to pin down the actual metabolic activity in a cell or tissue by identifying and quantifying all its metabolites, that is, its **metabolome.** This is no trivial task, as a cell may contain tens of thousands of different types of compounds, whose concentrations may range over many orders of magnitude. These substances include nonfood molecules such as toxins, preservatives, drugs, and their degradation products. Metabolites are typically detected through column chromatography, nuclear magnetic resonance (NMR) spectroscopy, or mass spectrometry (Section 4-5). In the example shown below, approximately 20 metabolites are visible in an ^{1}H NMR spectrum of a 10-μL sample of rat brain.



Metabolite profile of rat brain. [Courtesy Raghavendra Rao, University of Minnesota, Minneapolis.]

As has been done for genomics and proteomics and other areas of bioinformatics, metabolomic data are deposited in publicly accessible databases for retrieval and analysis. One hope for metabolomics is that disease diagnosis could be streamlined by obtaining a complete metabolic profile of a patient's urine or blood. Industrial applications include monitoring biological processes such as winemaking and bioremediation (using microorganisms to detoxify contaminated environments).

Question: Compare the metabolomic complexity of a single-celled prokaryote and that of a multicellular eukaryote.

An overview such as Figure 12-10 does not convey the true complexity of cellular metabolism, which takes place in a milieu crowded with multiple substrates, competing enzymes, and layers of regulatory mechanisms. Moreover, Figure 12-10 does not include any of the reactions involved in transmitting and decoding genetic information (these topics are covered in the final section of this book). However, a diagram such as Figure 12-10 is a useful tool for mapping the relationships among metabolic processes, and we will refer back to it in the coming chapters. Online databases provide additional information about metabolic pathways, enzymes, intermediates, and metabolic diseases (see Bioinformatics Project 6, Metabolic Enzymes, Microarrays, and Proteomics).

Human metabolism depends on vitamins

Humans lack many of the biosynthetic pathways that occur in plants and microorganisms and so rely on other species to provide certain raw materials. Some amino acids and unsaturated fatty acids are considered **essential** because the human body cannot synthesize them and must obtain them from food (Table 12-1; see also Box 8-B). **Vitamins** likewise are compounds that humans need but cannot make. Presumably, the pathways for synthesizing these substances, which require many specialized enzymes, are not necessary for heterotrophic organisms and have been lost through evolution.

TABLE 12-1 Some Essential Substances for Humans

Amino Acids	Fatty Acids		Other	
Isoleucine	Linoleate	$CH_3(CH_2)_4(CH{=}CHCH_2)_2(CH_2)_6COO^-$	Choline	$(CH_3)_3N^+CH_2CH_2OH$
Leucine	Linolenate	$CH_3CH_2(CH{=}CHCH_2)_3(CH_2)_6COO^-$		
Lysine				
Methionine				
Phenylalanine				
Threonine				
Tryptophan				
Valine				

The word *vitamin* comes from *vital amine,* a term coined by Casimir Funk in 1912 to describe organic compounds that are required in small amounts for normal health. It turns out that most vitamins are not amines, but the name has stuck. Table 12-2 lists the vitamins and their metabolic roles. Vitamins A, D, E, and K are lipids; their functions were described in Box 8-B. Many of the water-soluble vitamins are the precursors of coenzymes, which we will describe as we encounter them in the context of their particular metabolic reactions. Vitamins are a diverse group of compounds,

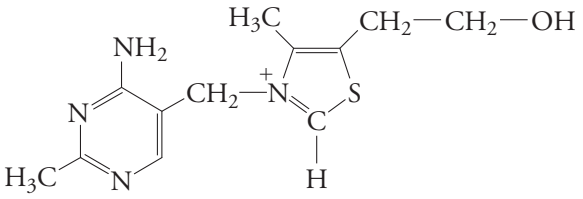
TABLE 12-2 Vitamins and Their Roles

Vitamin	Coenzyme Product	Biochemical Function	Human Deficiency Disease	Text Reference
Water-Soluble				
Ascorbic acid (C)	Ascorbate	Cofactor for hydroxylation of collagen	Scurvy	Box 5-D
Biotin (B_7)	Biocytin	Cofactor for carboxylation reactions	*	Section 13-1
Cobalamin (B_{12})	Cobalamin coenzymes	Cofactor for alkylation reactions	Anemia	Section 17-1
Folic acid	Tetrahydrofolate	Cofactor for one-carbon transfer reactions	Anemia	Section 18-2
Lipoic acid	Lipoamide	Cofactor for acyl transfer reactions	*	Section 14-1
Nicotinamide (niacin, B_3)	Nicotinamide coenzymes (NAD^+, $NADP^+$)	Cofactor for oxidation–reduction reactions	Pellagra	Fig. 3-3, Section 12-2
Pantothenic acid (B_5)	Coenzyme A	Cofactor for acyl transfer reactions	*	Fig. 3-3, Section 12-3
Pyridoxine (B_6)	Pyridoxal phosphate	Cofactor for amino-group transfer reactions	*	Section 18-1
Riboflavin (B_2)	Flavin coenzymes (FAD, FMN)	Cofactor for oxidation–reduction reactions	*	Fig. 3-3
Thiamine (B_1)	Thiamine pyrophosphate	Cofactor for aldehyde transfer reactions	Beriberi	Sections 12-2, 14-1
Fat-Soluble				
Vitamin A (retinol)		Light-absorbing pigment	Blindness	Box 8-B
Vitamin D		Hormone that promotes Ca^{2+} absorption	Rickets	Box 8-B
Vitamin E (tocopherol)		Antioxidant	*	Box 8-B
Vitamin K (phylloquinone)		Cofactor for carboxylation of blood coagulation proteins	Bleeding	Box 8-B

*Deficiency in humans is rare or unobserved.

whose discoveries and functional characterization have provided some of the more colorful stories in the history of biochemistry.

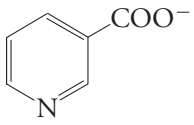
Many vitamins were discovered through studies of nutritional deficiencies. One of the earliest links between nutrition and disease was observed centuries ago in sailors suffering from scurvy, an illness caused by vitamin C deficiency (Box 5-D). A study of the disease beriberi led to the discovery of the first B vitamin. Beriberi, characterized by leg weakness and swelling, is caused by a deficiency of thiamine (vitamin B_1).



Thiamine (vitamin B_1)

Thiamine acts as a prosthetic group in some essential enzymes, including the one that converts pyruvate to acetyl-CoA. Rice husks are rich in thiamine, and individuals whose diet consists largely of polished (huskless) rice can develop beriberi. The disease was originally thought to be infectious, until the same symptoms were observed in chickens and prisoners fed a diet of polished rice. Thiamine deficiency can occur in chronic alcoholics and others with a limited diet and impaired nutrient absorption.

Niacin, a component of NAD^+ and $NADP^+$, was first identified as the factor missing in the vitamin-deficiency disease pellagra.



Niacin

The symptoms of pellagra, including diarrhea and dermatitis, can be alleviated by boosting the intake of the essential amino acid tryptophan, which humans can convert to niacin. Niacin deficiency was once common in certain populations whose diet consisted largely of maize (corn). This grain is low in tryptophan and its niacin is covalently bound to other molecules; hence, it is not easily absorbed during digestion. In South America, where maize originated, the kernels are traditionally prepared by soaking or boiling them in an alkaline solution, a treatment that releases niacin and prevents pellagra. Unfortunately, this food-preparation custom did not spread to other parts of the world that adopted maize farming.

Most vitamins are readily obtained from a balanced diet, although poor nutrition, particularly in impoverished parts of the world, still causes vitamin-deficiency diseases. Intestinal bacteria, as well as plant- and animal-derived foods, are the natural sources of vitamins. However, plants do not contain cobalamin, so individuals who follow a strict vegetarian diet are at higher risk for developing a cobalamin deficiency.

CONCEPT REVIEW

- Why are compounds such as glyceraldehyde-3-phosphate, pyruvate, and acetyl-CoA so important in metabolism?
- What role do cofactors such as NAD^+ and ubiquinone play in metabolic reactions?
- What is the importance of reoxidizing NADH and QH_2 by molecular oxygen?
- Summarize the main features of metabolic pathways.
- Explain the relationship between vitamins and coenzymes.

12-3 Free Energy Changes in Metabolic Reactions

KEY CONCEPTS

- The free energy change for a reaction depends on the equilibrium constant for the reaction and on the actual concentrations of the reacting species.
- A reaction with a large negative change in free energy can be coupled to another, unfavorable reaction.
- A reaction that breaks a phosphoanhydride bond in ATP occurs with a large change in free energy.
- Cells also use the free energy of other phosphorylated compounds, thioesters, reduced cofactors, and electrochemical gradients.
- Nonequilibrium reactions often serve as metabolic control points.

We have introduced the idea that catabolic reactions tend to release free energy and anabolic reactions tend to consume it (see Fig. 12-1), but, in fact, *all reactions in vivo occur with a net decrease in free energy; that is, ΔG is always less than zero* (free energy is discussed in Section 1-3). In a cell, metabolic reactions are not isolated but are linked, so the free energy of a thermodynamically favorable reaction can be used to pull a second, unfavorable reaction forward. How can free energy be transferred from one reaction to another? Free energy is not a substance or the property of a single molecule, so it is misleading to refer to a molecule or a bond within that molecule as having a large amount of free energy. Rather, *free energy is a property of a system, and it changes when the system undergoes a chemical reaction.*

The free energy change depends on reactant concentrations

The change in free energy of a system is related to the concentrations of the reacting substances. When a reaction such as $A + B \rightleftharpoons C + D$ is at equilibrium, the concentrations of the four reactants define the **equilibrium constant, K_{eq},** for the reaction:

$$K_{eq} = \frac{[C]_{eq}[D]_{eq}}{[A]_{eq}[B]_{eq}} \qquad \textbf{[12-1]}$$

(the brackets indicate the molar concentration of each substance). Recall that at equilibrium, the rates of the forward and reverse reactions are balanced, so there is no net change in the concentration of any reactant. Equilibrium does *not* mean that the concentrations of the reactants and products are equal.

When the system is not at equilibrium, the reactants experience a driving force to reach their equilibrium values. This force is the ***standard free energy change for the reaction, $\Delta G^{\circ\prime}$,*** *which is defined as*

$$\Delta G^{\circ\prime} = -RT \ln K_{eq} \qquad \textbf{[12-2]}$$

R is the gas constant (8.3145 $J \cdot K^{-1} \cdot mol^{-1}$) and T is the temperature in Kelvin. Recall from Section 1-3 that free energy has units of joules per mole (Box 12-C). Equation 12-2 can be used to calculate $\Delta G^{\circ\prime}$ from K_{eq} and vice versa (see Sample Calculation 12-1).

SAMPLE CALCULATION 12-1

PROBLEM

Calculate $\Delta G^{\circ\prime}$ for a reaction at 25°C when $K_{eq} = 5.0$.

SOLUTION

Use Equation 12-2:

$$\begin{aligned}\Delta G^{\circ\prime} &= -RT \ln K_{eq} \\ &= -(8.3145\ J \cdot K^{-1} \cdot mol^{-1})(298\ K)\ln 5.0 \\ &= -4000\ J \cdot mol^{-1} = -4.0\ kJ \cdot mol^{-1}\end{aligned}$$

PRACTICE PROBLEMS

1. Calculate $\Delta G^{\circ\prime}$ for a reaction at 25°C when $K_{eq} = 0.25$.
2. If the temperature for the reaction in Practice Problem 1 was raised to 37°C, how would $\Delta G^{\circ\prime}$ change?
3. If $\Delta G^{\circ\prime}$ for a reaction at 37°C is –10 $kJ \cdot mol^{-1}$, what is K_{eq}?

BOX 12-C BIOCHEMISTRY NOTE

What Is a Calorie?

Most biochemists express quantities using the International System of units (Box 1-A), which includes the joule, named after physicist James Prescott Joule. The joule is actually a derived unit that can be defined in terms of different kinds of work (energy is the capacity to do work). For example, a joule is equivalent to the work done by applying a force of one newton through a distance of one meter; that is, 1 J = 1 N · m.

The joule has largely replaced the calorie, which is the amount of heat required to increase the temperature of 1 g of water by 1°C. In practice, a calorie is a difficult thing to measure, but the term remains popular for referring to the energy content of food. However, a calorie is actually a fairly small quantity, so kilocalories (kcal), also known as large calories (Cal), are typically used. Thus, a nutrition label indicating that a tablespoon of peanut butter contains 95 calories should really say 95 Cal, 95 kcal, or 95,000 cal. To avoid confusion, calories can always be converted to joules: 1 cal = 4.1484 J and 1 J = 0.239 cal.

Question: How many joules are in one tablespoon of peanut butter?

By convention, measurements of standard free energy are valid under **standard conditions,** where the temperature is 25°C (298 K) and the pressure is 1 atm (these conditions are indicated by the degree symbol after ΔG). For a chemist, standard conditions specify an initial activity of 1 for each reactant (activity is the reactant's concentration corrected for its nonideal behavior). However, these conditions are impractical for biochemists since most biochemical reactions occur near neutral pH (where $[H^+] = 10^{-7}$ M rather than 1 M) and in aqueous solution (where $[H_2O] = 55.5$ M). The biochemical standard conditions are summarized in Table 12-3. Biochemists use a prime symbol to indicate the standard free energy change for a reaction under biochemical standard conditions. In most equilibrium expressions, $[H^+]$ and $[H_2O]$ are set to 1 so that these terms can be ignored. And because biochemical reactions typically involve dilute solutions of reactants, molar concentrations can be used instead of activities.

Like K_{eq}, $\Delta G^{\circ\prime}$ is a constant for a particular reaction. It may be a positive or negative value, and it indicates whether the reaction can proceed spontaneously ($\Delta G^{\circ\prime} < 0$) or not ($\Delta G^{\circ\prime} > 0$) under standard conditions. In a living cell, reactants and products are almost never present at standard-state concentrations and the temperature may not be 25°C, yet reactions do occur with some change in free energy. Thus, it is important to distinguish the standard free energy change of a reaction from its actual free energy change, ΔG. ΔG is a function of the actual concentrations of the reactants and the temperature (37°C or 310 K in humans). ΔG is related to the standard free energy change for the reaction:

$$\Delta G = \Delta G^{\circ\prime} + RT \ln \frac{[C][D]}{[A][B]} \qquad \textbf{[12-3]}$$

Here, the bracketed quantities represent the actual, nonequilibrium concentrations of the reactants. The concentration term in Equation 12-3 is sometimes called the **mass action ratio.**

When the reaction is at equilibrium, $\Delta G = 0$ and

$$\Delta G^{\circ\prime} = -RT \ln \frac{[C]_{eq}[D]_{eq}}{[A]_{eq}[B]_{eq}} \qquad \textbf{[12-4]}$$

which is equivalent to Equation 12-2. Note that Equation 12-3 shows that *the criterion for spontaneity for a reaction is ΔG, a property of the actual concentrations of the reactants, not the constant $\Delta G^{\circ\prime}$*. Thus, a reaction with a positive standard free energy change (a reaction that cannot occur when the reactants are present at standard

TABLE 12-3

Biochemical Standard State

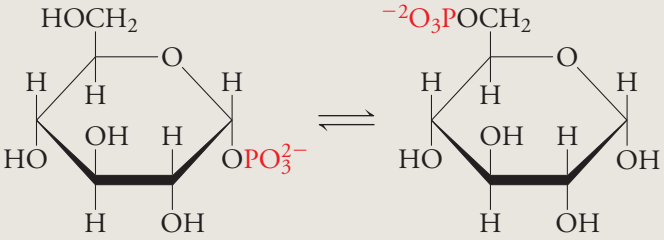
Temperature	25°C (298 K)
Pressure	1 atm
Reactant concentration	1 M
pH	7.0 ($[H^+] = 10^{-7}$ M)
Water concentration	55.5 M

concentrations) may proceed *in vivo*, depending on the concentrations of reactants in the cell (see Sample Calculation 12-2). Keep in mind that thermodynamic spontaneity does not imply a rapid reaction. Even a substance with a strong tendency to undergo reaction ($\Delta G \ll 0$) will usually not react until acted upon by an enzyme that catalyzes the reaction.

SAMPLE CALCULATION 12-2

PROBLEM

The standard free energy change for the reaction catalyzed by phosphoglucomutase is $-7.1\ \text{kJ} \cdot \text{mol}^{-1}$. Calculate the equilibrium constant for the reaction. Calculate ΔG at 37°C when the concentration of glucose-1-phosphate is 1 mM and the concentration of glucose-6-phosphate is 25 mM. Is the reaction spontaneous under these conditions?



SOLUTION

The equilibrium constant K_{eq} can be derived by rearranging Equation 12-2.

$$K_{eq} = e^{-\Delta G^{\circ\prime}/RT}$$
$$= e^{-(-7100\ \text{J}\cdot\text{mol}^{-1})/(8.3145\ \text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1})(298\ \text{K})}$$
$$= e^{2.87} = 17.6$$

At 37°C, $T = 310$ K.

$$\Delta G = \Delta G^{\circ\prime} + RT \ln \frac{[\text{glucose-6-phosphate}]}{[\text{glucose-1-phosphate}]}$$
$$= -7100\ \text{J} \cdot \text{mol}^{-1} + (8.3145\ \text{J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1})(310\ \text{K}) \ln(0.025/0.001)$$
$$= -7100\ \text{J} \cdot \text{mol}^{-1} + 8300\ \text{J} \cdot \text{mol}^{-1}$$
$$= +1200\ \text{J} \cdot \text{mol}^{-1} = +1.2\ \text{kJ} \cdot \text{mol}^{-1}$$

The reaction is not spontaneous because ΔG is greater than zero.

PRACTICE PROBLEMS

4. Calculate ΔG for the reaction shown here when the initial concentration of glucose-1-phosphate is 5 mM and the initial concentration of glucose-6-phosphate is 20 mM. Is the reaction spontaneous under these conditions?
5. At equilibrium, the concentration of glucose-6-phosphate is 35 mM. What is the concentration of glucose-1-phosphate?
6. Calculate the ratio of the concentration of glucose-6-phosphate to the concentration of glucose-1-phosphate that gives a free energy change of –2.0 $\text{kJ} \cdot \text{mol}^{-1}$.

Unfavorable reactions are coupled to favorable reactions

A biochemical reaction may at first seem to be thermodynamically forbidden because its free energy change is greater than zero. Yet the reaction can proceed *in vivo* when it is coupled to a second reaction whose value of ΔG is very large and negative so that the *net* change in free energy for the combined reactions is less than zero. *ATP is often involved in such coupled processes because its reactions occur with a relatively large negative change in free energy.*

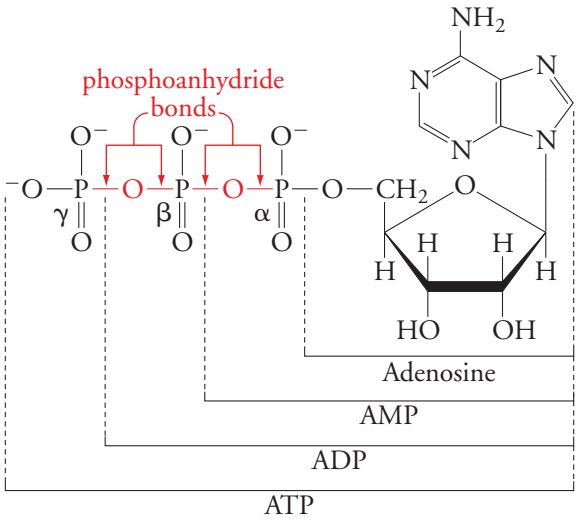


Figure 12-11 **Adenosine triphosphate.** The three phosphate groups are sometimes described by the Greek letters α, β, and γ. The linkage between the first (α) and second (β) phosphoryl groups, and between the second (β) and third (γ), is a phosphoanhydride bond. A reaction in which one or two phosphoryl groups are transferred to another compound (a reaction in which a phosphoanhydride bond is cleaved) has a large negative value of $\Delta G^{\circ\prime}$.

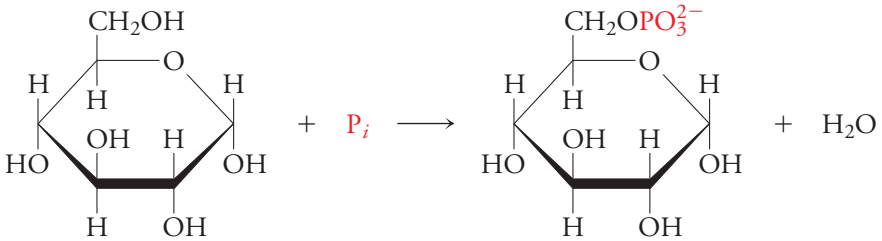
? How does hydrolysis of a phosphoanhydride bond affect the net charge of a nucleotide?

Adenosine triphosphate (ATP) contains two phosphoanhydride bonds **(Fig. 12-11)**. Cleavage of either of these bonds—that is, transfer of one or two of its phosphoryl groups to another molecule—is a reaction with a large negative standard free energy change (under physiological conditions, ΔG is even more negative). As a reference point, biochemists use the reaction in which a phosphoryl group is transferred to water—in other words, hydrolysis of the phosphoanhydride bond, such as

$$\text{ATP} + \text{H}_2\text{O} \rightarrow \text{ADP} + \text{P}_i$$

This is a spontaneous reaction with a $\Delta G^{\circ\prime}$ value of $-30\ \text{kJ} \cdot \text{mol}^{-1}$.

The following example illustrates the role of ATP in a coupled reaction. Consider the phosphorylation of glucose by inorganic phosphate (HPO_4^{2-} or P_i), a thermodynamically unfavorable reaction ($\Delta G^{\circ\prime} = +13.8\ \text{kJ} \cdot \text{mol}^{-1}$):



When this reaction is combined with the ATP hydrolysis reaction, the values of $\Delta G^{\circ\prime}$ for each reaction are added:

	$\Delta G^{\circ\prime}$
glucose + P_i → glucose-6-phosphate + H_2O	$+13.8\ \text{kJ} \cdot \text{mol}^{-1}$
ATP + H_2O → ADP + P_i	$-30.5\ \text{kJ} \cdot \text{mol}^{-1}$
glucose + ATP → glucose-6-phosphate + ADP	$-16.7\ \text{kJ} \cdot \text{mol}^{-1}$

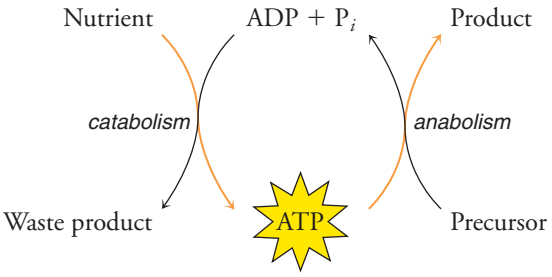
The net chemical reaction, the phosphorylation of glucose, is thermodynamically favorable ($\Delta G < 0$). *In vivo,* this reaction is catalyzed by hexokinase (introduced in Section 6-3), and a phosphoryl group is transferred from ATP directly to glucose. The ATP is not actually hydrolyzed, and there is no free phosphoryl group floating around the enzyme. However, writing out the two coupled reactions, as shown above, makes it easier to see what's going on thermodynamically.

Some biochemical processes appear to occur with the concomitant hydrolysis of ATP to ADP + P_i, for example, the operation of myosin and kinesin (Section 5-3) or the Na,K-ATPase ion pump (Section 9-3). But a closer look reveals that in all

these processes, ATP actually transfers a phosphoryl group to a protein. Later, the phosphoryl group is transferred to water, so the net reaction takes the form of ATP hydrolysis. The same ATP "hydrolysis" effect applies to some reactions in which the AMP moiety of ATP (rather than a phosphoryl group) is transferred to a substance, leaving inorganic pyrophosphate (PP_i). Cleavage of the phosphoanhydride bond of PP_i also has a large negative value of $\Delta G^{\circ\prime}$.

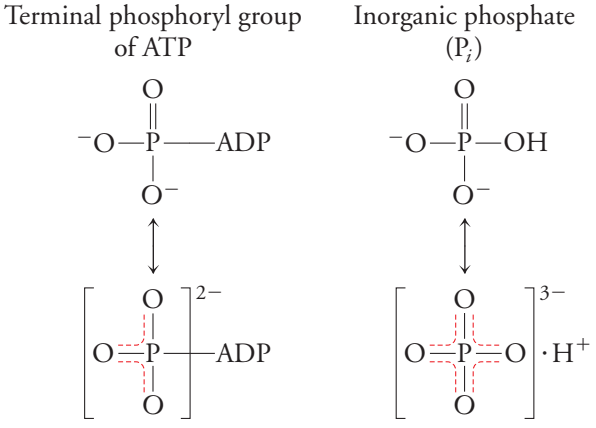
▶▶ WHAT'S so special about ATP?

Because ATP appears to drive many thermodynamically unfavorable reactions, it is tempting to think of ATP as an agent that transfers packets of free energy around the cell. This is one reason why ATP is commonly called the energy currency of the cell. The general role of ATP in linking exergonic ATP-producing processes to endergonic ATP-consuming processes can be diagrammed as



In this scheme, it appears that the "energy" of the catabolized nutrient is transferred to ATP, then the "energy" of ATP is transferred to another product in a biosynthetic reaction. However, free energy is not a tangible item, and there is nothing magic about ATP, as the question at the start of the chapter indicates. The two phosphoanhydride bonds of ATP are sometimes called "high-energy" bonds, but they are no different from other covalent bonds. All that matters is that *breaking these bonds is a process with a large negative free energy change.* Using the simple example of ATP hydrolysis, we can state that a large amount of free energy is released when ATP is hydrolyzed because the products of the reaction have less free energy than the reactants. It is worth examining two reasons why this is so.

1. *The ATP hydrolysis products are more stable than the reactants.* At physiological pH, ATP has three to four negative charges (its pK is close to 7), and the anionic groups repel each other. In the products ADP and P_i, separation of the charges relieves some of this unfavorable electrostatic repulsion.
2. *A compound with a phosphoanhydride bond experiences less resonance stabilization than its hydrolysis products.* **Resonance stabilization** reflects the degree of electron delocalization in a molecule and can be roughly assessed by the number of different ways of depicting the molecule's structure. There are fewer equivalent ways of arranging the bonds of the terminal phosphoryl group of ATP than there are in free P_i.



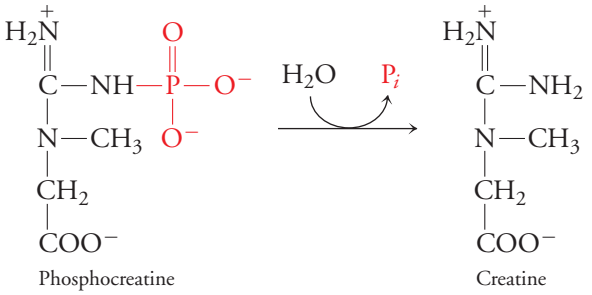
To summarize, *ATP functions as an energy currency because its reaction is highly exergonic ($\Delta G \ll 0$).* The favorable ATP reaction (ATP → ADP) can therefore pull

another, unfavorable reaction with it, provided that the sum of the free energy changes for both reactions is less than zero. In effect, the cell "spends" ATP to make another process happen.

Free energy can take different forms

ATP is not the only substance that functions as energy currency in the cell. Other compounds that participate in reactions with large negative changes in free energy can serve the same purpose. For example, a number of phosphorylated compounds other than ATP can give up their phosphoryl group to another molecule. Table 12-4 lists the standard free energy changes for some of these reactions in which the phosphoryl group is transferred to water.

Although hydrolysis of the bond linking the phosphate group to the rest of the molecule could be a wasteful process (the product would be free phosphate, P_i), the values listed in the table are a guide to how such compounds would behave in a coupled reaction, such as the hexokinase reaction described above. For example, phosphocreatine has a standard free energy of hydrolysis of -43.1 kJ · mol^{-1}:



Phosphocreatine Creatine

Creatine has lower free energy than phosphocreatine since it has two, rather than one, resonance forms; this resonance stabilization contributes to the large negative free energy change when phosphocreatine transfers its phosphoryl group to another compound. In muscles, phosphocreatine transfers a phosphoryl group to ADP to produce ATP (Box 12-D).

TABLE 12-4

Standard Free Energy Change for Phosphate Hydrolysis

Compound	$\Delta G^{\circ\prime}$ (kJ · mol^{-1})
Phosphoenolpyruvate	−61.9
1,3-Bisphosphoglycerate	−49.4
ATP → AMP + PP_i	−45.6
Phosphocreatine	−43.1
ATP → ADP + P_i	−30.5
Glucose-1-phosphate	−20.9
PP_i → 2 P_i	−19.2
Glucose-6-phosphate	−13.8
Glycerol-3-phosphate	−9.2

BOX 12-D BIOCHEMISTRY NOTE

Powering Human Muscles

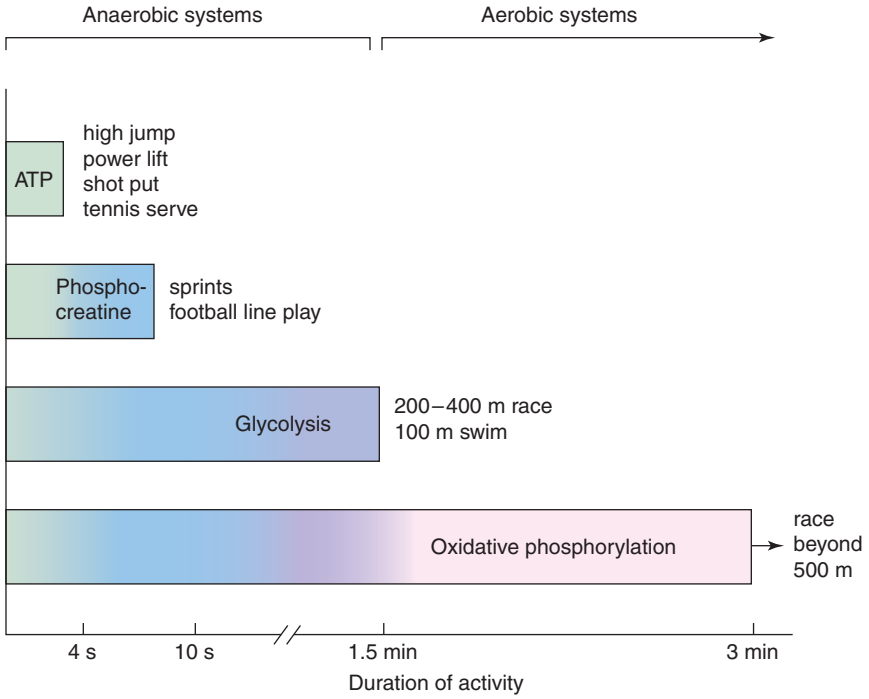
In resting muscles, when the demand for ATP is low, creatine kinase catalyzes the transfer of a phosphoryl group from ATP to creatine to produce phosphocreatine:

$$\text{ATP} + \text{creatine} \rightleftharpoons \text{phosphocreatine} + \text{ADP}$$

This reaction runs in reverse when ADP concentrations rise, as they do when muscle contraction converts ATP to ADP + P_i. Phosphocreatine therefore acts as a sort of phosphoryl-group reservoir to maintain the supply of ATP. Cells cannot stockpile ATP; its concentration remains remarkably stable (between 2 and 5 mM in most cells) under widely varying levels of demand. Without phosphocreatine, a muscle would exhaust its ATP supply before it could be replenished by other, slower processes.

Different types of physical activity make different demands on a muscle's ATP-generating mechanisms. A single burst of activity is powered by the available ATP. Activities lasting up to a few seconds require phosphocreatine to maintain the ATP supply. Phosphocreatine itself is limited, so continued muscle contraction must rely on ATP produced by catabolizing glucose (obtained from the muscle's store of glycogen) via glycolysis. The end product of this pathway is lactate, the conjugate base of a weak acid, and muscle pain sets in as the acid accumulates and the pH begins to drop. Up to this point, the muscle functions anaerobically (without the participation of O_2). To continue its activity, it must switch to aerobic (O_2-dependent) metabolism and further oxidize

glucose via the citric acid cycle. The muscle also catabolizes fatty acids, whose products also enter the citric acid cycle. Recall that the citric acid cycle generates reduced cofactors that must be reoxidized by molecular oxygen. Aerobic metabolism of glucose and fatty acids is slower than anaerobic glycolysis, but it generates considerably more ATP. Some forms of physical activity and the systems that power them are diagrammed here.



[Figure adapted from McArdle, W. D., Katch, F. I., and Katch, V. L., Exercise Physiology (2nd ed.), p. 348, Lea & Febiger (1986).]

A casual athlete can detect the shift from anaerobic to aerobic metabolism after about a minute and a half. In world-class athletes, the breakpoint occurs at about 150 to 170 seconds, which corresponds roughly to the finish line in a 1000-meter race.

The muscles of sprinters have a high capacity for anaerobic ATP generation, whereas the muscles of distance runners are better adapted to produce ATP aerobically. Such differences in energy metabolism are visibly manifest in the flight muscles of birds. Migratory birds such as geese, which power their long flights primarily with fatty acids, have large numbers of mitochondria to carry out oxidative phosphorylation. The reddish-brown color of the mitochondria gives the flight muscles a dark color. Birds that rarely fly, such as chickens, have fewer mitochondria and lighter-colored muscles. When these birds do fly, it is usually only a short burst of activity that is powered by anaerobic mechanisms.

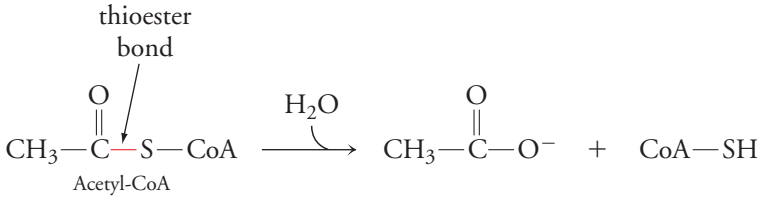
Question: Why do some athletes believe that creatine supplements boost their performance?

Like ATP, other nucleoside triphosphates have large negative standard free energies of hydrolysis. GTP rather than ATP serves as the energy currency for reactions that occur during cellular signaling (Section 10-2) and protein synthesis (Section 22-3). In the cell, nucleoside triphosphates are freely interconverted by reactions such as the one catalyzed by nucleoside diphosphate kinase, which transfers a phosphoryl group from ATP to a nucleoside diphosphate (NDP):

$$\text{ATP} + \text{NDP} \rightleftharpoons \text{ADP} + \text{NTP}$$

Because the reactants and products are energetically equivalent, $\Delta G^{\circ\prime}$ values for these reactions are near zero.

Another class of compounds that can release a large amount of free energy upon hydrolysis are **thioesters,** such as acetyl-CoA. Coenzyme A is a nucleotide derivative with a side chain ending in a sulfhydryl (SH) group (see Fig. 3-3a). An acyl or acetyl group (the "A" for which coenzyme A was named) is linked to the sulfhydryl group by a thioester bond. Hydrolysis of this bond has a $\Delta G^{\circ\prime}$ value of -31.5 kJ · mol^{-1}, comparable to that of ATP hydrolysis:



Hydrolysis of a thioester is more exergonic than the hydrolysis of an ordinary (oxygen) ester because thioesters have less resonance stability than oxygen esters, owing to the larger size of an S atom relative to an O atom. An acetyl group linked to coenzyme A can be readily transferred to another molecule because formation of the new linkage is powered by the favorable free energy change of breaking the thioester bond.

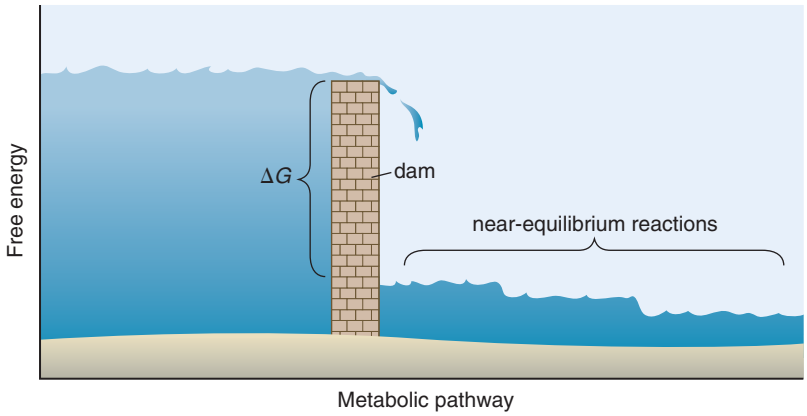
We have already seen that in oxidation–reduction reactions, cofactors such as NAD^+ and ubiquinone can collect electrons. The reduced cofactors are a form of energy currency because their subsequent reoxidation by another compound occurs with a negative change in free energy. Ultimately, the transfer of electrons from one reduced cofactor to another and finally to oxygen, the final electron acceptor in many cells, releases enough free energy to drive the synthesis of ATP.

Keep in mind that free energy changes occur not just as the result of chemical changes such as phosphoryl-group transfer or electron transfer. As decreed by the first law of thermodynamics (Section 1-3), *energy can take many forms.* We will see that ATP production in cells depends on the energy of an electrochemical gradient, that is, an imbalance in the concentration of a substance (in this case, protons) on the two sides of a membrane. The free energy change of dissipating this gradient (allowing the system to move toward equilibrium) is converted to the mechanical energy of an enzyme that synthesizes ATP. In photosynthetic cells, the chemical reactions required to generate carbohydrates are ultimately driven by the free energy changes of reactions in which light-excited molecules return to a lower-energy state.

Regulation occurs at the steps with the largest free energy changes

In a series of reactions that make up a metabolic pathway, some reactions have ΔG values near zero. These near-equilibrium reactions are not subject to a strong driving force to proceed in either direction. Rather, flux can go forward or backward, according to slight fluctuations in the concentrations of reactants and products. When the concentrations of metabolites change, the enzymes that catalyze these near-equilibrium reactions tend to act quickly to restore the near-equilibrium state.

Reactions with large changes in free energy have a longer way to go to reach equilibrium; these are the reactions that experience the greatest "urge" to proceed forward. However, the enzymes that catalyze these reactions do not allow the reaction to reach equilibrium because they work too slowly. Often the enzymes are already saturated with substrate, so the reactions cannot go any faster (when $[S] \gg K_M$, $v \approx V_{max}$; Section 7-2). The rates of these far-from-equilibrium reactions limit flux through the entire pathway because the reactions function like dams.



Cells can regulate flux through a pathway by adjusting the rate of a reaction with a large free energy change. This can be done by increasing the amount of enzyme that catalyzes that step or by altering the intrinsic activity of the enzyme through allosteric mechanisms (see Fig. 7-17). As soon as more metabolite has gotten past the dam, the near-equilibrium reactions go with the flow, allowing the pathway intermediates to move toward the final product. Most metabolic pathways do not have a single flow-control point, as the dam analogy might suggest. Instead, flux is typically controlled at several points to ensure that the pathway can work efficiently as part of the cell's entire metabolic network.

CONCEPT REVIEW

- Why must free energy changes be negative for reactions *in vivo*?
- What is the standard free energy change for a reaction and how is it related to the reaction's equilibrium constant?
- Distinguish ΔG and $\Delta G^{\circ\prime}$. How are they related?
- Why is it misleading to refer to ATP as a high-energy molecule?
- Explain why cleavage of one of ATP's phosphoanhydride bonds releases large amounts of free energy.
- How do phosphorylated compounds, thioesters, and reduced cofactors appear to transfer free energy? What other forms of energy do cells use?
- Why do cells control the metabolic reactions that have large free energy changes?

[SUMMARY]

12-1 Food and Fuel

- Polymeric food molecules such as starch, proteins, and triacylglycerols are broken down to their monomeric components (glucose, amino acids, and fatty acids), which are absorbed. These materials are stored as polymers in a tissue-specific manner.
- Metabolic fuels are mobilized from glycogen, fat, and proteins as needed.

12-2 Metabolic Pathways

- Series of reactions known as metabolic pathways break down and synthesize biological molecules. Several pathways make use of the same small molecule intermediates.
- During the oxidation of amino acids, monosaccharides, and fatty acids, electrons are transferred to carriers such as NAD^+ and ubiquinone. Reoxidation of the reduced cofactors drives the synthesis of ATP by oxidative phosphorylation.
- Metabolic pathways form a complex network, but not all cells or organisms carry out all possible metabolic processes. Humans rely on other organisms to supply vitamins and other essential materials.

12-3 Free Energy Changes in Metabolic Reactions

- The standard free energy change for a reaction is related to the equilibrium constant, but the actual free energy change is related to the actual cellular concentrations of reactants and products.
- A thermodynamically unfavorable reaction may proceed when it is coupled to a favorable process involving ATP, whose phosphoanhydride bonds release a large amount of free energy when cleaved.
- Other forms of cellular energy currency include phosphorylated compounds, thioesters, and reduced cofactors.
- Cells regulate metabolic pathways at the steps that are farthest from equilibrium.

GLOSSARY TERMS

chemoautotroph
photoautotroph
heterotroph
catabolism
anabolism
metabolism
lipoprotein
metabolic fuel
mobilization
phosphorolysis
diabetes mellitus
lysosome
proteasome
metabolic pathway
metabolite
glycolysis
citric acid cycle
oxidation
reduction
redox reaction
cofactor
coenzyme
oxidative phosphorylation
flux
transcriptomics
transcriptome
microarray (DNA chip)
proteomics
proteome
metabolomics
metabolome
essential compound
vitamin
equilibrium constant (K_{eq})
standard free energy change ($\Delta G^{\circ\prime}$)
standard conditions
mass action ratio
resonance stabilization
thioester

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METABOLIC ENZYMES, MICROARRAYS, AND PROTEOMICS

PROBLEMS

12-1 Food and Fuel

1. Classify the following organisms as chemoautotrophs, photoautotrophs, or heterotrophs:
(a) *Hydrogenobacter*, which converts molecular hydrogen and oxygen to water
(b) *Arabidopsis thaliana*, a green plant
(c) The nitrosifying bacteria, which oxidize NH_3 to nitrite
(d) *Saccharomyces cerevisiae*, yeast
(e) *Caenorhabditis elegans*, a nematode worm
(f) The *Thiothrix* bacteria, which oxidize hydrogen sulfide
(g) Cyanobacteria (erroneously termed "blue-green algae" in the past)

2. The purple nonsulfur bacteria obtain their cellular energy from a photosynthetic process that does not produce oxygen. These bacteria also require an organic carbon source. Using the terms in this chapter, coin a new term that describes the trophic strategy of this organism.

3. Digestion of carbohydrates begins in the mouth, where salivary amylases act on dietary starch. When the food is swallowed and enters the stomach, carbohydrate digestion ceases (it resumes in the small intestine). Why does carbohydrate digestion not occur in the stomach?

4. Pancreatic amylase, which is similar to salivary amylase, is secreted by the pancreas into the small intestine. The active site of pancreatic amylase accommodates five glucosyl residues and cleaves the glycosidic bond between the second and third residues. The enzyme cannot accommodate branched chains.
(a) What are the main products of amylose digestion?
(b) What are the products of amylopectin digestion?

5. Starch digestion is completed by the enzymes isomaltase (or α-dextrinase), which catalyzes the hydrolysis of α(1 → 6) glycosidic bonds, and maltase, which hydrolyzes α(1 → 4) bonds. Why are these enzymes needed in addition to α-amylase?

6. Monosaccharides, the products of polysaccharide and disaccharide digestion, enter the cells lining the intestine via a specialized transport system. What is the source of free energy for this transport process?

7. Unlike the monosaccharides described in Problem 6, sugar alcohols such as sorbitol (see Solution 11-28) are absorbed via passive diffusion. Why? What process occurs more rapidly, passive diffusion or passive transport?

8. Use what you know about the properties of alcohol (ethanol) to describe how it is absorbed in both the stomach and the small intestine. What effect does the presence of food have on the absorption of ethanol?

9. Nucleic acids that are present in food are hydrolyzed by digestive enzymes. What type of mechanism most likely mediates the entry of the reaction products into intestinal cells?

10. Hydrolysis of proteins begins in the stomach, catalyzed by the hydrochloric acid secreted into the stomach by parietal cells. Draw the reaction that shows the hydrolysis of a peptide bond.

11. How does the low pH of the stomach affect protein structure in such a way that the proteins are prepared for hydrolytic digestion?

12. Like the serine proteases (see Section 6-4), pepsin is made as a zymogen and is inactive at its site of synthesis, where the pH is 7. Pepsin becomes activated when secreted into the stomach, where it encounters a pH of ~2. Pepsinogen contains a "basic peptide" that blocks its active site at pH 7. The basic peptide dissociates from the active site at pH 2 and is cleaved, resulting in the formation of the active form of the enzyme. What amino acids residues are found in the active site of pepsin? Why does the basic

peptide bind tightly to the active site at pH 7 and why does it dissociate at the lower pH?

13. The cleavage of peptide bonds in the stomach is catalyzed both by hydrochloric acid (see Problem 10) and by the stomach enzyme pepsin. Peptide bond cleavage continues in the small intestine, catalyzed by the pancreatic enzymes trypsin and chymotrypsin. At what pH does pepsin function optimally; that is, at what pH is the V_{max} for pepsin greatest? Is the pH optimum for pepsin different from that for trypsin and chymotrypsin? Explain.

14. Scientists have recently discovered why the botulinum toxin survives the acidic environment of the stomach. The toxin forms a complex with a second nontoxic protein that acts as a shield to protect the botulinum toxin from being digested by stomach enzymes. Upon entry into the small intestine, the two proteins dissociate and the botulinum toxin is released. What is the likely interaction between the botulinum toxin and the nontoxic protein, and why does the complex form readily in the stomach but not in the small intestine?

15. Free amino acid transport from the intestinal lumen into intestinal cells requires Na^+ ions. Draw a diagram that illustrates amino acid transport into these cells.

16. In oral rehydration therapy (ORT), patients suffering from diarrhea are given a solution consisting of a mixture of glucose and electrolytes. Some formulations also contain amino acids. Why are electrolytes added to the mixture?

17. Triacylglycerol digestion begins in the stomach. Gastric lipase catalyzes hydrolysis of the fatty acid from the third glycerol carbon.

(a) Draw the reactants and products of this reaction.

(b) Conversion of the triacylglycerol to a diacylglycerol and a fatty acid promotes emulsification of fats in the stomach; that is, the products are more easily incorporated into micelles. Explain why.

18. Most of the fatty acids produced in the reaction described in Problem 17 form micelles and are absorbed as such, but a small percentage of fatty acids are free and are transported into the intestinal epithelial cells without the need for a transport protein. Explain why a transport protein is not required.

19. The cells lining the small intestine absorb cholesterol but not cholesteryl esters. Draw the reaction catalyzed by cholesteryl esterase that produces cholesterol from cholesteryl stearate.

20. Some cholesterol is converted back to cholesteryl esters in the epithelial cells lining the small intestine (the reverse of the reaction described in Problem 19). Both cholesterol and cholesteryl esters are packaged into particles called chylomicrons, which consist of lipid and protein. Use what you know about the physical properties of cholesterol and cholesteryl esters to describe their locations in the chylomicron particle.

21. **(a)** Consider the physical properties of a polar glycogen molecule and an aggregation of hydrophobic triacylglycerols. On a per-weight basis, why is fat a more efficient form of energy storage than glycogen?

(b) Explain why there is an upper limit to the size of a glycogen molecule but there is no upper limit to the amount of triacylglycerols that an adipocyte can store.

22. Glycogen can be expanded quickly, by adding glucose residues to its many branches, and degraded quickly, by simultaneously removing glucose from the ends of these branches. Are the enzymes that catalyze these processes specific for the reducing or nonreducing ends of the glycogen polymer? Explain.

23. The phosphorolysis reaction that removes glucose residues from glycogen yields as its product glucose-1-phosphate. Glucose-1-phosphate is isomerized to glucose-6-phosphate; then the phosphate group is removed in a hydrolysis reaction. Why is it necessary to remove the phosphate group before the glucose exits the cell to enter the circulation?

24. Hydrolytic enzymes encased within the membrane-bound lysosomes all work optimally at pH ~5. This feature serves as a cellular "insurance policy" in the event of lysosomal enzyme leakage into the cytosol. Explain.

12-2 Metabolic Pathways

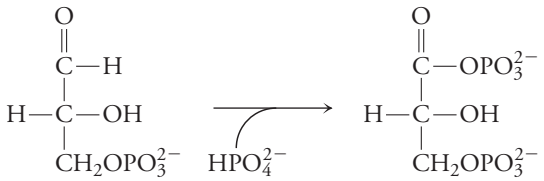
25. The common intermediates listed in the table below appear as reactants or products in several pathways. Place a checkmark in the box that indicates the appropriate pathway for each reactant.

	Glycolysis	Citric acid cycle	Fatty acid metabolism
Acetyl-CoA			
Glyceraldehyde-3-phosphate			
Pyruvate			

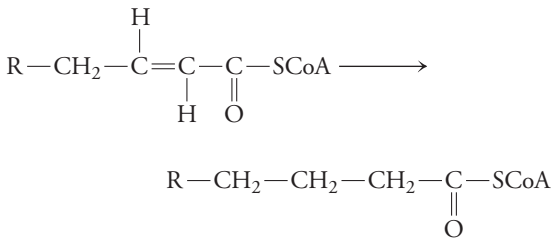
	Triacylglycerol synthesis	Photosynthesis	Transamination
Acetyl-CoA			
Glyceraldehyde-3-phosphate			
Pyruvate			

26. For each of the (unbalanced) reactions shown below, tell whether the reactant is being oxidized or reduced.

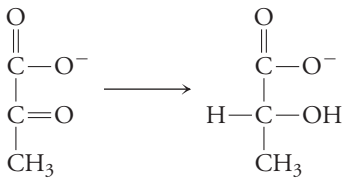
(a) A reaction from the catabolic glycolytic pathway



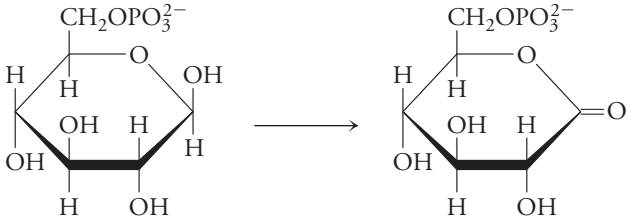
(b) A reaction from the fatty acid synthesis pathway



(c) A reaction associated with the catabolic glycolytic pathway



(d) A reaction associated with the anabolic pentose phosphate pathway



27. For each of the reactions shown in Problem 26, identify the cofactor as NAD^+, $NADP^+$, NADH, or NADPH.

28. A potential way to reduce the concentration of methane, a greenhouse gas, is to take advantage of sulfate-reducing bacteria. **(a)** Complete the chemical equation for methane consumption by these organisms:

$$CH_4 + SO_4^{2-} \rightarrow \_\_\_\_\_ + HS^- + H_2O$$

Identify the reaction component that undergoes **(b)** oxidation and **(c)** reduction.

29. Vitamin B_{12} is synthesized by certain gastrointestinal bacteria and is also found in foods of animal origin such as meat, milk, eggs, and fish. When vitamin B_{12}–containing foods are consumed, the vitamin is released from the food and binds to a salivary vitamin B_{12}–binding protein called haptocorrin. The haptocorrin–vitamin B_{12} complex passes from the stomach to the small intestine, where the vitamin is released from the haptocorrin and then binds to intrinsic factor (IF). The IF–vitamin B_{12} complex then enters the cells lining the intestine by receptor-mediated endocytosis. Using this information, make a list of individuals most at risk for vitamin B_{12} deficiency.

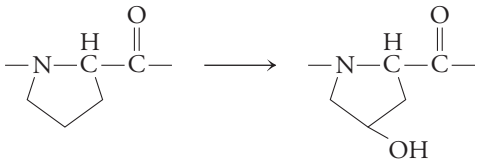
30. Hartnup disease is a hereditary disorder caused by a defective transporter for nonpolar amino acids.
(a) The symptoms of the disease (photosensitivity and neurological abnormalities) can be prevented through dietary adjustments. What sort of diet would be effective?
(b) Patients with Hartnup disease often exhibit pellagra-like symptoms. Explain.

31. A vitamin K–dependent carboxylase enzyme catalyzes the γ-carboxylation of specific glutamate residues in blood coagulation proteins.
(a) Draw the structure of a γ-carboxyglutamate residue.
(b) Why does this post-translational modification assist coagulation proteins in binding the Ca^{2+} ions required for blood clotting?

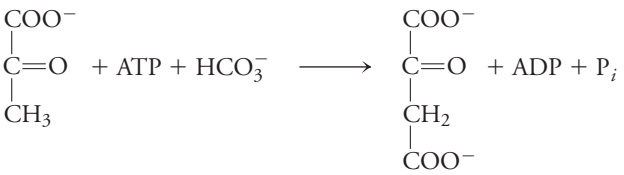
32. Would you expect vitamin A to be more easily absorbed from raw or from cooked carrots? Explain.

33. Refer to Table 12-2 and identify the vitamin required to accomplish each of the following reactions:

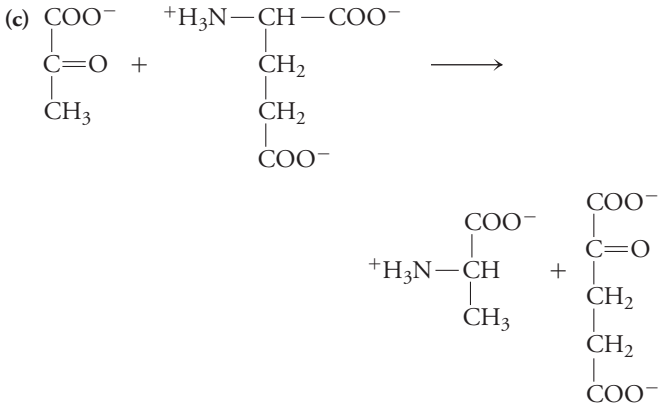
(a)



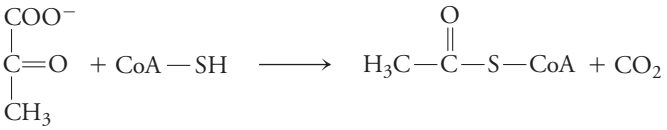
(b)



(c)



(d)



34. Why is niacin technically not a vitamin?

12-3 Free Energy Changes in Metabolic Reactions

35. Consider two reactions: A $\rightleftharpoons$ B and C $\rightleftharpoons$ D. K_{eq} for the A $\rightleftharpoons$ B reaction is 10, and K_{eq} for the C $\rightleftharpoons$ D reaction is 0.1. You place 1 mM A in tube 1 and 1 mM C in tube 2 and allow the reactions to reach equilibrium. Without doing any calculations, determine whether the concentration of B in tube 1 will be greater than or less than the concentration of D in tube 2.

36. Calculate the $\Delta G^{\circ\prime}$ values for the reactions described in Problem 35. Assume a temperature of 37°C.

37. For the reaction E $\rightleftharpoons$ F, $K_{eq} = 1$.
(a) Without doing any calculations, what can you conclude about the $\Delta G^{\circ\prime}$ value for the reaction?
(b) You place 1 mM F in a tube and allow the reaction to reach equilibrium. Determine the final concentrations of E and F.

38. Refer to the hypothetical reaction described in Problem 37. Determine the direction the reaction will proceed if you place 5 mM E and 2 mM F in a test tube. What are the final concentrations of E and F?

39. Calculate the ΔG value for the A $\rightleftharpoons$ B reaction described in Problem 35 when the concentrations of A and B are 0.9 mM and 0.1 mM, respectively. In which direction will the reaction proceed?

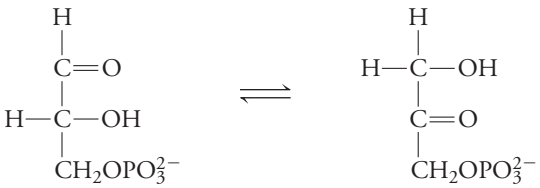
40. Calculate the ΔG value for the C $\rightleftharpoons$ D reaction described in Problem 35 when the concentrations of C and D are 0.9 mM and 0.1 mM, respectively. In which direction will the reaction proceed?

41. **(a)** The $\Delta G^{\circ\prime}$ value for a hypothetical reaction is 10 kJ · mol^{-1}. Compare the K_{eq} for this reaction with the K_{eq} for a reaction whose $\Delta G^{\circ\prime}$ value is twice as large.
(b) Carry out the same exercise for a hypothetical reaction whose $\Delta G^{\circ\prime}$ value is −10 kJ · mol^{-1}.

42. Use the standard free energies provided in Table 12-4 to calculate the $\Delta G^{\circ\prime}$ for the isomerization of glucose-1-phosphate to glucose-6-phosphate.
(a) Is this reaction spontaneous under standard conditions?
(b) Is the reaction spontaneous when the concentration of glucose-6-phosphate is 5 mM and the concentration of glucose-1-phosphate is 0.1 mM?

43. Calculate ΔG for the hydrolysis of ATP under cellular conditions, where [ATP] = 3 mM, [ADP] = 1 mM, and [P_i] = 5 mM.

44. The standard free energy change for the reaction catalyzed by triose phosphate isomerase is 7.9 kJ · mol^{-1}.



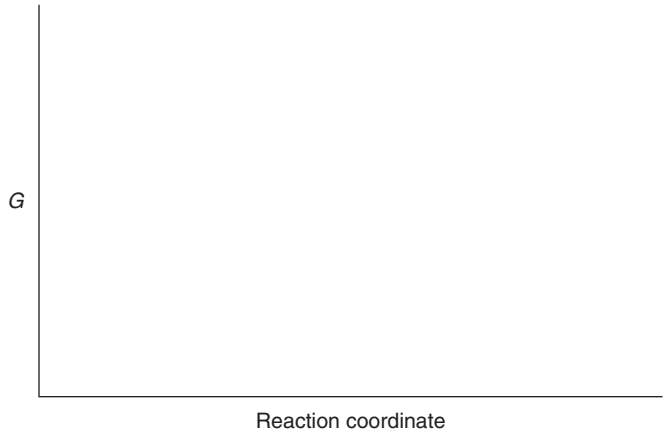
Glyceraldehyde-3-phosphate ⇌ Dihydroxyacetone phosphate

(a) Calculate the equilibrium constant for the reaction.
(b) Calculate ΔG at 37°C when the concentration of glyceraldehyde-3-phosphate is 0.1 mM and the concentration of dihydroxyacetone phosphate is 0.5 mM.
(c) Is the reaction spontaneous under these conditions? Would the reverse reaction be spontaneous?

45. An apple contains about 72 Calories. Express this quantity in terms of ATP equivalents (that is, how many ATP → ADP + P_i reactions?).

46. A large hot chocolate with whipped cream purchased at a national coffee chain contains 760 calories. Express this quantity in terms of ATP equivalents (see Problem 45).

47. Use the graph below to sketch the free energy changes for **(a)** the glucose + P_i → glucose-6-phosphate reaction, **(b)** the ATP + H_2O → ADP + P_i reaction, and **(c)** the coupled reaction (see Section 12-3).



48. Some studies (but not all) show that creatine supplementation increases performance in high-intensity exercises lasting less than 30 seconds. Would you expect creatine supplements to affect endurance exercise?

49. The $\Delta G^{\circ\prime}$ for the hydrolysis of ATP under standard conditions at pH 7 and in the presence of magnesium ions is −30.5 kJ · mol^{-1}.
(a) How would this value change if ATP hydrolysis was carried out at a pH of less than 7? Explain.
(b) How would this value change if magnesium ions were not present?

50. The $\Delta G^{\circ\prime}$ for the formation of UDP–glucose from glucose-1-phosphate and UTP is about zero. Yet the production of UDP–glucose is highly favorable. What is the driving force for this reaction?

glucose-1-phosphate + UTP ⇌ UDP–glucose + PP_i

51. **(a)** The complete oxidation of glucose releases a considerable amount of energy. The $\Delta G^{\circ\prime}$ for the reaction shown below is −2850 kJ · mol^{-1}.

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$$

How many moles of ATP could be produced under standard conditions from the oxidation of one mole of glucose, assuming about 33% efficiency?
(b) The oxidation of palmitate, a 16-carbon saturated fatty acid, releases 9781 kJ · mol^{-1}.

$$C_{16}H_{32}O_2 + 23\ O_2 \rightarrow 16\ CO_2 + 16\ H_2O$$

How many moles of ATP could be produced under standard conditions from the oxidation of one mole of palmitate, assuming 33% efficiency?
(c) Calculate the number of ATP molecules produced per carbon for glucose and palmitate. Explain the reason for the difference.

52. A moderately active adult female weighing 125 pounds must consume 2200 Calories of food daily.
(a) If this energy is used to synthesize ATP, calculate the number of moles of ATP that would be synthesized each day under standard conditions (assuming 33% efficiency).
(b) Calculate the number of grams of ATP that would be synthesized each day. The molar mass of ATP is 505 g · mol^{-1}. What is the mass of ATP in pounds? (2.2 kg = 1 lb)
(c) There is approximately 40 g of ATP in the adult 125-lb female. Considering this fact and your answer to part (b), suggest an explanation that is consistent with these findings.

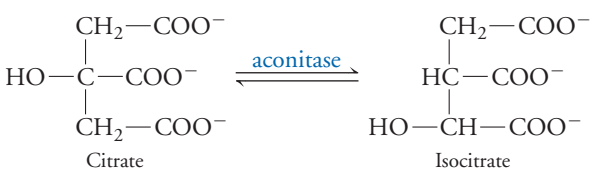
53. Calculate how many apples (see Solution 45) would be required to provide the amount of ATP calculated in Problem 52.

54. Calculate how many large hot chocolate drinks (see Solution 46) would be required to provide the amount of ATP calculated in Problem 52.

55. Which of the compounds listed in Table 12-4 could be involved in a reaction coupled to the synthesis of ATP from ADP + P_i?

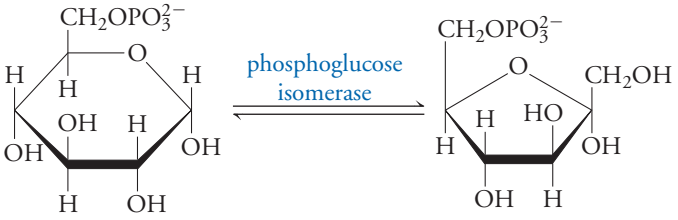
56. Which of the compounds listed in Table 12-4 could be involved in a reaction coupled to the hydrolysis of ATP to ADP + P_i?

57. Citrate is isomerized to isocitrate in the citric acid cycle (Chapter 14). The reaction is catalyzed by the enzyme aconitase. The $\Delta G^{\circ\prime}$ of the reaction is 5 kJ · mol^{-1}. The kinetics of the reaction are studied *in vitro,* where 1 M citrate and 1 M isocitrate are added to an aqueous solution of the enzyme at 25°C.
(a) What is the K_{eq} for the reaction?
(b) What are the equilibrium concentrations of the reactant and product?
(c) What is the preferred direction of the reaction under standard conditions?
(d) The aconitase reaction is the second step of an eight-step pathway and occurs in the direction shown in the figure. How can you reconcile these facts with your answer to part (c)?



Citrate ⇌ Isocitrate

58. The equilibrium constant for the conversion of glucose-6-phosphate to fructose-6-phosphate is 0.41. The reaction is reversible and is catalyzed by the enzyme phosphoglucose isomerase.



Glucose-6-phosphate Fructose-6-phosphate

(a) What is the $\Delta G^{\circ\prime}$ for this reaction? Would this reaction proceed in the direction written under standard conditions?
(b) What is the ΔG for this reaction at 37°C when the concentration of glucose-6-phosphate is 2.0 mM and the concentration of the fructose-6-phosphate is 0.5 mM? Would the reaction proceed in the direction written under these cellular conditions?

59. The conversion of glutamate to glutamine is unfavorable. In order for this transformation to occur in the cell, it must be coupled to the hydrolysis of ATP. Consider two possible mechanisms:

$$\text{Mechanism 1: glutamate} + NH_3 \rightleftharpoons \text{glutamine}$$
$$ATP + H_2O \rightleftharpoons ADP + P_i$$

$$\text{Mechanism 2: glutamate} + ATP \rightleftharpoons \gamma\text{-glutamylphosphate} + ADP$$
$$\gamma\text{-glutamylphosphate} + H_2O + NH_3 \rightleftharpoons \text{glutamine} + P_i$$

Write the overall equation for the reaction for each mechanism. Is one mechanism more likely than the other? Or are both mechanisms equally feasible for the conversion of glutamate to glutamine? Explain.

60. The phosphorylation of glucose to glucose-6-phosphate is the first step of glycolysis (Chapter 13). The phosphorylation of glucose by phosphate is described by the following equation:

$$\text{glucose} + P_i \rightleftharpoons \text{glucose-6-phosphate} + H_2O$$
$$\Delta G^{\circ\prime} = +13.8\ \text{kJ} \cdot \text{mol}^{-1}.$$

(a) Calculate the equilibrium constant for the above reaction.
(b) What would the equilibrium concentration of glucose-6-phosphate be under cellular conditions of [glucose] = [P_i] = 5 mM if glucose was phosphorylated according to the reaction above? Does this reaction provide a feasible route for the production of glucose-6-phosphate for the glycolytic pathway?
(c) One way to increase the amount of product is to increase the concentrations of the reactants. This would decrease the mass action ratio (see Equation 12-3) and would theoretically make the reaction as written more favorable. If the cellular concentration of phosphate is 5 mM, what concentration of glucose would be required to achieve a glucose-6-phosphate concentration of 250 μM? Is this strategy physiologically feasible, given that the solubility of glucose in aqueous medium is less than 1 M?
(d) Another way to promote the formation of glucose-6-phosphate is to couple the phosphorylation of glucose to the hydrolysis of ATP as shown in Section 12-3. Calculate K_{eq} for the reaction in which glucose is converted to glucose-6-phosphate with concomitant ATP hydrolysis.
(e) When the ATP-dependent phosphorylation of glucose is carried out, what concentration of glucose is needed to achieve a 250-μM intracellular concentration of glucose-6-phosphate when the concentrations of ATP and ADP are 5.0 mM and 1.25 mM, respectively?
(f) Which route is more feasible to accomplish the phosphorylation of glucose to glucose-6-phosphate: the direct phosphorylation by P_i or the coupling of this phosphorylation to ATP hydrolysis? Explain.

61. Fructose-6-phosphate is phosphorylated to fructose-1,6-bisphosphate as part of the glycolytic pathway. The phosphorylation of fructose-6-phosphate by phosphate is described by the following equation:

$$\text{fructose-6-phosphate} + P_i \rightleftharpoons \text{fructose-1,6-bisphosphate}$$
$$\Delta G^{\circ\prime} = 47.7\ \text{kJ} \cdot \text{mol}^{-1}.$$

(a) What is the ratio of fructose-1,6-bisphosphate to fructose-6-phosphate at equilibrium if the concentration of phosphate in the cell is 5 mM?
(b) Suppose that the phosphorylation of fructose-6-phosphate is coupled to the hydrolysis of ATP.

$$ATP + H_2O \rightleftharpoons ADP + P_i \qquad \Delta G^{\circ\prime} = -30.5\ \text{kJ} \cdot \text{mol}^{-1}$$

Write the new equation that describes the phosphorylation of fructose-6-phosphate coupled with ATP hydrolysis. Calculate the $\Delta G^{\circ\prime}$ for the reaction.
(c) What is the ratio of fructose-1,6-bisphosphate to fructose-6-phosphate at equilibrium for the reaction you wrote in part (b) if the equilibrium concentration of ATP = 3 mM and [ADP] = 1 mM?
(d) Write a concise paragraph that summarizes your findings above.
(e) One can envision two mechanisms for coupling ATP hydrolysis to the phosphorylation of fructose-6-phosphate, yielding the same overall reaction:

Mechanism 1: ATP is hydrolyzed as fructose-6-phosphate is transformed to fructose-1,6-bisphosphate:

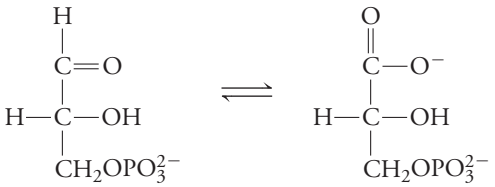
$$\text{fructose-6-phosphate} + P_i \rightleftharpoons \text{fructose-1,6-bisphosphate}$$
$$ATP + H_2O \rightleftharpoons ADP + P_i$$

Mechanism 2: ATP transfers its γ-phosphate directly to fructose-6-phosphate in one step, producing fructose-1,6-bisphosphate.

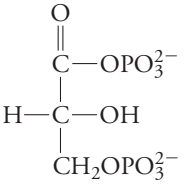
$$\text{fructose-6-phosphate} + ATP + H_2O \rightleftharpoons \text{fructose-1,6-bisphosphate} + ADP$$

Choose one of the above mechanisms as the more biochemically feasible and provide a rationale for your choice.

62. Glyceraldehyde-3-phosphate (GAP) is eventually converted to 3-phosphoglycerate (3PG) in the glycolytic pathway.



Glyceraldehyde-3-phosphate 3-Phosphoglycerate



1,3-Bisphosphoglycerate (1,3-BPG)

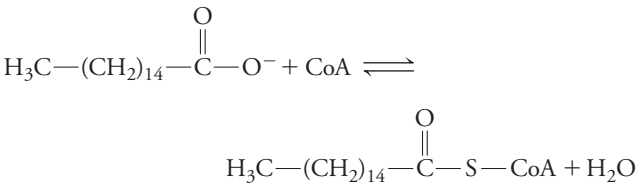
Consider these two scenarios:

I. GAP is oxidized to 1,3-BPG ($\Delta G^{\circ\prime} = 6.7$ kJ · mol^{-1}), which is subsequently hydrolyzed to yield 3PG ($\Delta G^{\circ\prime} = -49.3$ kJ · mol^{-1})

II. GAP is oxidized to 1,3-BPG, which then transfers its phosphate to ADP, yielding ATP ($\Delta G^{\circ\prime} = -18.8$ kJ · mol^{-1}).

Write the overall equations for the two scenarios. Which is more likely to occur in the cell, and why?

63. Palmitate is activated in the cell by forming a thioester bond to coenzyme A. The $\Delta G^{\circ\prime}$ for the synthesis of palmitoyl-CoA from palmitate and coenzyme A is 31.5 kJ · mol^{-1}.



(a) What is the ratio of products to reactants at equilibrium for the reaction? Is the reaction favorable? Explain.
(b) Suppose the synthesis of palmitoyl-CoA were coupled with ATP hydrolysis. The standard free energy for the hydrolysis of the ATP to ADP is listed in Table 12-4. Write the new equation for the activation of palmitate when coupled with ATP hydrolysis to ADP. Calculate $\Delta G^{\circ\prime}$ for the reaction. What is the ratio of products to reactants at equilibrium for the reaction? Is the reaction favorable? Compare your answer to the answer you obtained in part (a).
(c) Suppose the reaction described in part (a) were coupled with ATP hydrolysis to AMP; the standard free energy for the hydrolysis of ATP to AMP is listed in Table 12-4. Write the new equation for the activation of palmitate when coupled with ATP hydrolysis to AMP. Calculate $\Delta G^{\circ\prime}$ for the reaction. What is the ratio of products to reactants at equilibrium for the reaction? Is the reaction favorable? Compare your answer to the answer you obtained in part (b).
(d) Pyrophosphate, PP_i, is hydrolyzed to 2 P_i, as shown in Table 12-4. The activation of palmitate, as described in part (c), is coupled to the hydrolysis of pyrophosphate. Write the equation for this coupled reaction and calculate the $\Delta G^{\circ\prime}$. What is the ratio of products to reactants at equilibrium for the reaction? Is the reaction favorable? Compare your answer to the answers you obtained in parts (b) and (c).

64. DNA containing broken phosphodiester bonds ("nicks") can be repaired by the action of a ligase enzyme. Formation of a new phosphodiester bond in DNA requires the free energy of ATP phosphoanhydride bond cleavage. In the ligase-catalyzed reaction, ATP is hydrolyzed to AMP:

$$\text{ATP} + \text{nicked bond} \xrightleftharpoons{\text{ligase}} \text{AMP} + PP_i + \text{phosphodiester bond}$$

The equilibrium constant expression for this reaction can be rearranged to define a constant, C, as follows:

$$K_{eq} = \frac{[\text{phosphodiester bond}][\text{AMP}][PP_i]}{[\text{nick}][\text{ATP}]}$$

$$\frac{[\text{nick}]}{[\text{phosphodiester bond}]} = \frac{[\text{AMP}][PP_i]}{K_{eq}[\text{ATP}]}$$

$$C = \frac{[PP_i]}{K_{eq}[\text{ATP}]}$$

$$\frac{[\text{nick}]}{[\text{phosphodiester bond}]} = C[\text{AMP}]$$

Researchers have determined the ratio of nicked bonds to phosphodiester bonds at various concentrations of AMP.

(a) Using the data provided, construct a plot of [nick]/[phosphodiester bond] versus [AMP] and determine the value of C from the plot.

[AMP] (mM)	[nick]/[phosphodiester bond]
10	4.0×10^{-5}
15	4.3×10^{-5}
20	5.47×10^{-5}
25	6.67×10^{-5}
30	8.67×10^{-5}
35	9.47×10^{-5}
40	9.30×10^{-5}
45	1.0×10^{-4}
50	1.13×10^{-4}

(b) Determine the value of K_{eq} for the reaction, given that the concentrations of PP_i and ATP were held constant at 1.0 mM and 14 μM, respectively.
(c) What is the value of $\Delta G^{\circ\prime}$ for the reaction?
(d) What is the value of $\Delta G^{\circ\prime}$ for the following reaction?

$$\text{nicked bond} \rightleftharpoons \text{phosphodiester bond}$$

Note that the $\Delta G^{\circ\prime}$ for the hydrolysis of ATP to AMP and PP_i is -48.5 kJ · mol^{-1} in the presence of 10 mM Mg^{2+}, the conditions used in these experiments.
(e) The $\Delta G^{\circ\prime}$ for the hydrolysis of a typical phosphomonoester to yield P_i and an alcohol is -13.8 kJ · mol^{-1}. Compare the stability of the phosphodiester bond in DNA to the stability of a typical phosphomonoester bond.

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Hanson, R. W., The role of ATP in metabolism, *Biochem. Ed.* **17,** 86–92 (1989). [Provides an excellent explanation of why ATP is an energy transducer rather than an energy store.]

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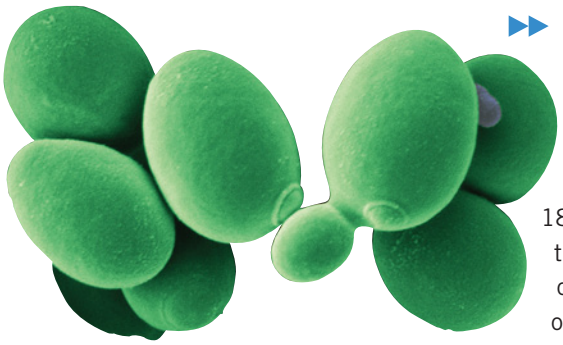
chapter 13

GLUCOSE METABOLISM



[SciMAT/Photo Researchers, Inc.]

▸▸ HOW do yeast transform sugars into other substances?

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

THIS CHAPTER IN CONTEXT

Part 1 Foundations

Part 2 Molecular Structure and Function

Part 3 Metabolism

13 Glucose Metabolism

Part 4 Genetic Information

Do You Remember?

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

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

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

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

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

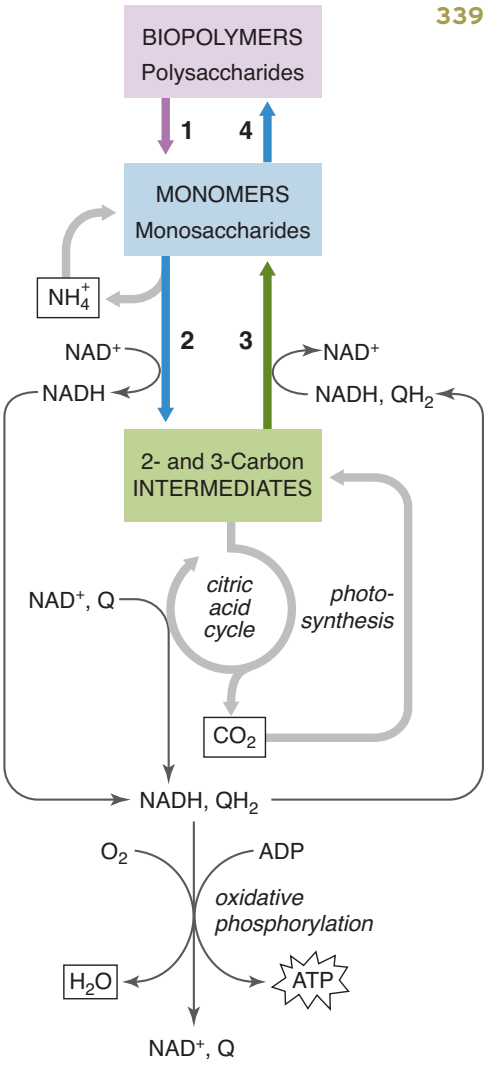


Figure 13-1 Glucose metabolism in context. (1) The polysaccharide glycogen is degraded to glucose, which is then catabolized by the glycolytic pathway (2) to the three-carbon intermediate pyruvate. Gluconeogenesis (3) is the pathway for the synthesis of glucose from smaller precursors. Glucose can then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

13-1 Glycolysis

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

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

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

KEY CONCEPTS

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

See Guided Exploration. Glycolysis overview.

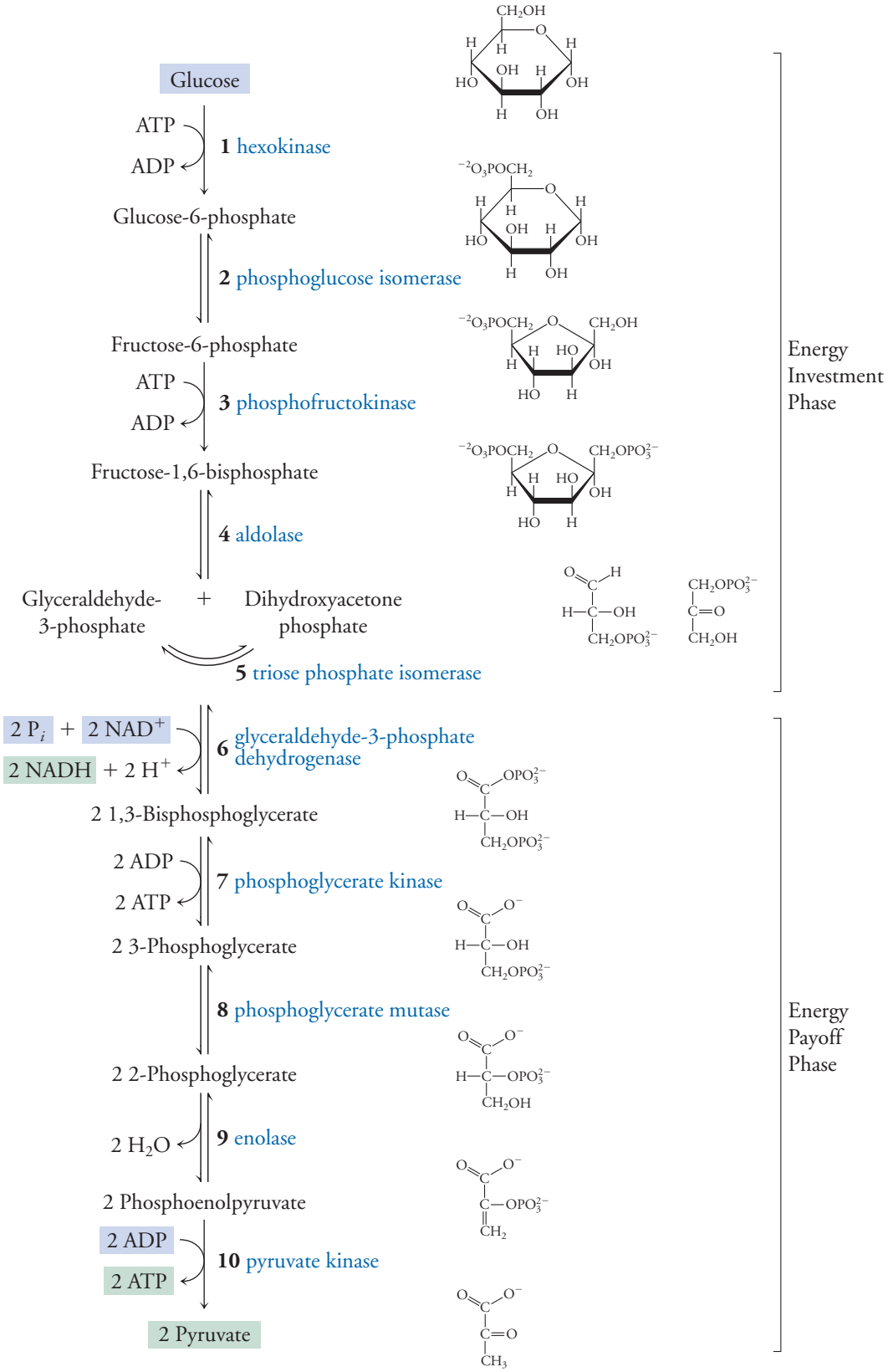


Figure 13-2 The reactions of glycolysis. The substrates, products, and enzymes corresponding to the 10 steps of the pathway are shown. Shading indicates the substrates (purple) and products (green) of the pathway as a whole. ⊕ **See Animated Figure. Overview of glycolysis.**

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

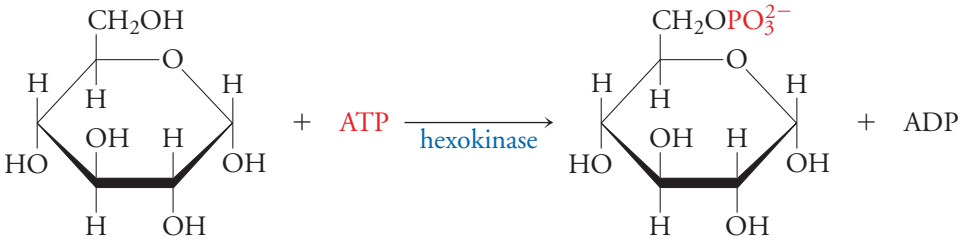
As you examine each of the reactions of glycolysis described in the following pages, note how the reaction substrates are converted to products by the action of an enzyme (and note how the enzyme's name often reveals its purpose). Pay attention also to the free energy change of each reaction.

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

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

1. Hexokinase

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

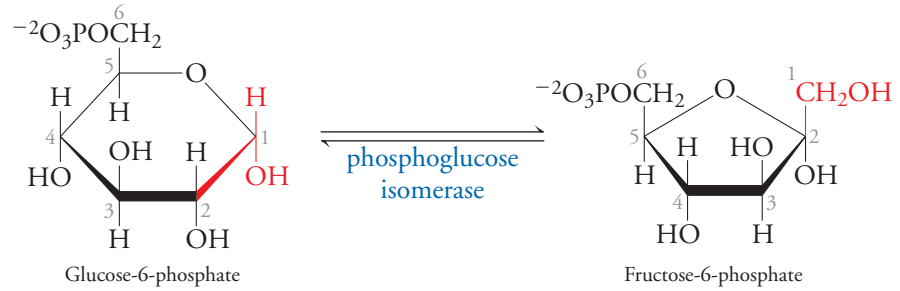


A **kinase** is an enzyme that transfers a phosphoryl group from ATP (or another nucleoside triphosphate) to another substance.

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

2. Phosphoglucose Isomerase

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



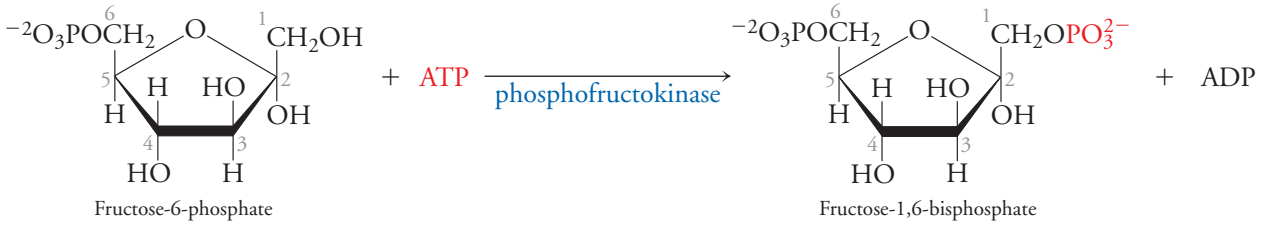
Because fructose is a six-carbon ketose (Section 11-1), it forms a five-membered ring.

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

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

3. Phosphofructokinase

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

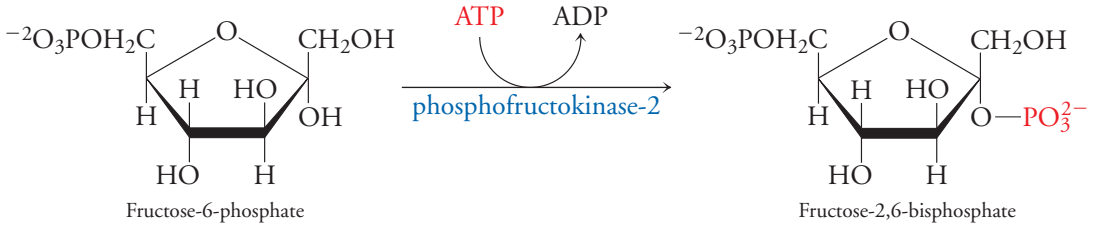


Fructose-6-phosphate — Fructose-1,6-bisphosphate

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

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

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



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

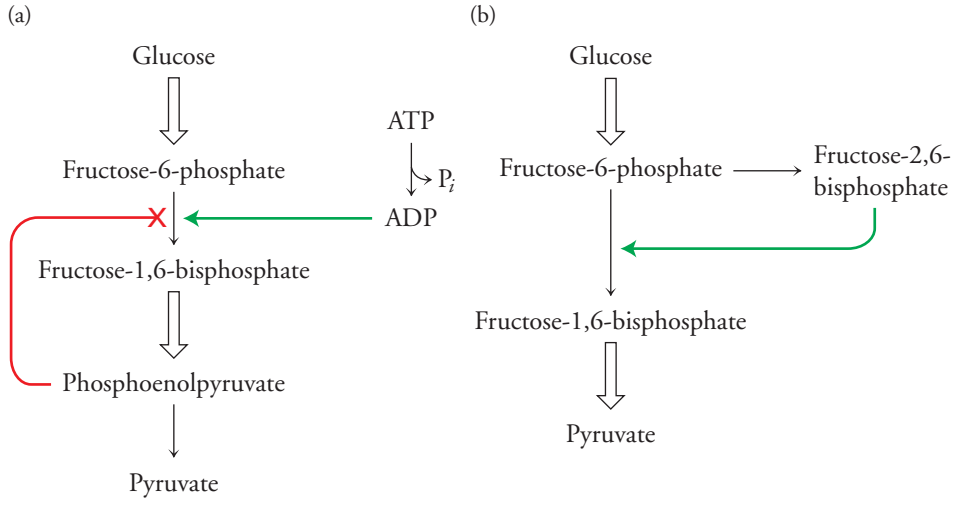


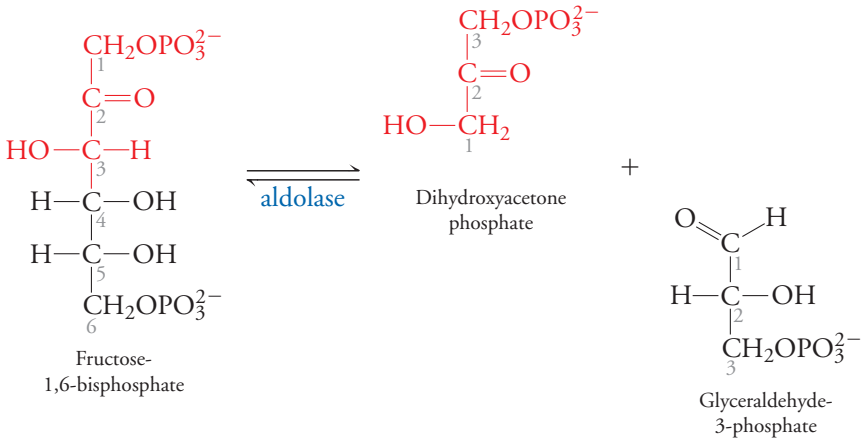
Figure 13-3 Regulation of phosphofructokinase. (a) Regulation in bacteria. ADP, produced when ATP is consumed elsewhere in the cell, stimulates the activity of phosphofructokinase (green arrow). Phosphoenolpyruvate, a late intermediate of glycolysis, inhibits phosphofructokinase (red symbol), thereby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

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

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

4. Aldolase

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

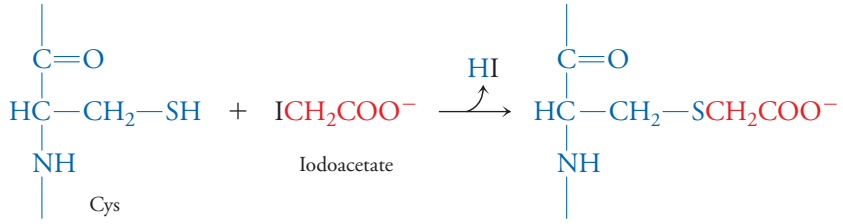


Fructose-1,6-bisphosphate

Glyceraldehyde-3-phosphate

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

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



Cys

Iodoacetate

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

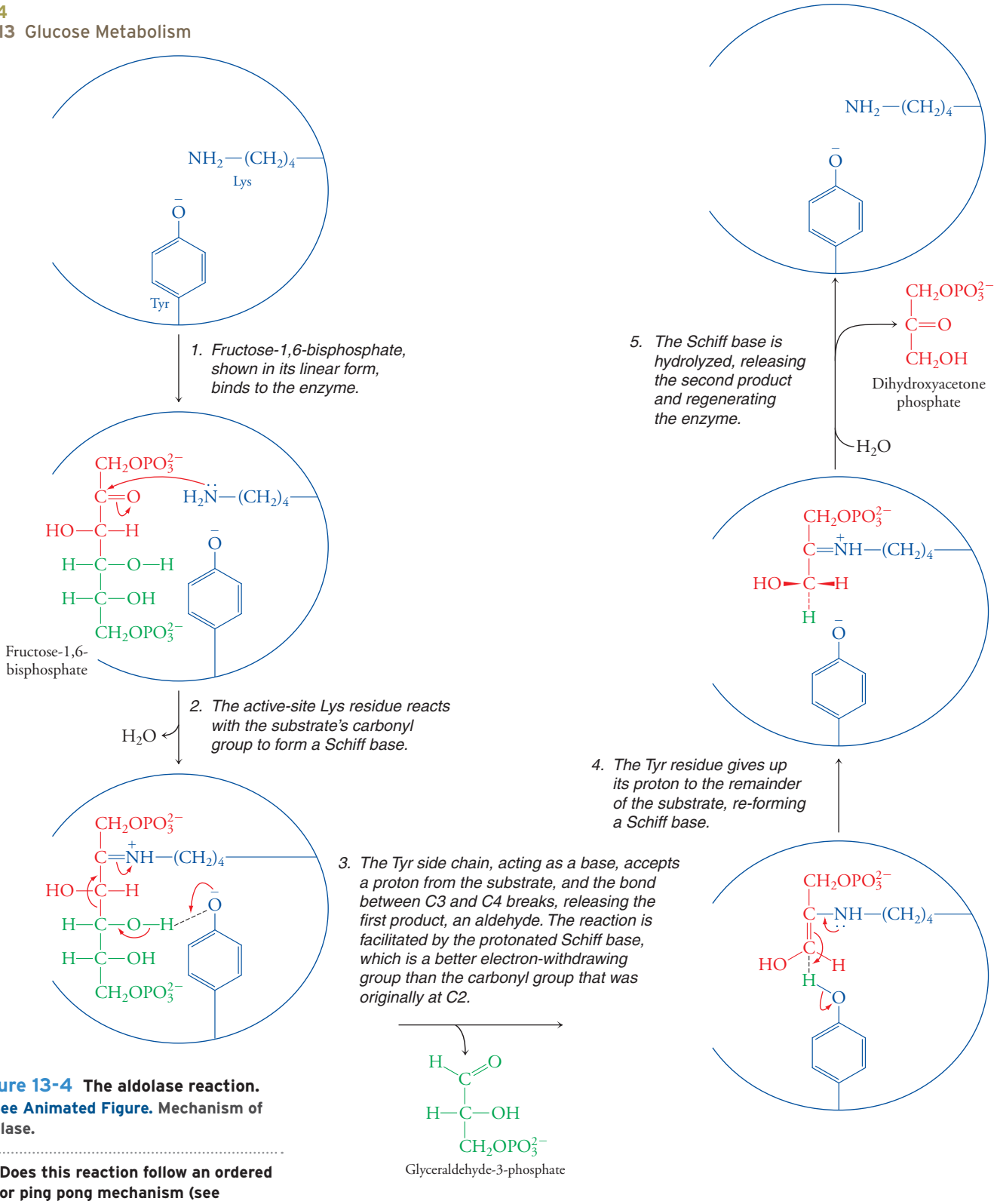


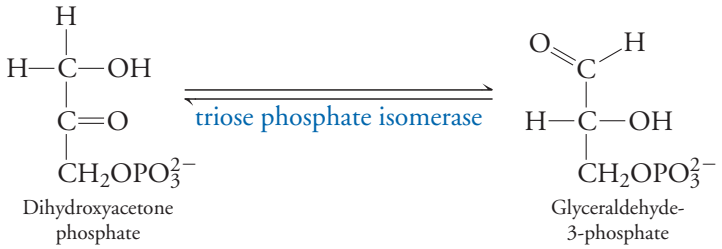
Figure 13-4 **The aldolase reaction.** **See Animated Figure.** **Mechanism of aldolase.**

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

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

5. Triose Phosphate Isomerase

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

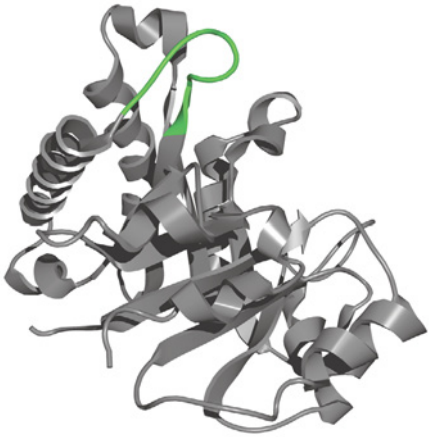


Dihydroxyacetone phosphate

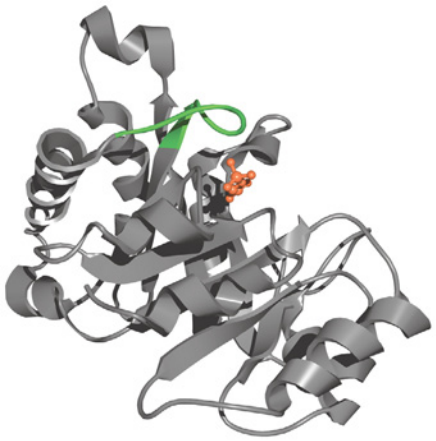
Glyceraldehyde-3-phosphate

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

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



(a)



(b)

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

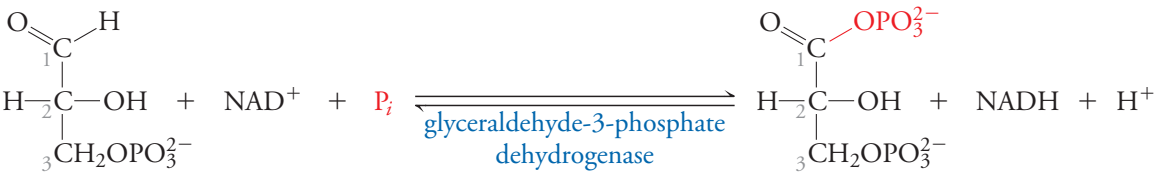
Reactions 6–10 are the energy-payoff phase of glycolysis

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

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

6. Glyceraldehyde-3-Phosphate Dehydrogenase

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



Glyceraldehyde-3-phosphate

1,3-Bisphosphoglycerate

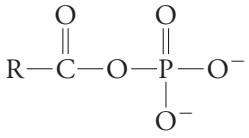
Unlike the kinases that catalyze Reactions 1 and 3, glyceraldehyde-3-phosphate dehydrogenase does not use ATP as a phosphoryl-group donor; it adds inorganic

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

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

7. Phosphoglycerate Kinase

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



An acyl phosphate

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

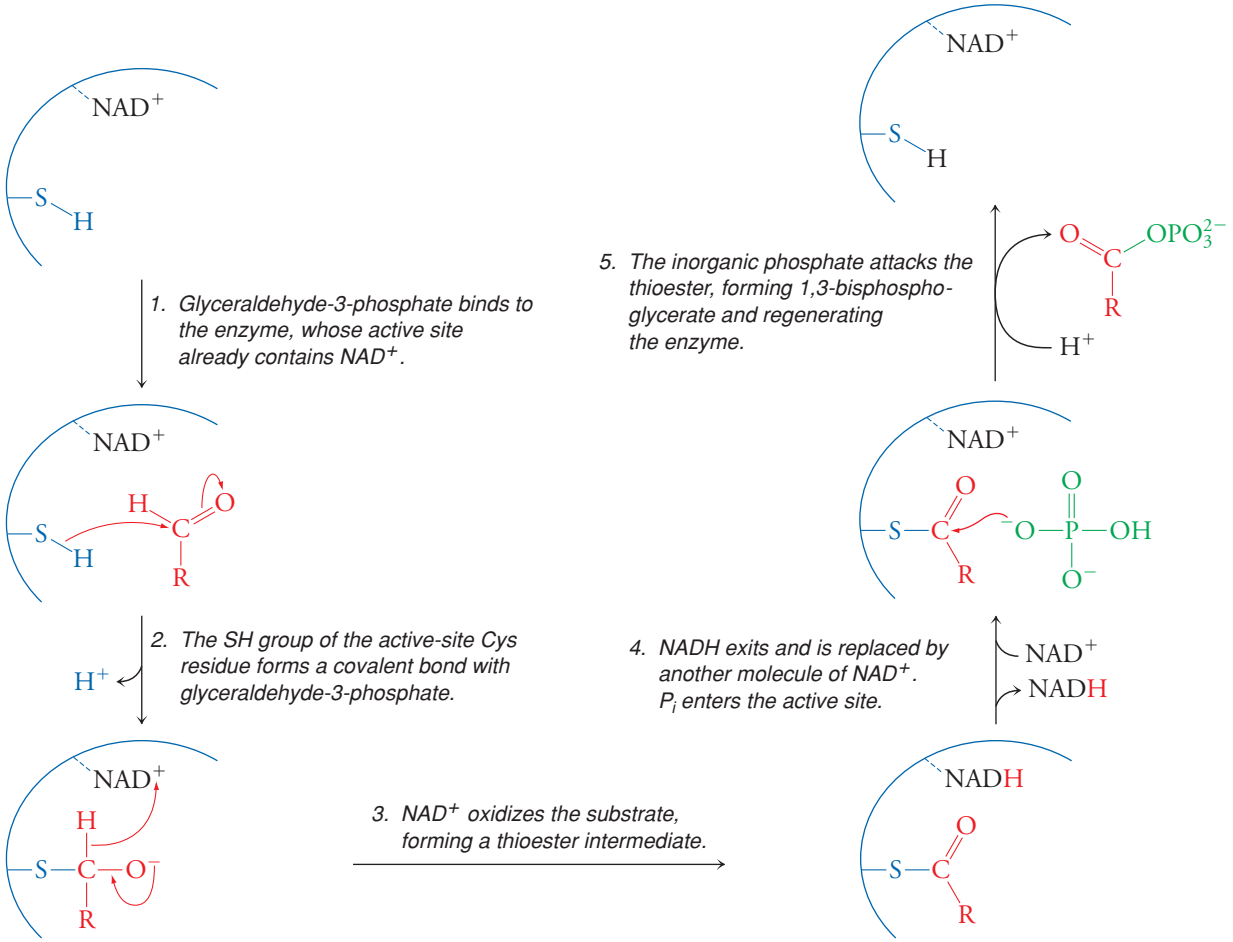
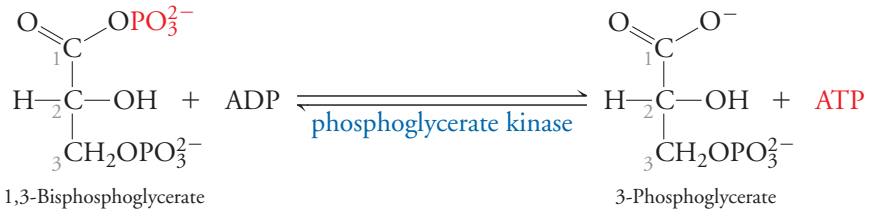


Figure 13-6 **The glyceraldehyde-3-phosphate dehydrogenase reaction.** ⊕ **See Animated Figure. Mechanism of glyceraldehyde-3-phosphate dehydrogenase.**

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

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



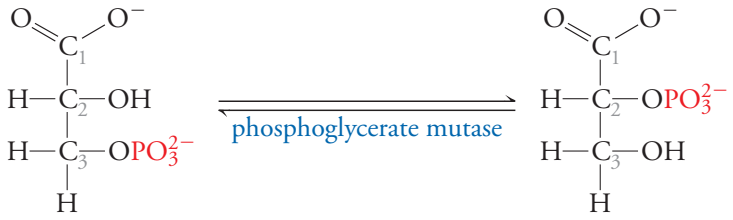
1,3-Bisphosphoglycerate — 3-Phosphoglycerate

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

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

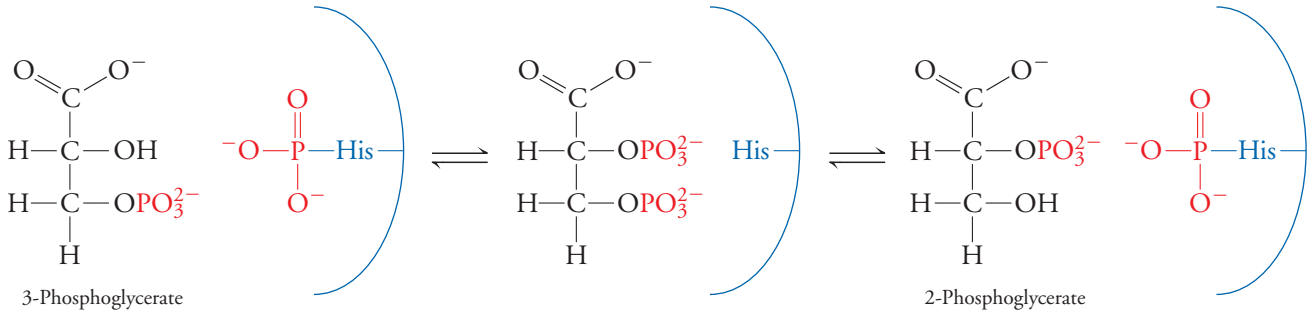
8. Phosphoglycerate Mutase

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



3-Phosphoglycerate — 2-Phosphoglycerate

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

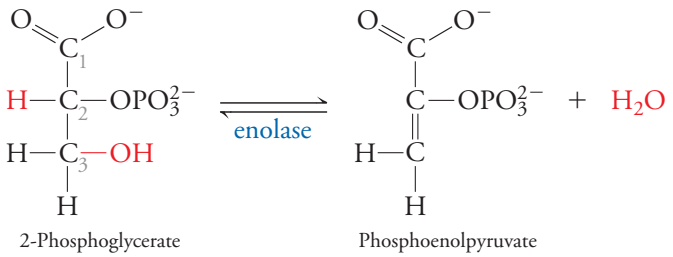


3-Phosphoglycerate — 2-Phosphoglycerate

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

9. Enolase

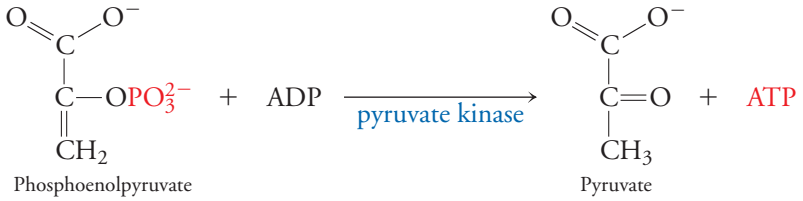
Enolase catalyzes a dehydration reaction, in which water is eliminated:



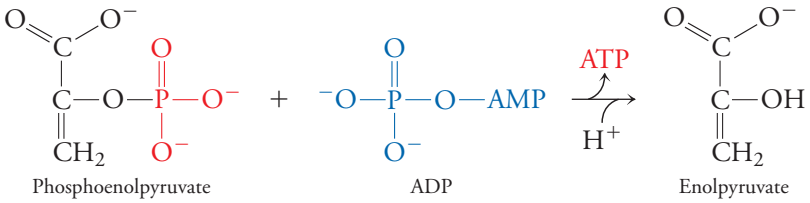
The enzyme active site includes an Mg^{2+} ion that apparently coordinates with the OH group at C3 and makes it a better leaving group. Fluoride ion and P_i can form a complex with the Mg^{2+} and thereby inhibit the enzyme. In early studies demonstrating the inhibition of glycolysis by F^-, 2-phosphoglycerate,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

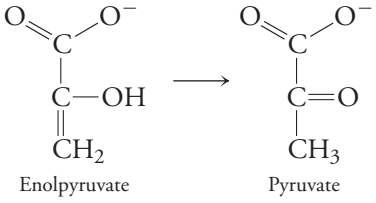
10. Pyruvate Kinase

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



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





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

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

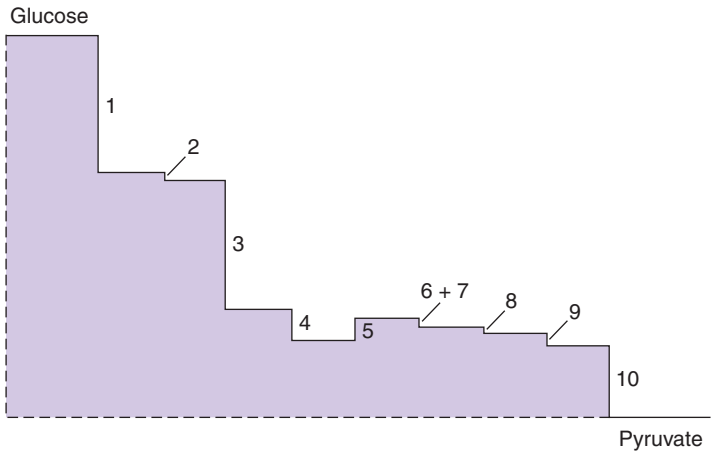


Figure 13-7 Graphical representation of the free energy changes of glycolysis. Three steps have large negative values of ΔG; the remaining steps are near equilibrium ($\Delta G \approx 0$). The height of each step corresponds to its ΔG value in heart muscle, and the numbers correspond to glycolytic enzymes.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

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

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

BOX 13-A BIOCHEMISTRY NOTE

Catabolism of Other Sugars

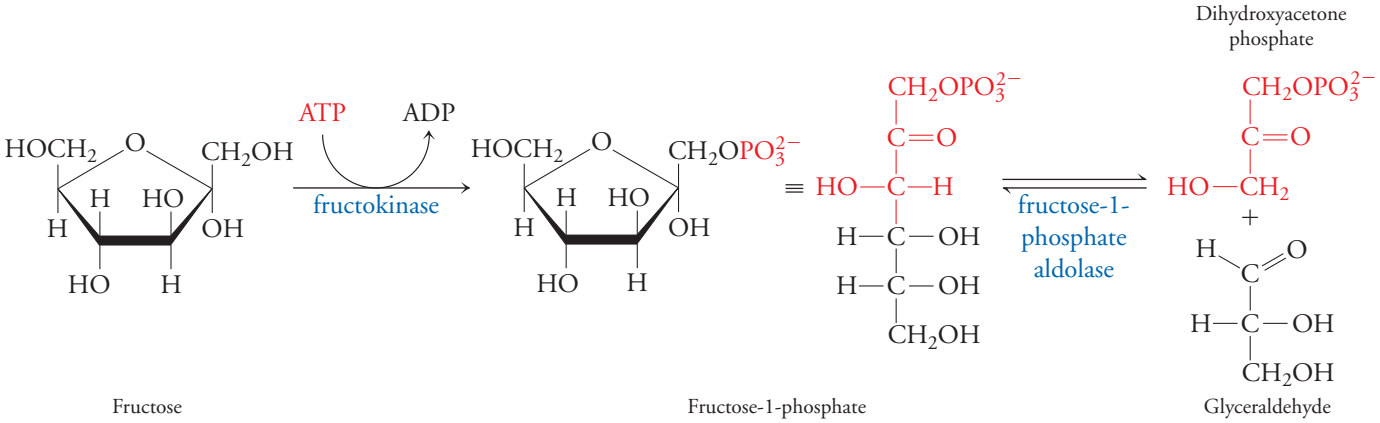
A typical human diet contains many carbohydrates other than glucose and its polymers. For example, lactose, a disaccharide of glucose and galactose, is present in milk and food derived from it (Section 11-2). Lactose is cleaved in the intestine by the enzyme lactase, and the two monosaccharides are absorbed,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

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

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

(*continued on next page*)

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



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

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

Pyruvate is converted to other substances

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

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

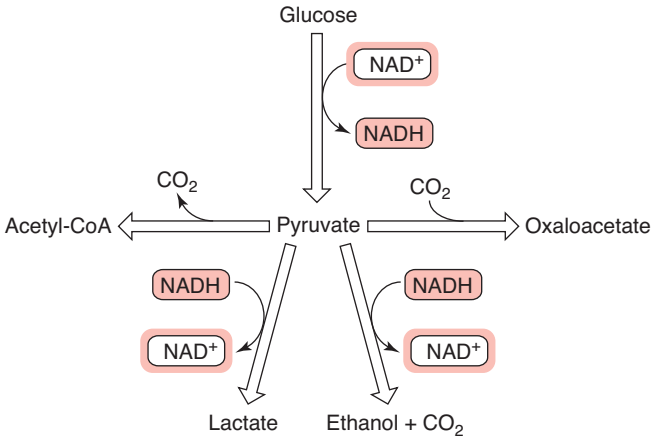
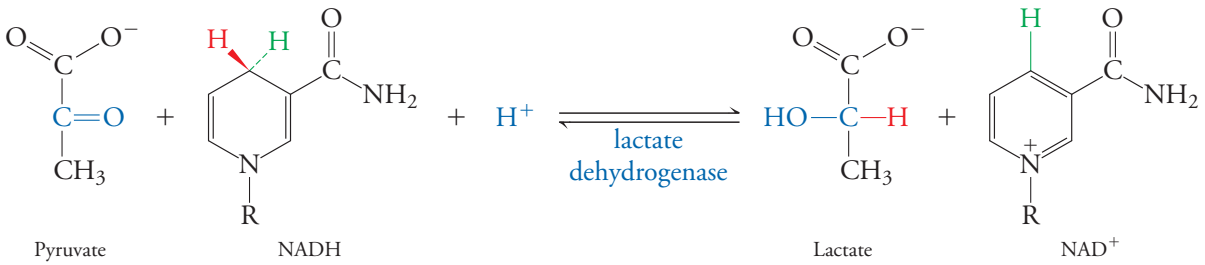


Figure 13-8 Fates of pyruvate. Pyruvate may be converted to a two-carbon acetyl group linked to the carrier coenzyme A. Acetyl-CoA may be further broken down by the citric acid cycle or used to synthesized fatty acids. In muscle, pyruvate is reduced to lactate to regenerate NAD^+ for glycolysis. Yeast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

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

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



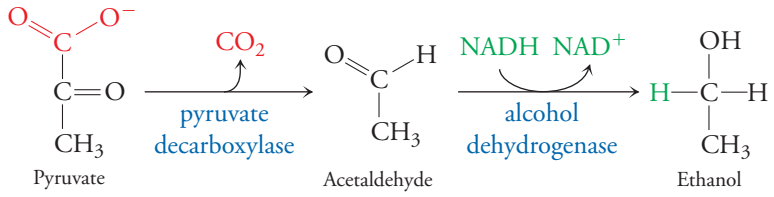
This reaction, sometimes called the eleventh step of glycolysis, allows the muscle to function anaerobically for a minute or so (see Box 12-D). The net reaction for anaerobic glucose catabolism is

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

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

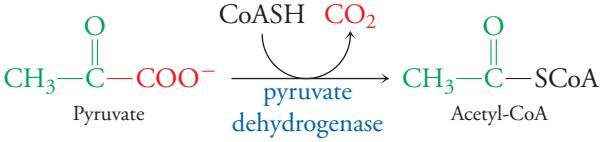
Organisms such as yeast growing under anaerobic conditions can regenerate NAD^+ by producing alcohol. In the mid-1800s, Louis Pasteur called this process **fermentation,** meaning life without air, although yeast also ferment sugars in the presence of O_2. And, to answer the question posed at the start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

▶▶ HOW do yeast transform sugars into other substances?



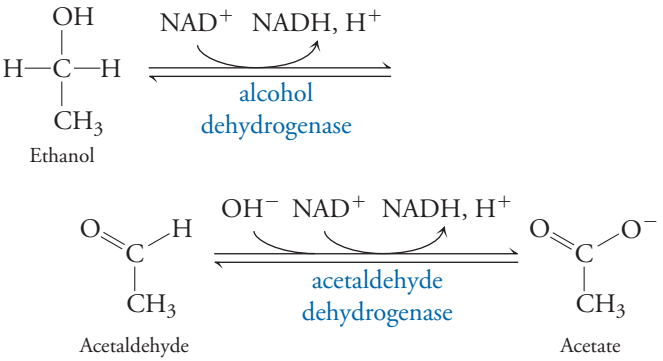
Ethanol is considered to be a waste product of sugar metabolism; its accumulation is toxic to other organisms (Box 13-B), including the yeast that produce it. This is one reason why the alcohol content of yeast-fermented beverages such as wine is limited to about 13%. "Hard" liquor must be distilled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

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



Alcohol Metabolism

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



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

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

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

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

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

Questions:

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

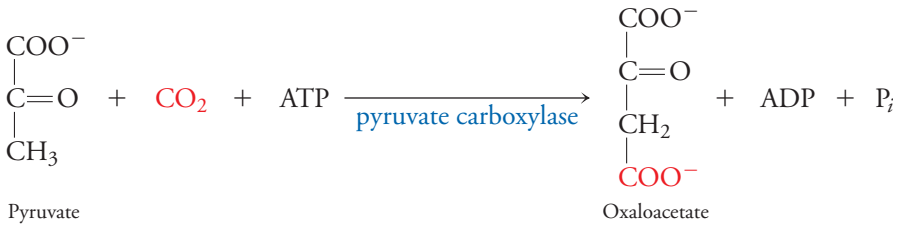
TABLE 13-1 Standard Free Energy Changes for Glucose Catabolism

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

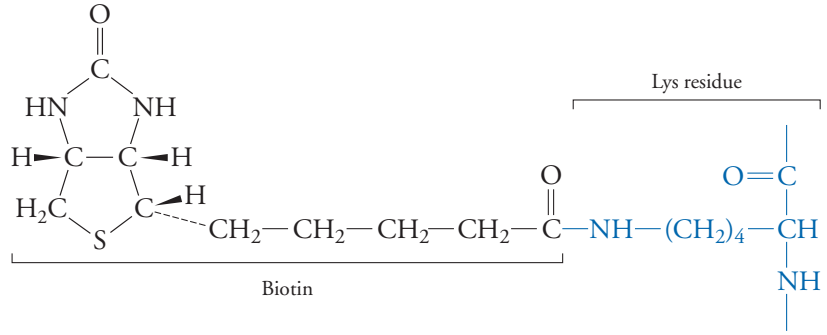
The resulting acetyl-CoA is a substrate for the citric acid cycle (Chapter 14). The complete oxidation of the six glucose carbons to CO_2 releases much more free energy than the conversion of glucose to lactate (Table 13-1). Much of this energy is recovered in the synthesis of ATP by the reactions of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

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

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



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



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

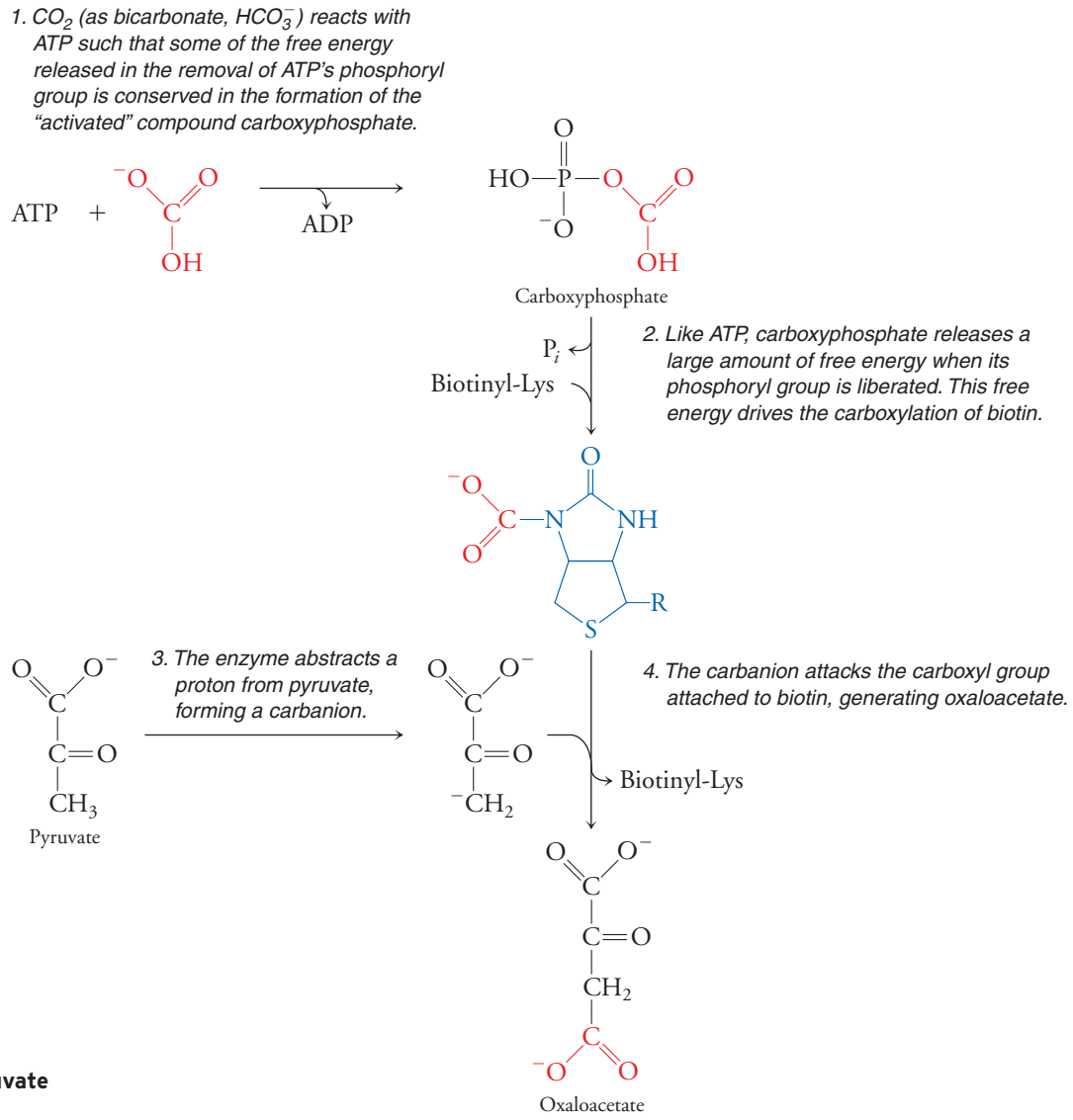


Figure 13-9 **The pyruvate carboxylase reaction.**

CONCEPT REVIEW

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

13-2 Gluconeogenesis

KEY CONCEPTS

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

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

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

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

Four gluconeogenic enzymes plus some glycolytic enzymes convert pyruvate to glucose

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

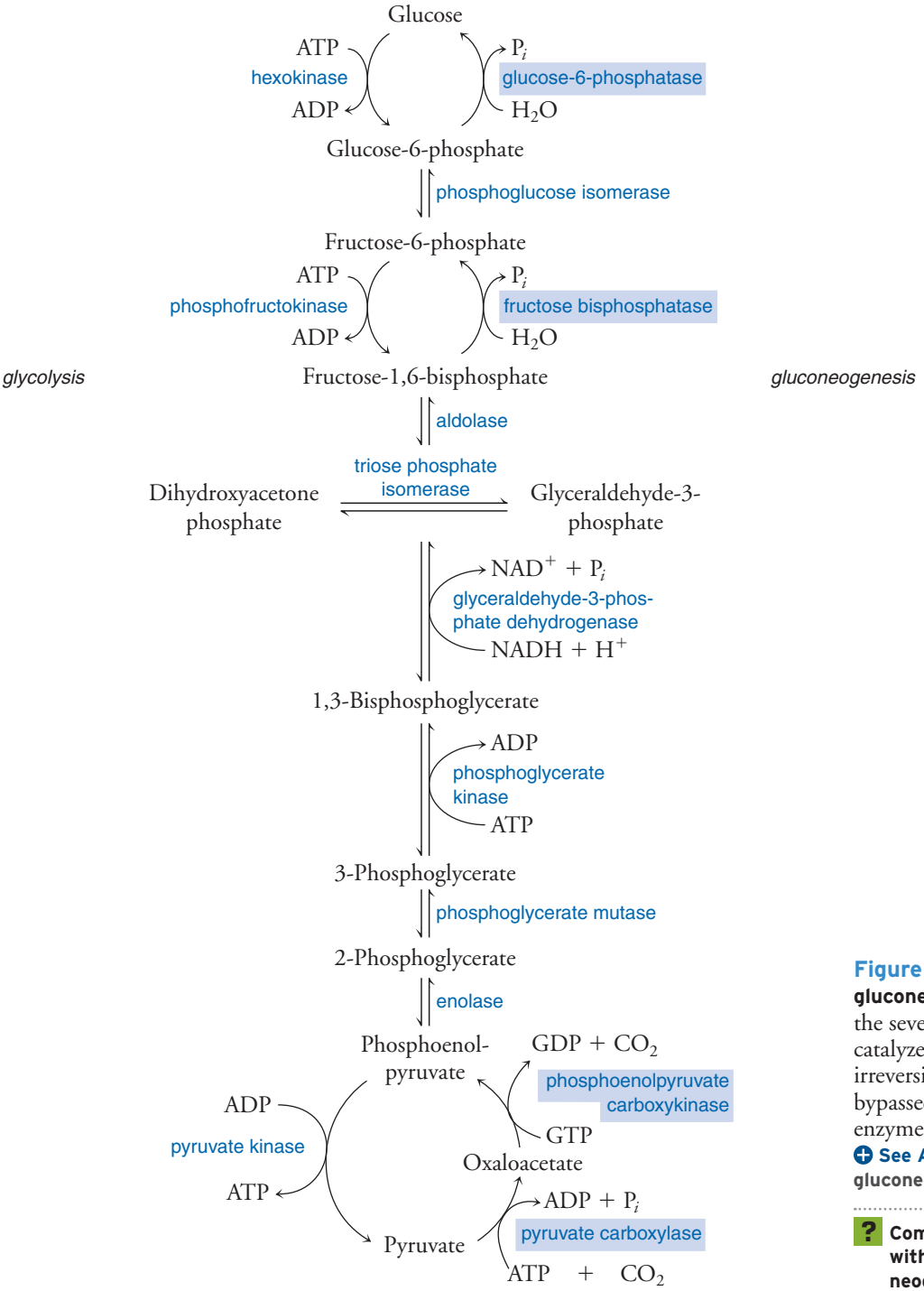
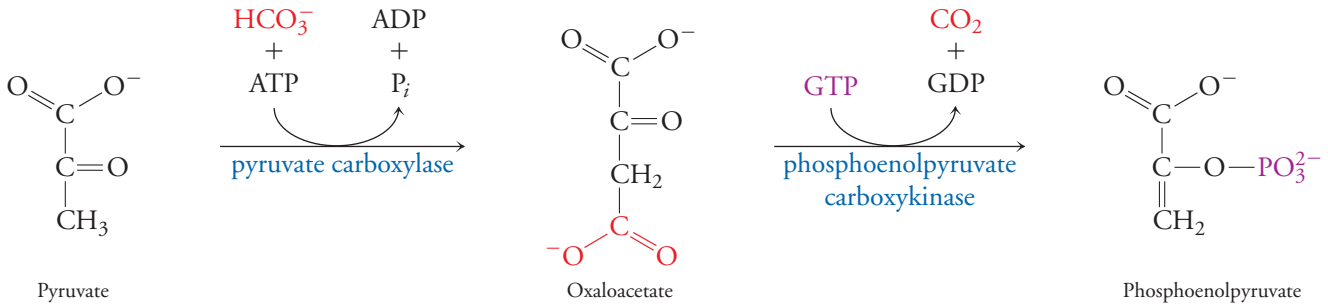


Figure 13-10 The reactions of gluconeogenesis. The pathway uses the seven glycolytic enzymes that catalyze reversible reactions. The three irreversible reactions of glycolysis are bypassed in gluconeogenesis by the four enzymes that are highlighted in blue. **See Animated Figure. Comparison of gluconeogenesis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

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

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



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

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

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

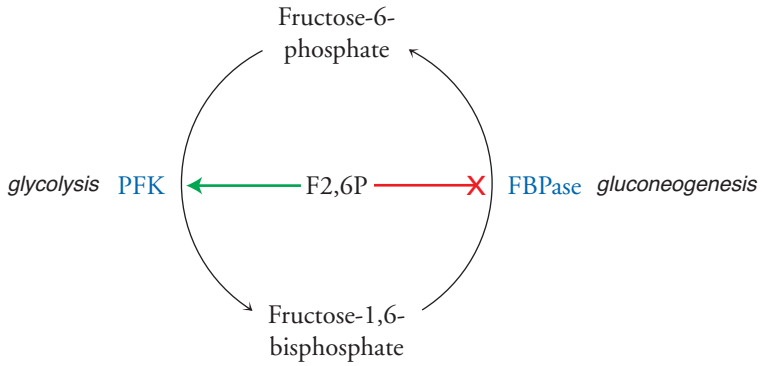
The final three reactions of gluconeogenesis require two enzymes unique to this pathway. The first step undoes the phosphofructokinase reaction, the irreversible reaction that is the major control point of glycolysis. In gluconeogenesis, the enzyme fructose bisphosphatase hydrolyzes the C1 phosphate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

Gluconeogenesis is regulated at the fructose bisphosphatase step

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

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

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



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

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

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

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

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

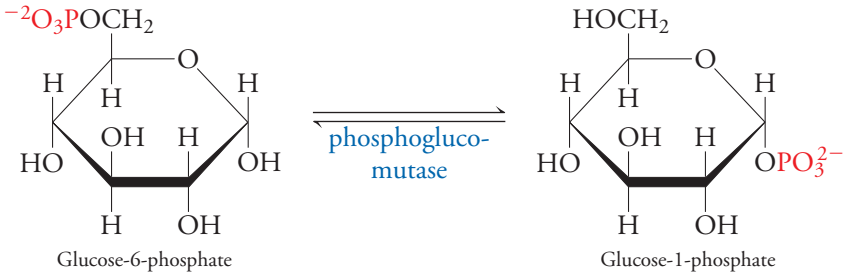
13-3 Glycogen Synthesis and Degradation

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

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

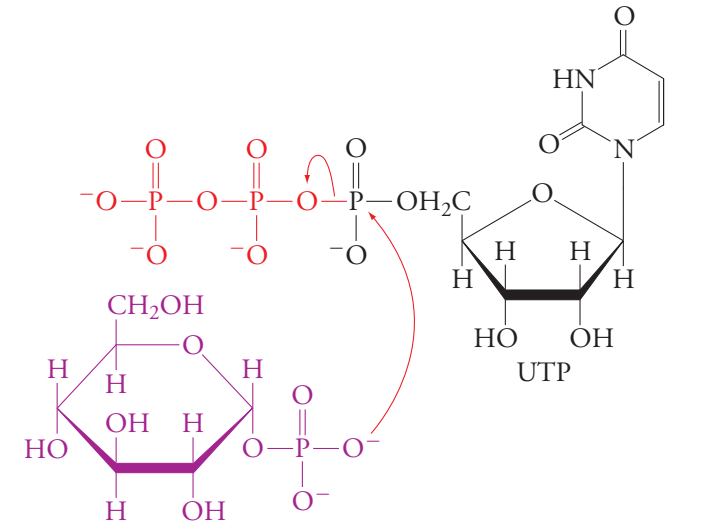
Glycogen synthesis consumes the free energy of UTP

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



Glucose-6-phosphate Glucose-1-phosphate

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

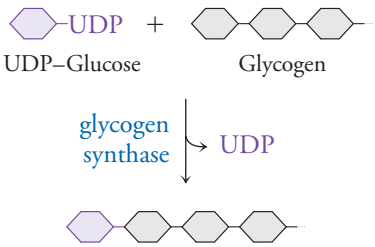


UDP–glucose

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

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

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



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

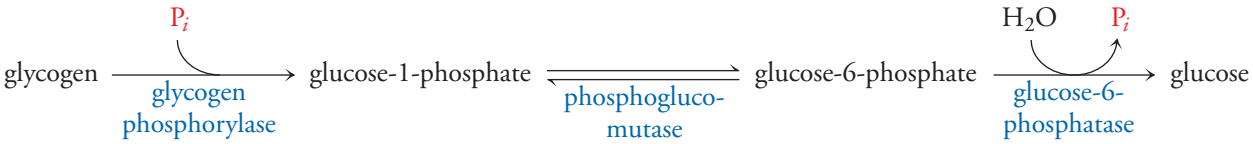
The steps of glycogen synthesis can be summarized as follows:

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

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

Glycogen phosphorylase catalyzes glycogenolysis

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



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

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

CONCEPT REVIEW

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

Glycogen Storage Diseases

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

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

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

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

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

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

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

Questions:

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

13-4 The Pentose Phosphate Pathway

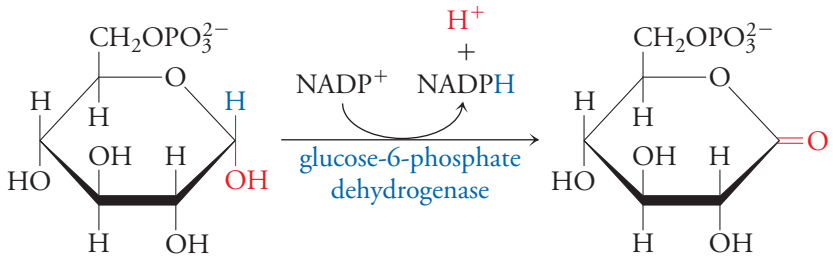
KEY CONCEPTS

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

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

The oxidative reactions of the pentose phosphate pathway produce NADPH

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

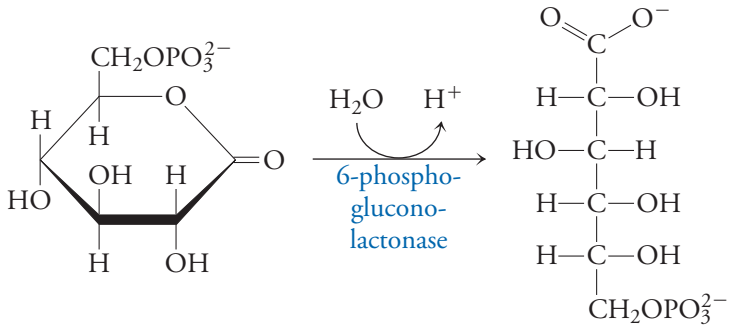


Glucose-6-phosphate

6-Phosphoglucono-δ-lactone

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

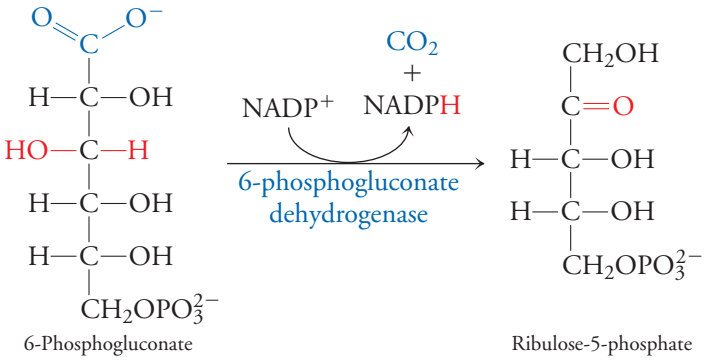
The lactone intermediate is hydrolyzed to 6-phosphogluconate by the action of 6-phosphogluconolactonase, although this reaction can also occur in the absence of the enzyme:



6-Phosphoglucono-δ-lactone

6-Phosphogluconate

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

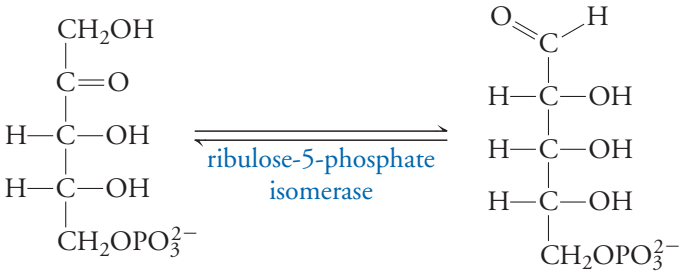


6-Phosphogluconate — Ribulose-5-phosphate

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

Isomerization and interconversion reactions generate a variety of monosaccharides

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

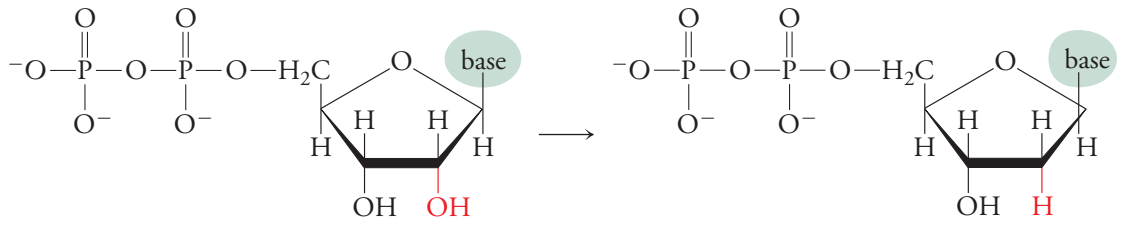


Ribulose-5-phosphate — Ribose-5-phosphate

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

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

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



NDP — dNDP

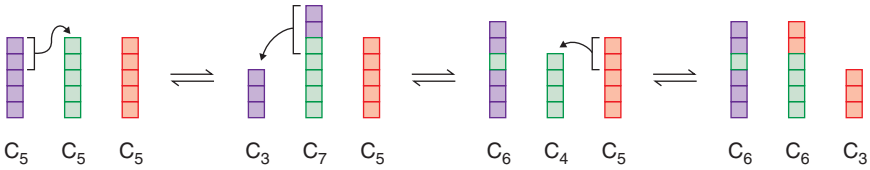


Figure 13-11 Rearrangements of the products of the pentose phosphate pathway. Three of the five-carbon products of the oxidative phase of the pentose phosphate pathway are converted to two fructose-6-phosphate and one glyceraldehyde-3-phosphate by reversible reactions involving the transfer of two- and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

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

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

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

CONCEPT REVIEW

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

A summary of glucose metabolism

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

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

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

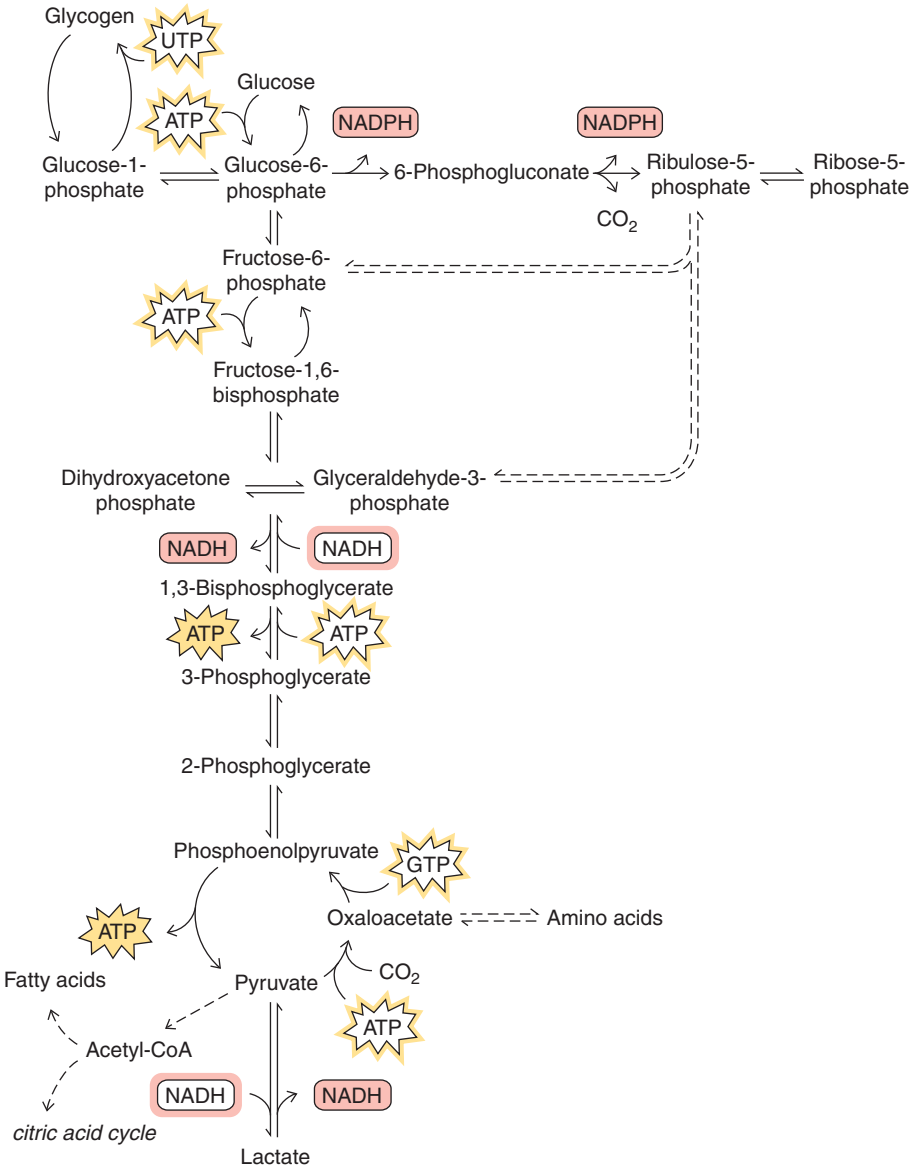


Figure 13-12 Summary of glucose metabolism. This diagram includes the pathways of glycogen synthesis and degradation, glycolysis, gluconeogenesis, and the pentose phosphate pathway. Dotted lines are used where the individual reactions are not shown. Filled gold symbols indicate ATP production; shadowed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

SUMMARY

13-1 Glycolysis

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

13-2 Gluconeogenesis

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

13-3 Glycogen Synthesis and Degradation

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

13-4 The Pentose Phosphate Pathway

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

GLOSSARY TERMS

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

PROBLEMS

13-1 Glycolysis

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

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

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

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

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

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

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

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

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

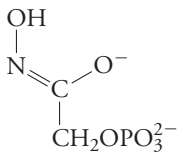
10. Refer to Figure 7-16. Why does the conformational change that results when Arg 162 changes places with Glu 161 result in a form of the enzyme that has a low affinity for its substrate?

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

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

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

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



Phosphoglycohydroxamate

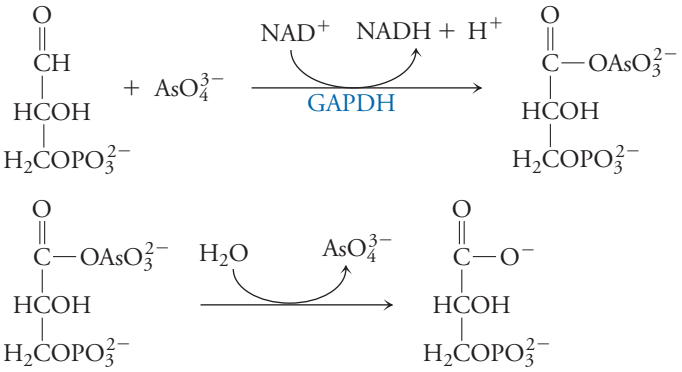
15. What is the ratio of glyceraldehyde-3-phosphate (GAP) to dihydroxyacetone phosphate (DHAP) in cells at 37°C under nonequilibrium conditions? Considering your answer to this question, how do you account for the fact that the conversion of DHAP to GAP occurs readily in cells?

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

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

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

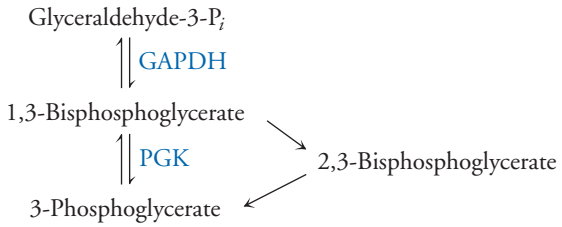
17. Arsenate, AsO_4^{3-}, acts as a phosphate analog and can replace phosphate in the GAPDH reaction. The product of this reaction is 1-arseno-3-phosphoglycerate. It is unstable and spontaneously hydrolyzes to form 3-phosphoglycerate, as shown below. What is the effect of arsenate on cells undergoing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



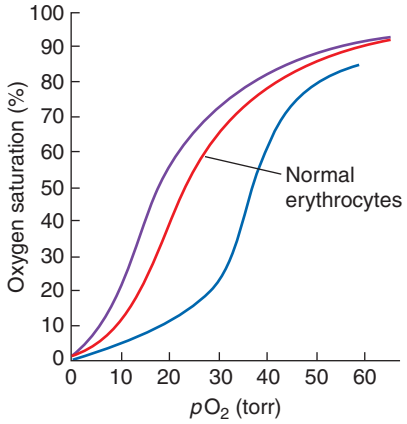
18. In several species of bacteria, activity of GADPH is controlled by the NADH/NAD^+ ratio. Does the activity of GAPDH increase or decrease when the NADH/NAD^+ ratio increases? Explain. Assume that only the forward direction of the reaction is relevant.

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

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



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



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

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

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

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

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

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

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

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

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

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

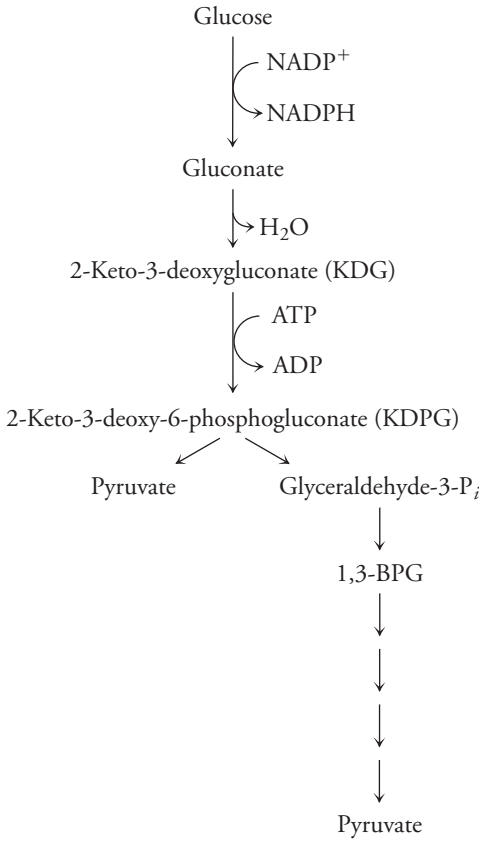
29. Drinking methanol can cause blindness and death, depending on the dosage. The causative agent is formaldehyde derived from methanol.

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

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

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

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

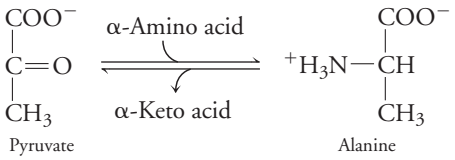


(a) What is the ATP yield per mole of glucose for this pathway?
(b) Describe (in general) what kinds of reactions would need to follow the Entner–Doudoroff pathway in this organism.

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

13-2 Gluconeogenesis

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



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

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

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

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

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

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

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

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

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

13-3 Glycogen Synthesis and Degradation

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

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

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

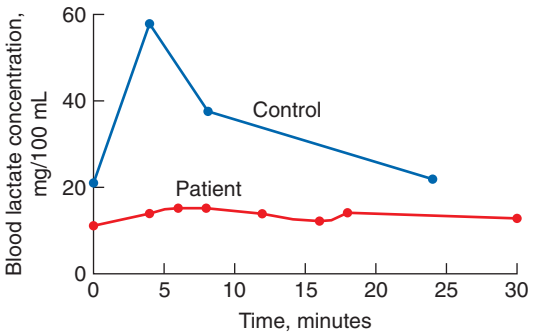
46. The equation for the degradation of glycogen is shown below.
(a) What is the ratio of $[P_i]/[G1P]$ under standard conditions?
(b) What is the value of ΔG under cellular conditions when the $[P_i]/[G1P]$ ratio is 50:1?

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

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

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

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

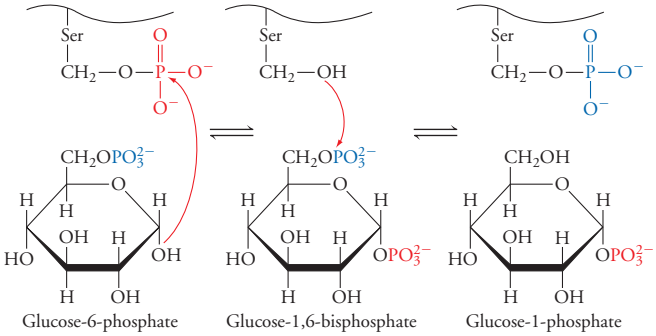


50. Does a patient with McArdle's disease (see Problems 47–49) suffer from hypoglycemia, hyperglycemia, or neither?

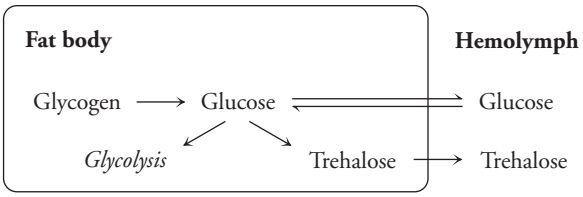
51. Patients with von Gierke's disease (type I glycogen storage disease) have a deficiency of glucose-6-phosphatase. One of the most prominent symptoms of the disease is a protruding abdomen due to an enlarged liver. Explain why the liver is enlarged in patients with von Gierke's disease.

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

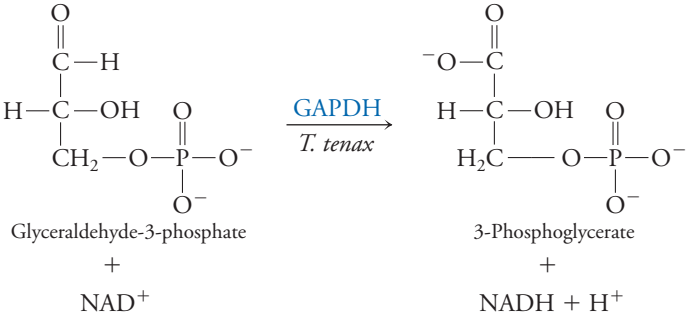
53. The mechanism of the phosphoglucomutase enzyme is similar to that of the plant mutase described in Problem 22 and is shown here. On occasion, the glucose-1,6-bisphosphate dissociates from the enzyme. Why does the dissociation of glucose-1,6-bisphosphate inhibit the enzyme?



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



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



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

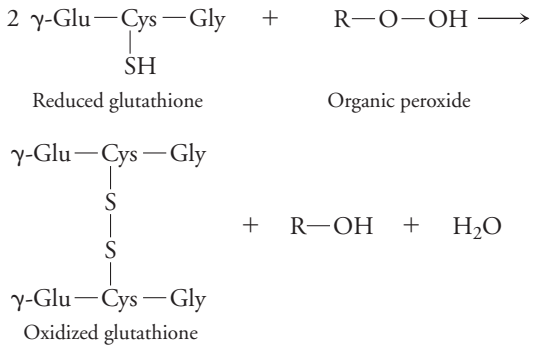
(a) Explain why individuals with fructose intolerance exhibit hypoglycemia (low blood sugar).
(b) Administering glycerol and dihydroxyacetone phosphate does not alleviate the hypoglycemia, but administering galactose does relieve the hypoglycemia. Explain.

13-4 The Pentose Phosphate Pathway

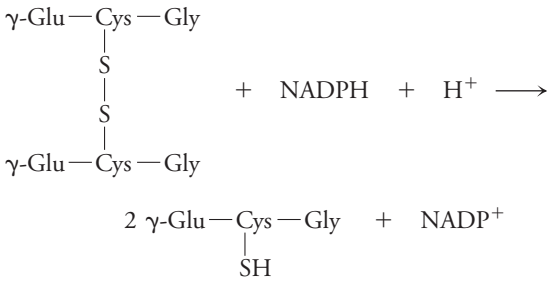
57. Most metabolic pathways include an enzyme-catalyzed reaction that commits a metabolite to continue through the pathway.
(a) Identify the first committed step of the pentose phosphate pathway. Explain your reasoning.
(b) Hexokinase catalyzes an irreversible reaction at the start of glycolysis. Does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

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

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



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



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

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

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

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

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

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

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

SELECTED READINGS

Brosnan, J. T., Comments on metabolic needs for glucose and the role of gluconeogenesis, *Eur. J. Clin. Nutr.* **53,** S107–S111 (1999). [A very readable review that discusses possible reasons why carbohydrates are used universally as metabolic fuels, why glucose is stored as glycogen, and why the pentose phosphate pathway is important.]

Greenberg, C. C., Jurczak, M. J., Danos, A. M., and Brady, M. J., Glycogen branches out: new perspectives on the role of glycogen metabolism in the integration of metabolic pathways, *Am. J. Physiol. Endocrinol. Metab.* **291,** E1–E8 (2006). [Describes the roles of glycogen in the liver and muscles.]

Özen, H., Glycogen storage diseases: New perspectives, *World J. Gastroenterology* **13,** 2541–2553 (2007). [Describes the symptoms, biochemistry, and treatment of the major forms of these diseases.]

Roach, P. J., Depaoli-Roach, A. A., Hurley, T. D., and Tagliabracci, V. S., Glycogen and its metabolism: Some new developments and old themes. *Biochem. J.* **441,** 763–787 (2012). [Includes discussions of the hormone-mediated regulation of key enzymes of glycogen synthesis and breakdown.]

chapter 14

THE CITRIC ACID CYCLE

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

▸▸ WHERE does exhaled CO_2 come from?

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

THIS CHAPTER IN CONTEXT

Part 1 Foundations

Part 2 Molecular Structure and Function

Part 3 Metabolism

14 The Citric Acid Cycle

Part 4 Genetic Information

Do You Remember?

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

The **citric acid cycle** is a pathway that occupies a central place in the metabolism of most cells. It converts two-carbon groups, in the form of acetyl-CoA, into CO_2 and therefore represents the final stage in the oxidation of metabolic fuels—not just carbohydrates but also fatty acids and amino acids (Fig. 14-1). As the carbons become fully oxidized to CO_2, their energy is conserved and subsequently used to produce ATP. The eight reactions of the citric acid cycle take place in the cytosol of prokaryotes and in the mitochondria of eukaryotes.

Unlike a linear pathway such as glycolysis (see Fig. 13-2) or gluconeogenesis (see Fig. 13-10), the citric acid cycle always returns to its starting position, essentially behaving as a multistep catalyst. However, it is still possible to follow the chemical transformations that occur at each step.

An examination of the citric acid cycle also illustrates an important feature of metabolic pathways in general, namely, that a pathway is less like an element of plumbing and more like a web or network. In other words, the pathway does not function like a simple pipeline, where one substance enters one end and another emerges from the other end. Instead, the pathway's intermediates can participate in many reactions, serving as both precursors and products of a large variety of biological molecules.

Although the carbon atoms that enter the citric acid cycle may be derived from amino acids, fatty acids, or carbohydrates, we will use pyruvate, the end product of glycolysis, as the starting point for our study of the citric acid cycle. We will examine the eight reactions of the citric acid cycle and discuss how this sequence of reactions might have evolved. Finally, we will consider the citric acid cycle as a multifunctional pathway with links to other metabolic processes.

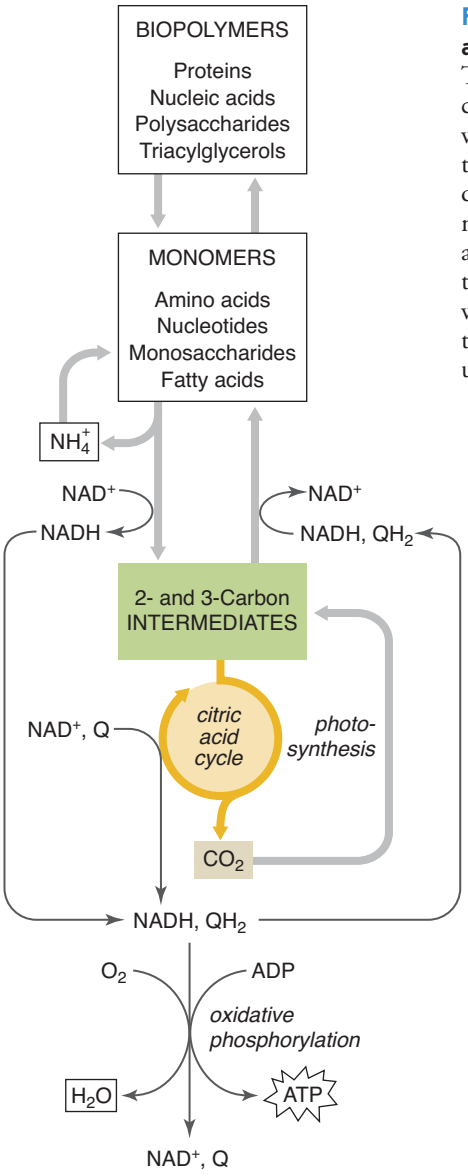


Figure 14-1 The citric acid cycle in context. The citric acid cycle is a central metabolic pathway whose starting material is two-carbon acetyl units derived from amino acids, monosaccharides, and fatty acids. These are oxidized to the waste product CO_2, with the reduction of the cofactors NAD^+ and ubiquinone (Q).

14-1 The Pyruvate Dehydrogenase Reaction

The end product of glycolysis is the three-carbon compound pyruvate. In aerobic organisms, these carbons are ultimately oxidized to 3 CO_2 (although the oxygen atoms come not from molecular oxygen but from water and phosphate). The first molecule of CO_2 is released when pyruvate is decarboxylated to an acetyl unit. The second and third CO_2 molecules are products of the citric acid cycle.

KEY CONCEPT

- The pyruvate dehydrogenase complex includes three types of enzymes that collectively remove a carboxylate group from pyruvate and produce acetyl-CoA and NADH.

The pyruvate dehydrogenase complex contains multiple copies of three different enzymes

The decarboxylation of pyruvate is catalyzed by the pyruvate dehydrogenase complex. In eukaryotes, this enzyme complex, and the enzymes of the citric acid cycle itself, are located inside the mitochondrion (an organelle surrounded by a double membrane and whose interior is called the **mitochondrial matrix**). Accordingly, pyruvate produced by glycolysis in the cytosol must first be transported into the mitochondria.

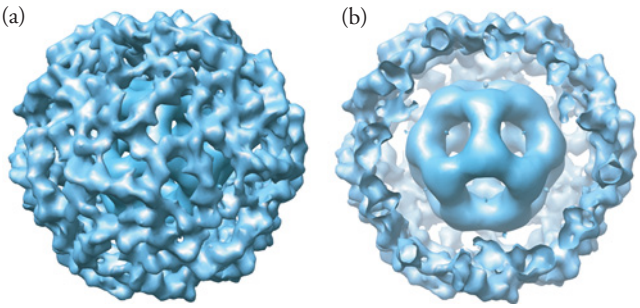


Figure 14-2 Model of the pyruvate dehydrogenase complex from *B. stearothermophilus*. These images are based on cryoelectron microscope studies of the pyruvate dehydrogenase complex. (a) Surface view. (b) Cutaway view showing the core of 60 E2 subunits. In this model the outer shell contains only E3; in the native pyruvate dehydrogenase complex, the outer shell contains both E1 and E3, which occupy similar positions. The space between the two layers of protein is about 75–90 Å. [Courtesy Jacqueline L. S. Milne and Sriram Subramaniam, National Cancer Institute, National Institutes of Health.]

For convenience, the three kinds of enzymes that make up the pyruvate dehydrogenase complex are called E1, E2, and E3. Together *they catalyze the oxidative decarboxylation of pyruvate and the transfer of the acetyl unit to coenzyme A*:

$$\text{pyruvate} + \text{CoA} + \text{NAD}^+ \rightarrow \text{acetyl-CoA} + \text{CO}_2 + \text{NADH}$$

The structure of coenzyme A, a nucleotide derivative containing the vitamin pantothenate, is shown in Figure 3-3a.

In *E. coli*, the pyruvate dehydrogenase complex contains 60 protein subunits (24 E1, 24 E2, and 12 E3) and has a mass of about 4600 kD. In mammals and some other bacteria, the enzyme complex is even larger, with 42–48 E1, 60 E2, and 6–12 E3 plus additional proteins that hold the complex together and regulate its enzymatic activity. The pyruvate dehydrogenase complex from *Bacillus stearothermophilus* consists of a core of 60 E2 subunits that form a dodecahedron (a 12-sided polyhedron) surrounded by an outer protein shell (Fig. 14-2).

Pyruvate dehydrogenase converts pyruvate to acetyl-CoA

The operation of the pyruvate dehydrogenase complex requires several coenzymes, whose functional roles in the five-step reaction are described below.

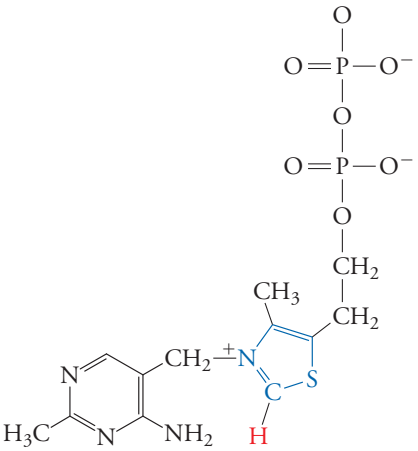
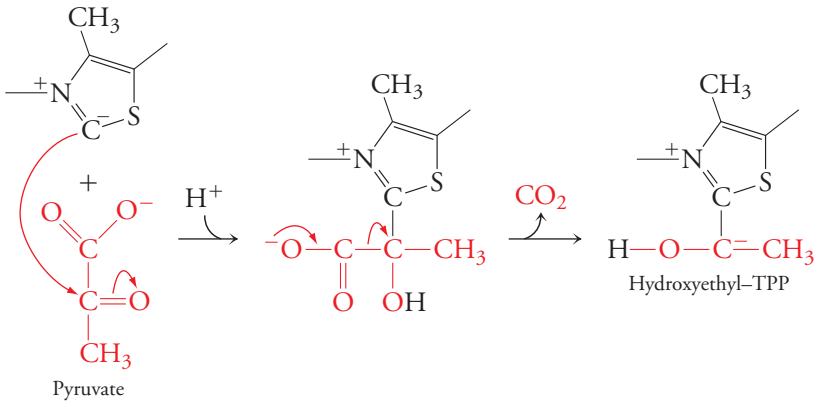
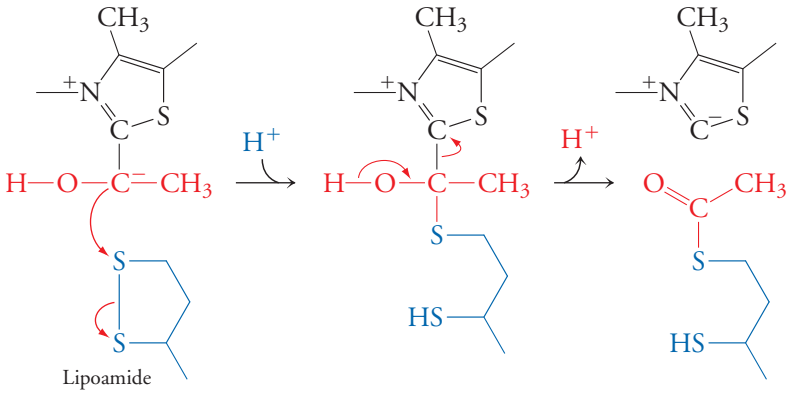


Figure 14-3 Thiamine pyrophosphate (TPP). This cofactor is the phosphorylated form of thiamine, also known as vitamin B_1 (see Section 12-2). The central thiazolium ring (blue) is the active portion. An acidic proton (red) dissociates, and the resulting carbanion is stabilized by the nearby positively charged nitrogen. TPP is a cofactor for several different decarboxylases.

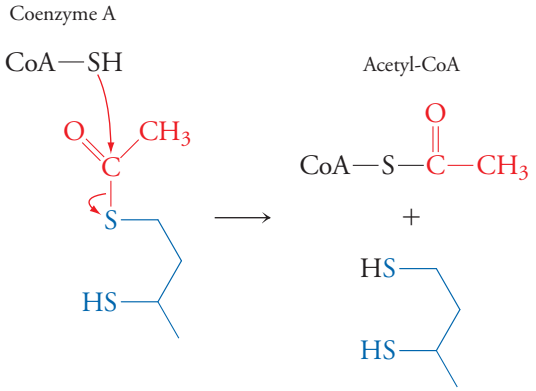
1. In the first step, which is catalyzed by E1 (also called pyruvate dehydrogenase), pyruvate is decarboxylated. This reaction requires the cofactor thiamine pyrophosphate (TPP; Fig. 14-3). TPP attacks the carbonyl carbon of pyruvate, and the departure of CO_2 leaves a hydroxyethyl group attached to TPP. This carbanion is stabilized by the positively charged thiazolium ring group of TPP:



2. The hydroxyethyl group is then transferred to E2 of the pyruvate dehydrogenase complex. The hydroxyethyl acceptor is a lipoamide prosthetic group (Fig. 14-4). The transfer reaction regenerates the TPP cofactor of E1 and oxidizes the hydroxyethyl group to an acetyl group:

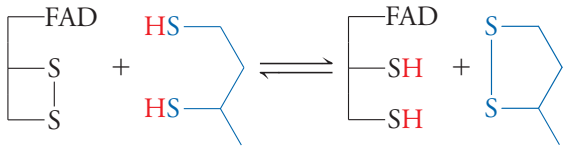


3. Next, E2 transfers the acetyl group to coenzyme A, producing acetyl-CoA and leaving a reduced lipoamide group.

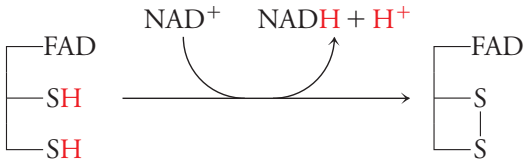


Recall that acetyl-CoA is a thioester, a form of energy currency (see Section 12-3). Some of the free energy released in the oxidation of the hydroxyethyl group to an acetyl group is conserved in the formation of acetyl-CoA.

4. The final two steps of the reaction restore the pyruvate dehydrogenase complex to its original state. E3 reoxidizes the lipoamide group of E2 by transferring electrons to a Cys–Cys disulfide group in the enzyme.



5. Finally, NAD^+ reoxidizes the reduced Cys sulfhydryl groups. This electron-transfer reaction is facilitated by an FAD prosthetic group (the structure of FAD, a nucleotide derivative, is shown in Fig. 3-3c).



During the five-step reaction (summarized in **Fig. 14-5**), the long lipoamide group of E2 acts as a swinging arm that visits the active sites of E1, E2, and E3 within the multienzyme complex. The arm picks up an acetyl group from an E1 subunit and transfers it to coenzyme A in an E2 active site. The arm then swings to an E3 active site, where it is reoxidized. Some other multienzyme complexes also include swinging arms, often attached to hinged protein domains to maximize their mobility.

A **multienzyme complex** such as the pyruvate dehydrogenase complex can carry out a multistep reaction sequence efficiently because *the product of one reaction can quickly become the substrate for the next reaction without diffusing away or reacting*

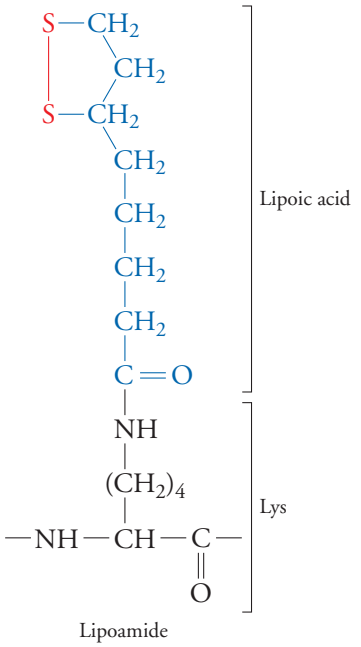
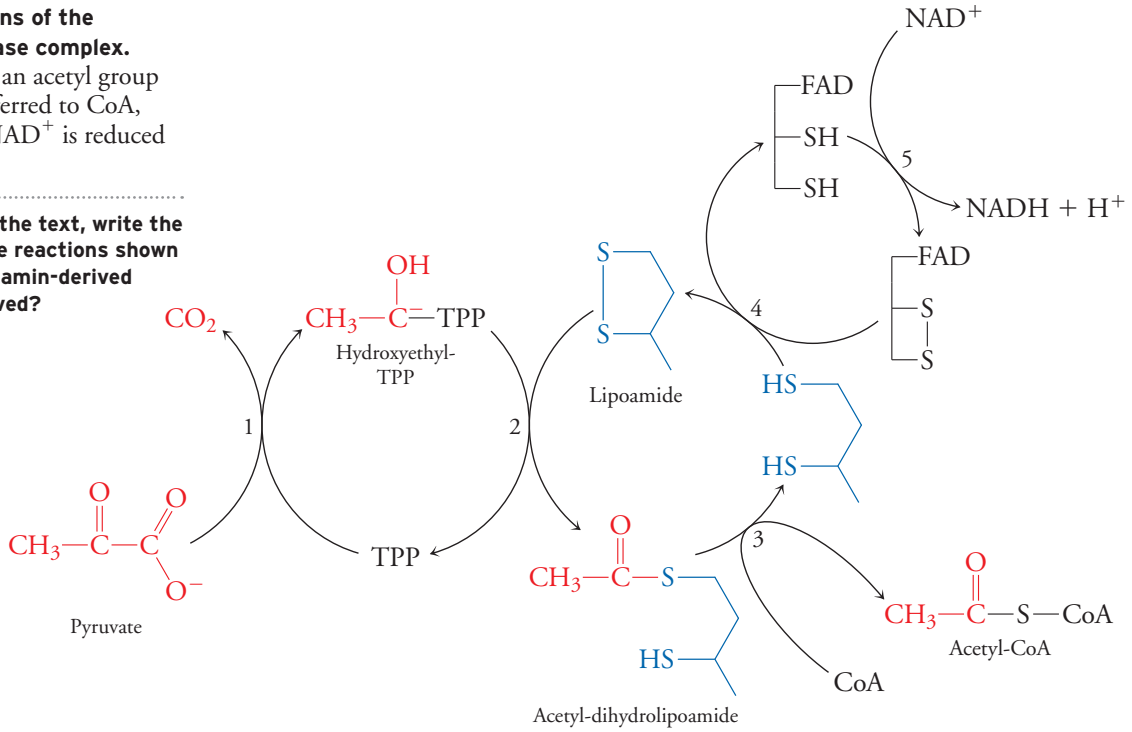


Figure 14-4 Lipoamide. This prosthetic group consists of lipoic acid (a vitamin) linked via an amide bond to the ε-amino group of a protein Lys residue. The active portion of the 14-Å-long lipoamide is the disulfide bond (red), which can be reversibly reduced.

Figure 14-5 Reactions of the pyruvate dehydrogenase complex. In these five reactions, an acetyl group from pyruvate is transferred to CoA, CO_2 is released, and NAD^+ is reduced to NADH.

? Without looking at the text, write the net equation for the reactions shown here. How many vitamin-derived cofactors are involved?



with another substance. There is also evidence that the individual enzymes of glycolysis and the citric acid cycle associate loosely with each other so that the close proximity of their active sites can increase flux through their respective pathways.

Flux through the pyruvate dehydrogenase complex is regulated by product inhibition: Both NADH and acetyl-CoA act as inhibitors. The activity of the complex is also regulated by hormone-controlled phosphorylation and dephosphorylation, which suits its function as the gatekeeper for the entry of a metabolic fuel into the citric acid cycle.

CONCEPT REVIEW

- Describe the functional importance of the coenzymes that participate in the reactions carried out by the pyruvate dehydrogenase complex.
- What is the advantage of a multienzyme complex?

14-2 The Eight Reactions of the Citric Acid Cycle

KEY CONCEPTS

- The citric acid cycle is a set of eight reactions in which an acetyl group is condensed with oxaloacetate, two CO_2 are lost, and oxaloacetate is regenerated.
- Each round of the citric acid cycle generates three NADH, one QH_2, and one GTP or ATP.
- Flux through the citric acid cycle is regulated primarily by feedback inhibition at three steps.
- The citric acid cycle likely evolved by the combination of oxidative and reductive pathways.

See Guided Exploration. Citric acid cycle overview.

An acetyl-CoA molecule derived from pyruvate is a product of carbohydrate catabolism as well as a product of amino acid catabolism, since the carbon skeletons of many amino acids are broken down to pyruvate. Acetyl-CoA is also a direct product of the degradation of certain amino acids and of fatty acids. In some tissues, the bulk of acetyl-CoA is derived from the catabolism of fatty acids rather than carbohydrates or amino acids.

Whatever its source, acetyl-CoA enters the citric acid cycle for further oxidation. This process is highly exergonic, and free energy is conserved at several steps in the form of a nucleotide triphosphate (GTP) and reduced cofactors. *For each acetyl group that enters the citric acid cycle, two molecules of fully oxidized CO_2 are produced, representing a loss of four pairs of electrons.* These electrons are transferred to 3 NAD^+ and 1 ubiquinone (Q) to produce 3 NADH and 1 QH_2. The net equation for the citric acid cycle is therefore

$$\text{acetyl-CoA} + \text{GDP} + P_i + 3\,NAD^+ + Q \rightarrow 2\,CO_2 + \text{CoA} + \text{GTP} + 3\,\text{NADH} + QH_2$$

In this section we examine the sequence of eight enzyme-catalyzed reactions of the citric acid cycle, focusing on a few interesting reactions. The entire pathway is summarized in **Figure 14-6**.

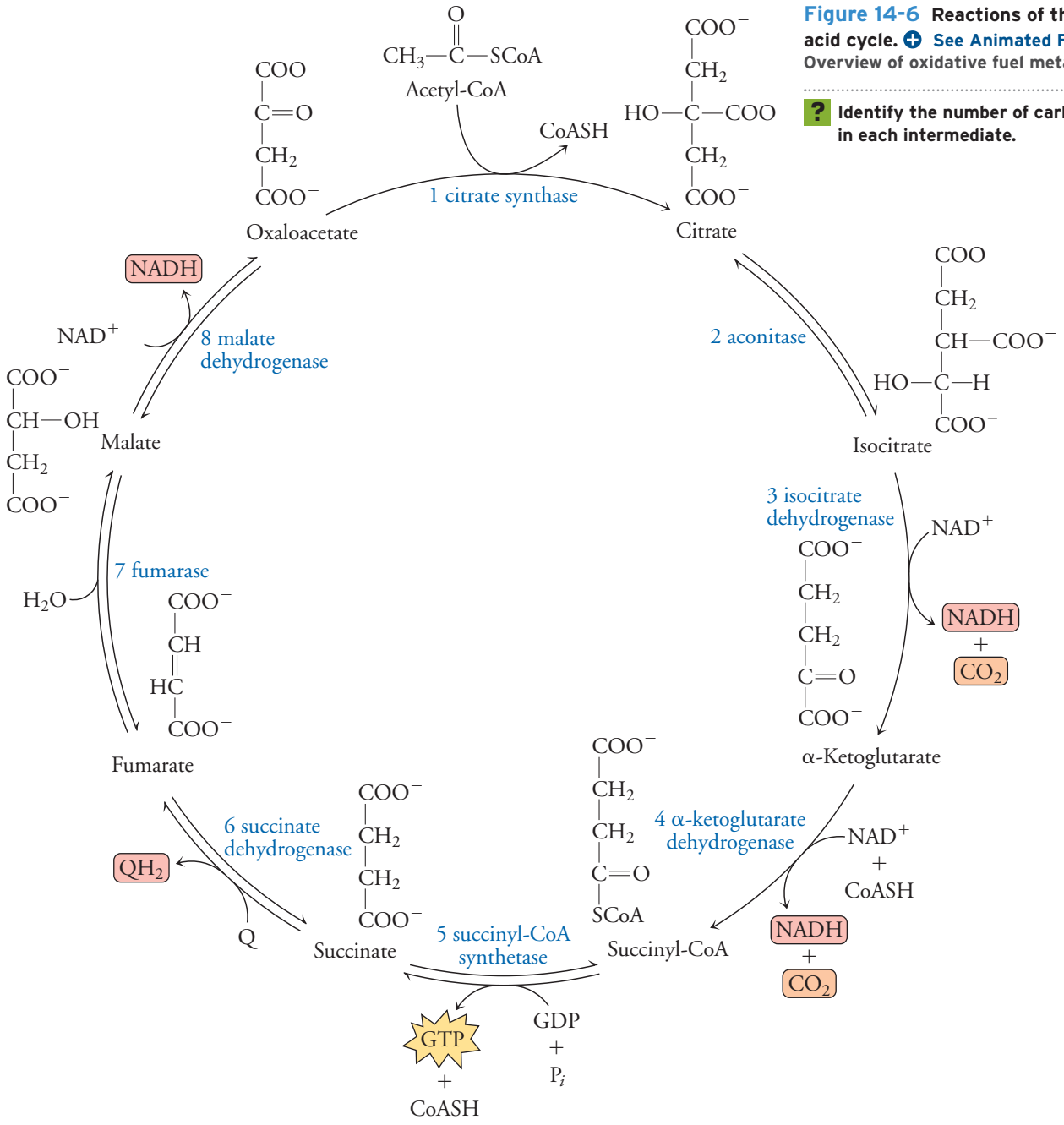
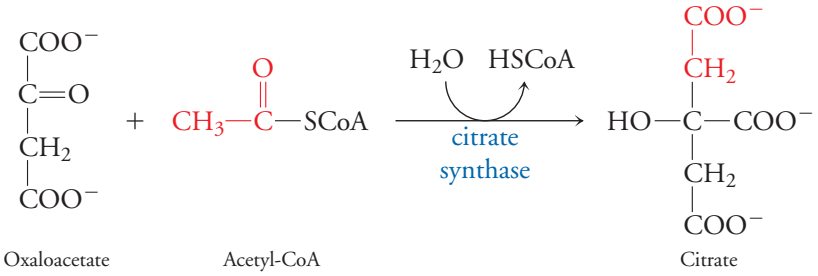


Figure 14-6 **Reactions of the citric acid cycle.** ⊕ **See Animated Figure.** Overview of oxidative fuel metabolism.

? **Identify the number of carbon atoms in each intermediate.**

1. Citrate synthase adds an acetyl group to oxaloacetate

In the first reaction of the citric acid cycle, the acetyl group of acetyl-CoA condenses with the four-carbon compound oxaloacetate to produce the six-carbon compound citrate:



Citrate synthase is a dimer that undergoes a large conformational change upon substrate binding (Fig. 14-7).

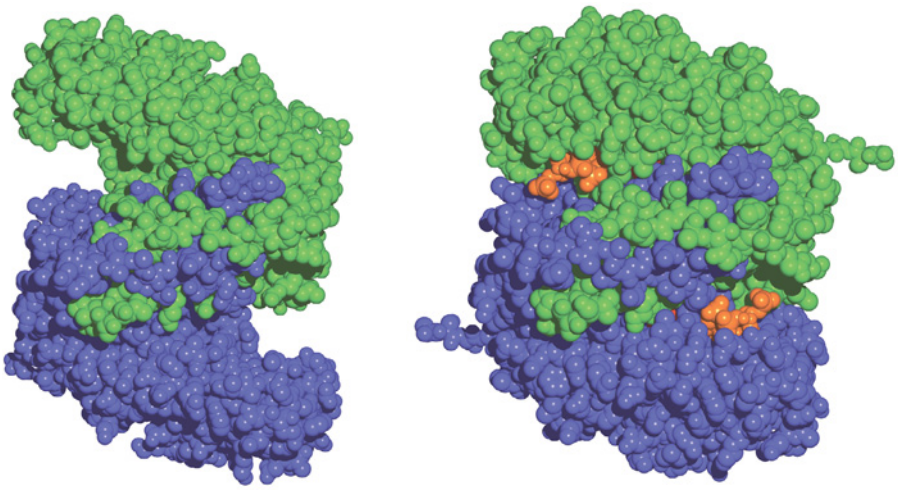


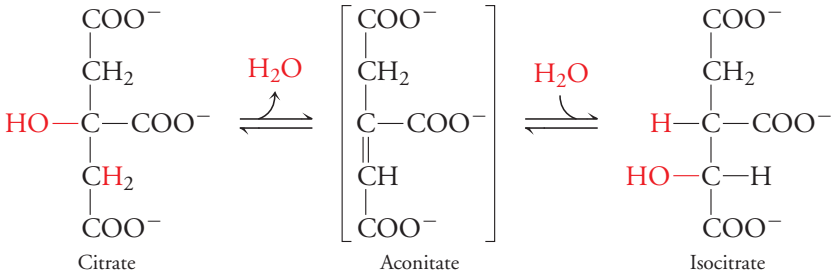
Figure 14-7 Conformational changes in citrate synthase. (a) The enzyme in the absence of substrates. The two subunits of the dimeric enzyme are colored blue and green. (b) When oxaloacetate (red, mostly buried) binds, each subunit undergoes a conformational change that creates a binding site for acetyl-CoA (an acetyl-CoA analog is shown here in orange). This conformational change explains why oxaloacetate must bind to the enzyme before acetyl-CoA can bind. [Structure of chicken citrate synthase alone (pdb 5CSC) determined by D.-I. Liao, M. Karpusas, and S. J. Remington; structure of citrate synthase with oxaloacetate and carboxymethyl-CoA (pdb 5CTS) determined by M. Karpusas, B. Branchaud, and S. J. Remington.] **See Interactive Exercise. Conformational changes in citrate synthase.**

Citrate synthase is one of the few enzymes that can synthesize a carbon–carbon bond without using a metal ion cofactor. Its mechanism is shown in **Figure 14-8**. The first reaction intermediate may be stabilized by the formation of low-barrier hydrogen bonds, which are stronger than ordinary hydrogen bonds (see Section 6-3). The coenzyme A released during the final step can be reused by the pyruvate dehydrogenase complex or used later in the citric acid cycle to synthesize the intermediate succinyl-CoA.

The reaction catalyzed by citrate synthase is highly exergonic ($\Delta G^{\circ\prime} = -31.5$ kJ · mol^{-1}, equivalent to the free energy of breaking the thioester bond of acetyl-CoA). We will see later why the efficient operation of the citric acid cycle requires that this step have a large free energy change.

2. Aconitase isomerizes citrate to isocitrate

The second enzyme of the citric acid cycle catalyzes the reversible isomerization of citrate to isocitrate:



The enzyme is named after the reaction intermediate.

Citrate is a symmetrical molecule, yet only one of its two carboxymethyl arms (—CH_2—COO^-) undergoes dehydration and rehydration during the aconitase reaction. This stereochemical specificity long puzzled biochemists, including Hans Krebs, who first described the citric acid cycle (also known as the Krebs cycle).

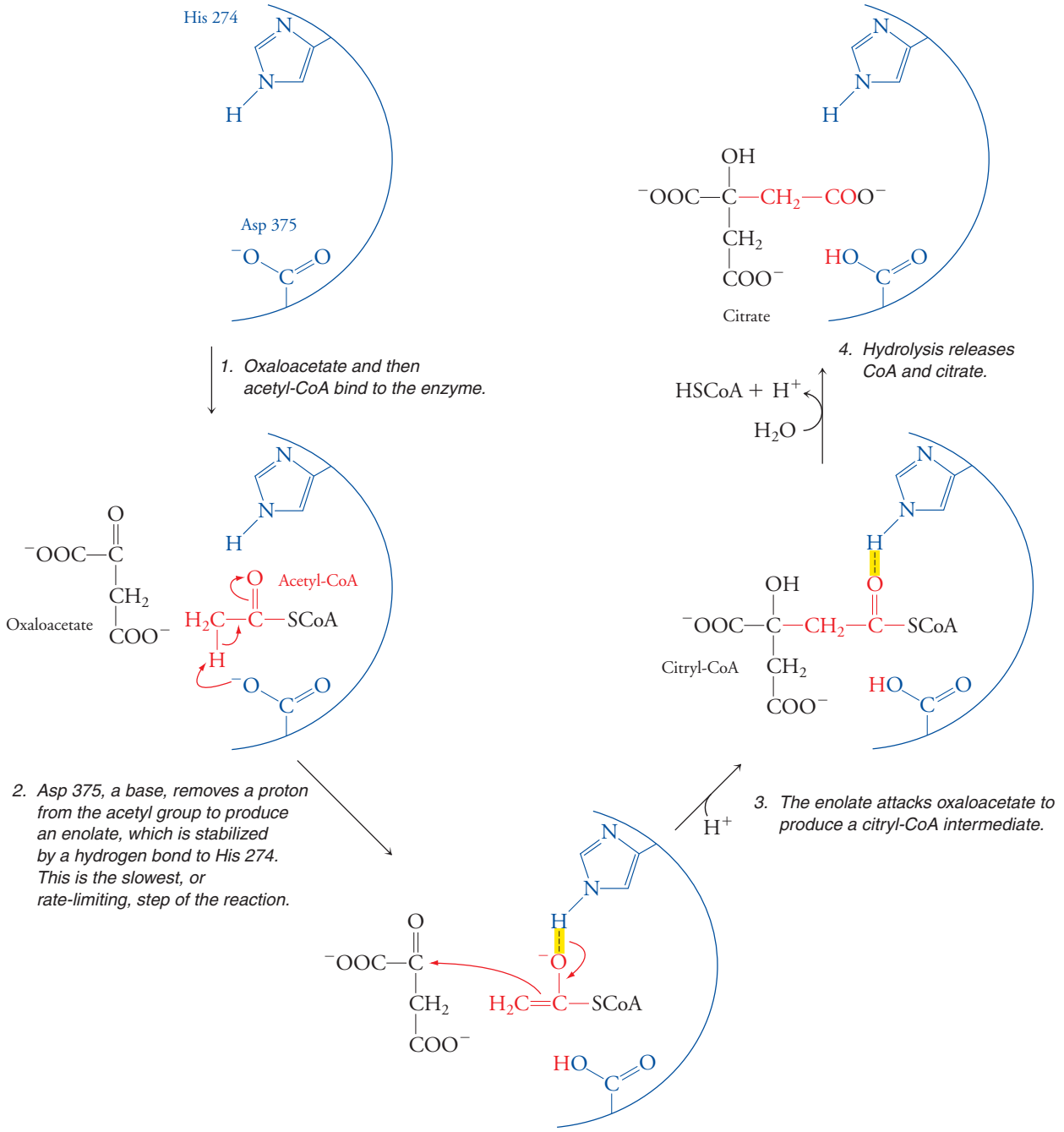


Figure 14-8 **The citrate synthase reaction.**

Eventually, Alexander Ogston pointed out that although citrate is symmetrical, its two carboxymethyl groups are no longer identical when it is bound to an asymmetrical enzyme (Fig. 14-9). In fact, a three-point attachment is not even necessary for an enzyme to distinguish two groups in a molecule such as citrate, which are related by mirror symmetry. You can prove this yourself with a simple organic chemistry model kit. By now you should appreciate that biological systems, including enzyme, are inherently chiral (also see Section 4-1).

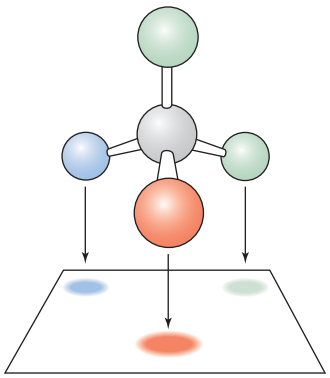
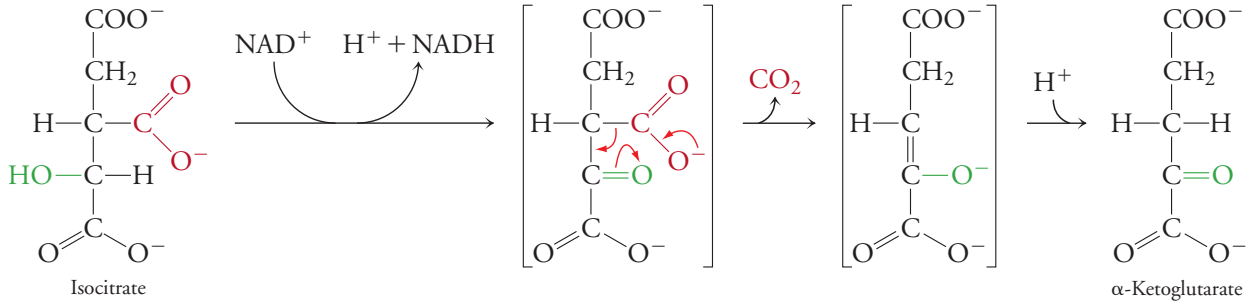


Figure 14-9 **Stereochemistry of citrate synthase.** The three-point attachment of citrate to the enzyme allows only one carboxymethyl group (shown in green) to react.

3. Isocitrate dehydrogenase releases the first CO_2

The third reaction of the citric acid cycle is the oxidative decarboxylation of isocitrate to α-ketoglutarate. The substrate is first oxidized in a reaction accompanied by the reduction of NAD^+ to NADH. Then the carboxylate group β to the ketone function (that is, two carbon atoms away from the ketone) is eliminated as CO_2.

An Mn^{2+} ion in the active site helps stabilize the negative charges of the reaction intermediate.

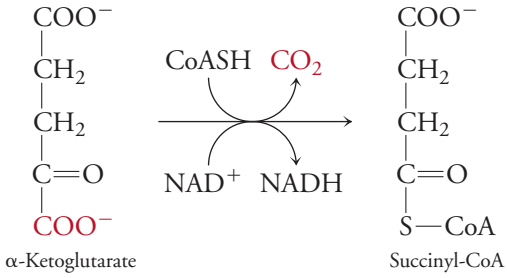


▶▶ WHERE does exhaled CO_2 come from?

The CO_2 molecules generated by isocitrate dehydrogenase—along with the CO_2 generated in the following reaction and the CO_2 produced by the decarboxylation of pyruvate—diffuse out of the cell and are carried in the bloodstream to the lungs, where they are breathed out. Note that these CO_2 molecules are produced through oxidation–reduction reactions: The carbons are oxidized, while NAD^+ is reduced. As described at the start of the chapter, O_2 is not directly involved in this process.

4. α-Ketoglutarate dehydrogenase releases the second CO_2

α-Ketoglutarate dehydrogenase, like isocitrate dehydrogenase, catalyzes an oxidative decarboxylation reaction. It also transfers the remaining four-carbon fragment to CoA:



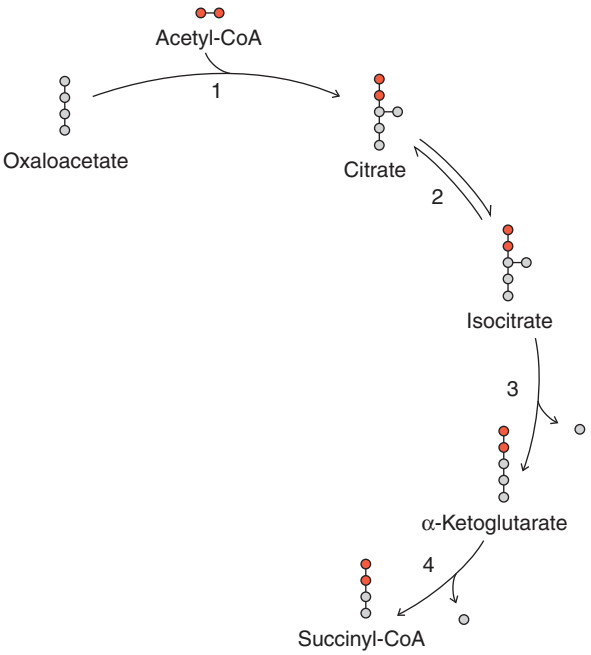


Figure 14-10 Fates of carbon atoms in the citric acid cycle. The two carbon atoms that are lost as CO_2 in the reactions catalyzed by isocitrate dehydrogenase (step 3) and α-ketoglutarate dehydrogenase (step 4) are not the same carbons that entered the cycle as acetyl-CoA (red). The acetyl carbons become part of oxaloacetate and are lost in subsequent rounds of the cycle.

The free energy of oxidizing α-ketoglutarate is conserved in the formation of the thioester succinyl-CoA. α-Ketoglutarate dehydrogenase is a multienzyme complex that resembles the pyruvate dehydrogenase complex in both structure and enzymatic mechanism. In fact, the same E3 enzyme is a member of both complexes.

The isocitrate dehydrogenase and α-ketoglutarate dehydrogenase reactions both release CO_2. These two carbons are not the ones that entered the citric acid cycle as acetyl-CoA; those acetyl carbons are released in subsequent rounds of the cycle (Fig. 14-10). *However, the net result of each round of the citric acid cycle is the loss of two carbons as CO_2 for each acetyl-CoA that enters the cycle.*

5. Succinyl-CoA synthetase catalyzes substrate-level phosphorylation

The thioester succinyl-CoA releases a large amount of free energy when it is cleaved ($\Delta G^{\circ\prime} = -32.6$ kJ · mol^{-1}). This is enough free energy to drive the synthesis of a nucleoside triphosphate from a nucleoside diphosphate and P_i ($\Delta G^{\circ\prime} = 30.5$ kJ · mol^{-1}). The change in free energy for the net reaction is near zero, so the reaction is reversible. In fact, the enzyme is named for the reverse reaction. Succinyl-CoA synthetase in the mammalian citric acid cycle generates GTP,

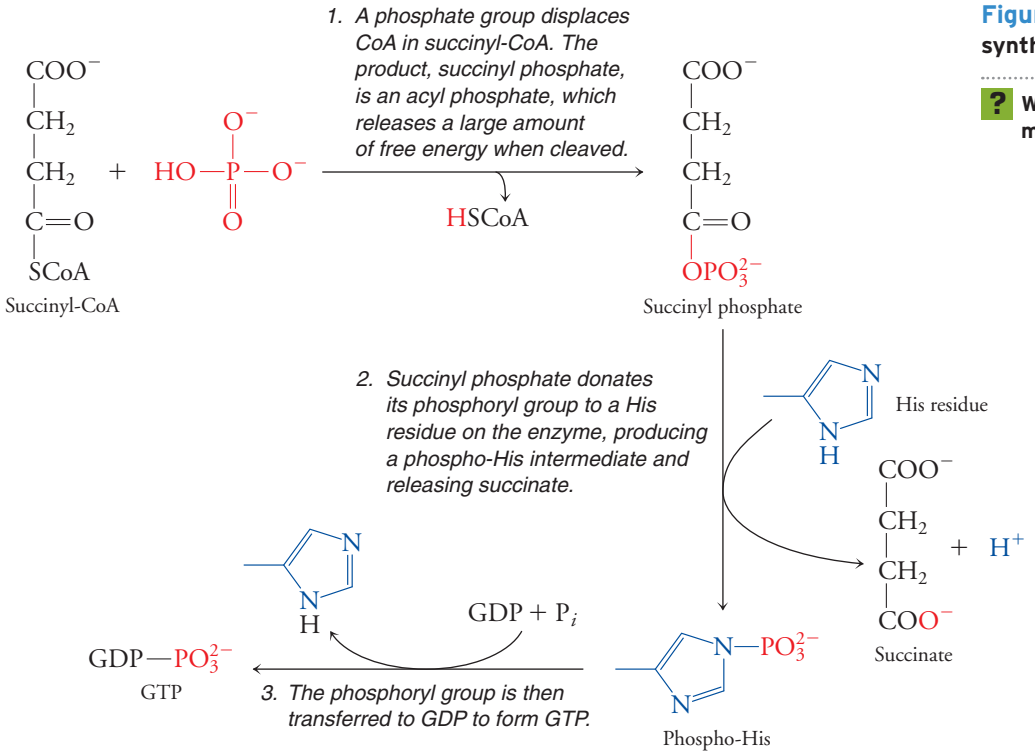


Figure 14-11 **The succinyl-CoA synthetase reaction.**

? **What is the fate of the free CoA molecule?**

whereas the plant and bacterial enzymes generate ATP (recall that GTP is energetically equivalent to ATP). An exergonic reaction coupled to the transfer of a phosphoryl group to a nucleoside diphosphate is termed **substrate-level phosphorylation** to distinguish it from oxidative phosphorylation (Section 15-4) and photophosphorylation (Section 16-2), which are more indirect ways of synthesizing ATP.

How does succinyl-CoA synthetase couple thioester cleavage to the synthesis of a nucleoside triphosphate? The reaction is a series of phosphoryl-group transfers that involve an active-site His residue **(Fig. 14-11)**. The phospho-His reaction intermediate must move a large distance to shuttle the phosphoryl group between the succinyl group and the nucleoside diphosphate **(Fig. 14-12)**.

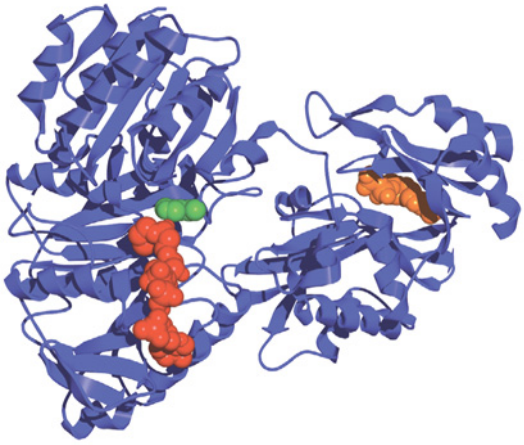
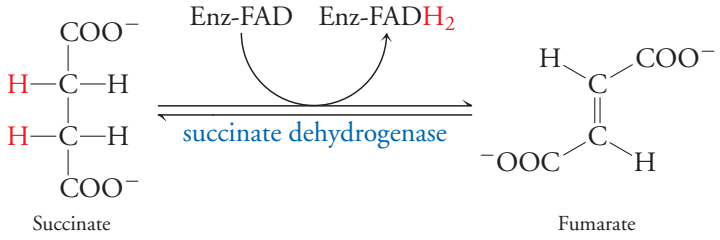


Figure 14-12 **Substrate binding in succinyl-CoA synthetase.** Succinyl-CoA (represented by coenzyme A, red) binds to the enzyme, and its succinyl group is phosphorylated. The succinyl phosphate then transfers its phosphoryl group to the His 246 side chain (green). A protein loop containing the phospho-His side chain must undergo a large movement because the nucleoside diphosphate awaiting phosphorylation (ADP, orange) binds to a site about 35 Å away. [Structure of *E. coli* succinyl-CoA synthetase (pdb 1CQI) determined by M. A. Joyce, M. E. Fraser, M. N. G. James, W. A. Bridger, and W. T. Wolodko.]

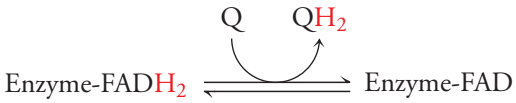
6. Succinate dehydrogenase generates ubiquinol

The final three reactions of the citric acid cycle convert succinate back to the cycle's starting substrate, oxaloacetate. Succinate dehydrogenase catalyzes the reversible dehydrogenation of succinate to fumarate. This oxidation–reduction reaction requires an FAD prosthetic group, which is reduced to $FADH_2$ during the reaction:



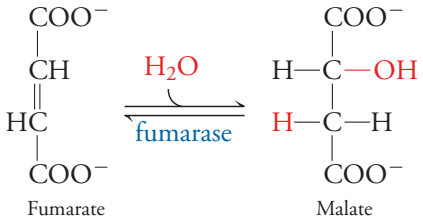
To regenerate the enzyme, the $FADH_2$ group must be reoxidized. Since succinate dehydrogenase is embedded in the inner mitochondrial membrane (it is the only one of the eight citric acid cycle enzymes that is not soluble in the mitochondrial

matrix), it can be reoxidized by the lipid-soluble electron carrier ubiquinone (see Section 12-2) rather than by the soluble cofactor NAD^+. Ubiquinone (abbreviated Q) acquires two electrons to become ubiquinol (QH_2).



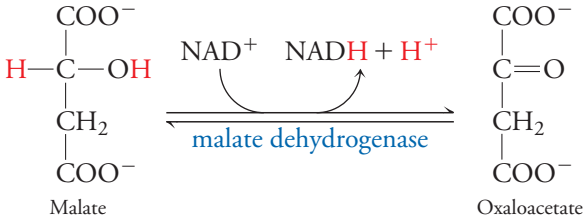
7. Fumarase catalyzes a hydration reaction

In the seventh reaction, fumarase (also known as fumarate hydratase) catalyzes the reversible hydration of a double bond to convert fumarate to malate:



8. Malate dehydrogenase regenerates oxaloacetate

The citric acid cycle concludes with the regeneration of oxaloacetate from malate in an NAD^+-dependent oxidation reaction:



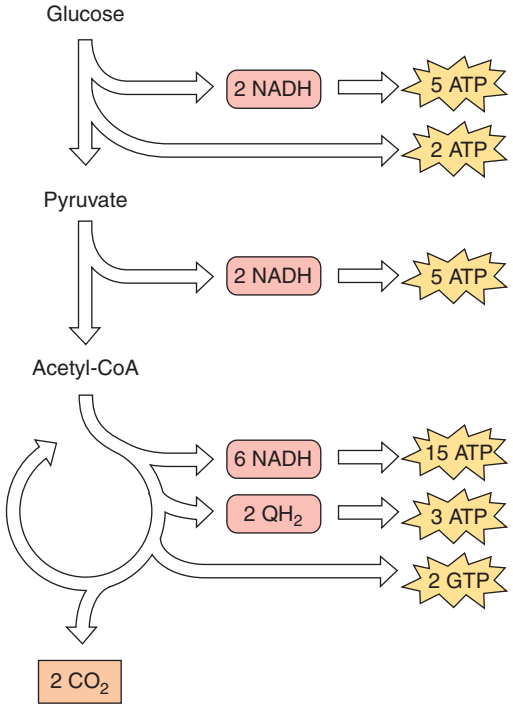
The standard free energy change for this reaction is $+29.7\ kJ \cdot mol^{-1}$, indicating that the reaction has a low probability of occurring as written. However, the product oxaloacetate is a substrate for the next reaction (Reaction 1 of the citric acid cycle). The highly exergonic—and therefore highly favorable—citrate synthase reaction helps pull the malate dehydrogenase reaction forward. This is the reason for the apparent waste of free energy released by cleaving the thioester bond of acetyl-CoA in the first reaction of the citric acid cycle.

The citric acid cycle is an energy-generating catalytic cycle

Because the eighth reaction of the citric acid cycle returns the system to its original state, *the entire pathway acts in a catalytic fashion to dispose of carbon atoms derived from amino acids, carbohydrates, and fatty acids.* Albert Szent-Györgyi discovered the catalytic nature of the pathway by observing that small additions of organic compounds such as succinate, fumarate, and malate stimulated O_2 uptake in a tissue preparation. Because the O_2 consumption was much greater than would be required for the direct oxidation of the added substances, he inferred that the compounds acted catalytically.

We now know that oxygen is consumed during oxidative phosphorylation, the process that reoxidizes the reduced cofactors (NADH and QH_2) that are produced by the citric acid cycle. Although the citric acid cycle generates one molecule of GTP (or ATP), considerably more ATP is generated when the reduced cofactors are reoxidized by O_2. Each NADH yields approximately 2.5 ATP, and each QH_2 yields approximately 1.5 ATP (we will see in Section 15-4 why these values are not whole numbers). Every acetyl unit that enters the citric acid cycle can therefore generate a

total of 10 ATP equivalents. The energy yield of a molecule of glucose, which generates two acetyl units, can be calculated:



A muscle operating anaerobically produces only 2 ATP per glucose, but under aerobic conditions when the citric acid cycle is fully functional, each glucose molecule generates about 32 ATP equivalents. This general phenomenon is called the **Pasteur effect,** after Louis Pasteur, who first observed that the rate of glucose consumption by yeast cells decreased dramatically when the cells were shifted from anaerobic to aerobic growth conditions.

The citric acid cycle is regulated at three steps

Flux through the citric acid cycle is regulated primarily at the cycle's three metabolically irreversible steps: those catalyzed by citrate synthase (Reaction 1), isocitrate dehydrogenase (Reaction 3), and α-ketoglutarate dehydrogenase (Reaction 4). The major regulators are shown in **Figure 14-13**.

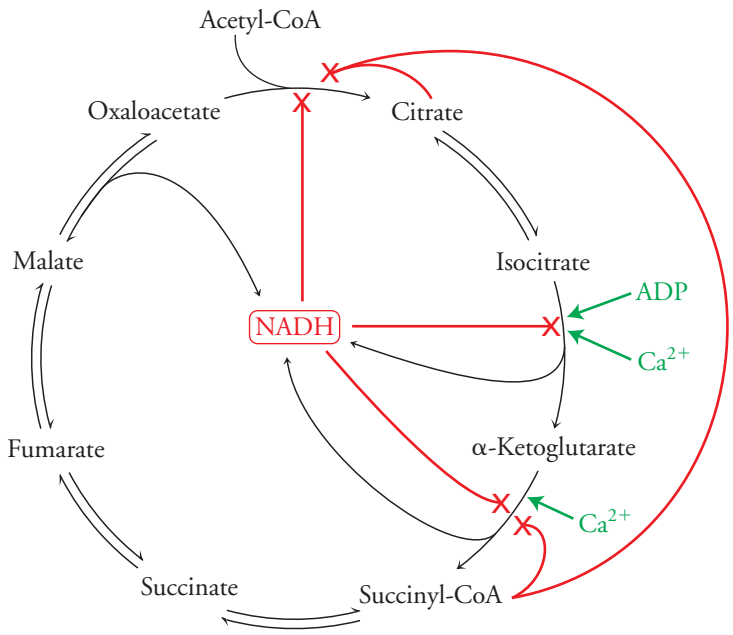


Figure 14-13 Regulation of the citric acid cycle. Inhibition is represented by red symbols, activation by green symbols.

Neither acetyl-CoA nor oxaloacetate is present at concentrations high enough to saturate citrate synthase, so flux through the first step of the citric acid cycle depends largely on the substrate concentrations. The product of the reaction, citrate, inhibits citrate synthase (citrate also inhibits phosphofructokinase, thereby decreasing the supply of acetyl-CoA produced by glycolysis). Succinyl-CoA, the product of Reaction 4, inhibits the enzyme that produces it. It also acts as a feedback inhibitor by competing with acetyl-CoA in Reaction 1.

The activity of isocitrate dehydrogenase is inhibited by its reaction product, NADH. NADH also inhibits α-ketoglutarate dehydrogenase and citrate synthase. Both dehydrogenases are activated by Ca^{2+} ions, which generally signify the need to generate cellular free energy. ADP, also representing the need for more ATP, activates isocitrate dehydrogenase. For reasons that are not entirely clear, many cancer cells develop mutations in isocitrate dehydrogenase (Box 14-A).

The citric acid cycle probably evolved as a synthetic pathway

A circular pathway such as the citric acid cycle must have evolved from a linear set of preexisting biochemical reactions. Clues to its origins can be found by examining

BOX 14-A CLINICAL CONNECTION

Mutations in Citric Acid Cycle Enzymes

Possibly because the citric acid cycle is a central metabolic pathway, severe defects in any of its components are expected to be incompatible with life. However, researchers have documented mutations in the genes for several of the cycle's enzymes, including α-ketoglutarate dehydrogenase, succinyl-CoA synthetase, and succinate dehydrogenase. These defects, which are all rare, typically affect the central nervous system, causing symptoms such as movement disorders and neurodegeneration. A rare form of fumarase deficiency results in brain malformation and developmental disabilities. Individuals who have a different fumarase defect show a higher risk of developing leiomyomas, noncancerous tumors such as uterine fibroids. A small percentage of these do become malignant. The other citric acid cycle enzyme mutations are also associated with cancer. One possible explanation is that a defective enzyme contributes to **carcinogenesis** (the development of cancer, or uncontrolled cell growth) by directly interfering with the cell's vital pathways for energy metabolism. Another possibility is that a defective enzyme causes the accumulation of particular metabolites, which are responsible for altering the cell's developmental fate.

Fumarase appears to be linked to cancer through the second mechanism. Normal cells respond to a drop in oxygen availability (hypoxia) by activating transcription factors known as hypoxia-inducible factors (HIFs). These proteins interact with DNA to turn on the expression of genes for glycolytic enzymes and a growth factor that promotes the development of new blood vessels. When the fumarase gene is defective, fumarate accumulates and inhibits a protein that destabilizes HIFs. As a result, the fumarase deficiency promotes glycolysis (an anaerobic pathway) and the growth of blood vessels. These two adaptations would favor tumors, whose growth, although characteristically rapid, may be limited by the availability of oxygen and other nutrients delivered by the bloodstream.

Defects in isocitrate dehydrogenase also promote cancer in an indirect fashion. Many cancerous cells exhibit a mutation in one of the two genes for the enzyme, suggesting that the unaltered copy is necessary for maintaining the normal activity of the citric acid cycle, while the mutated copy plays a role in carcinogenesis. Interestingly, the mutation usually converts an active-site Arg residue to His, indicating some strong selective pressure for a gain-of-function mutation (most mutations in proteins lead to loss of function). The mutated isocitrate dehydrogenase no longer carries out the usual reaction (converting isocitrate to α-ketoglutarate) but instead converts α-ketoglutarate to 2-hydroxyglutarate in an NADPH-dependent manner. The mechanism whereby 2-hydroxyglutarate contributes to carcinogenesis is not clear, but its involvement is bolstered by the observation that individuals who harbor other mutations that lead to 2-hydroxyglutarate accumulation have an increased risk of developing brain tumors.

Questions:

1. How would a fumarase deficiency affect the levels of pyruvate, fumarate, and malate?
2. Why does a succinate dehydrogenase deficiency produce the same symptoms as a fumarase deficiency?
3. Why would a deficiency of succinate dehydrogenase lead to a shortage of free coenzyme A?
4. Individuals who are deficient in fumarase develop lactic acidosis. Explain.
5. Draw the structure of 2-hydroxyglutarate, the product of the reaction catalyzed by the mutated isocitrate dehydrogenase.
6. Describe the single-nucleotide changes that could convert the arginine in isocitrate dehydrogenase to histidine.
7. How does operation of the mutated isocitrate dehydrogenase affect the cell's supply of reduced cofactors?

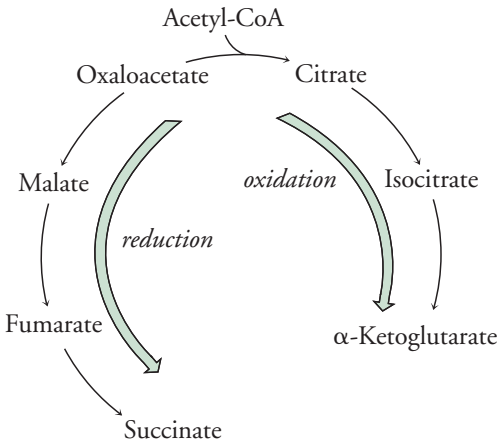


Figure 14-14 Pathways that might have given rise to the citric acid cycle. The pathway starting from oxaloacetate and proceeding to the right is an oxidative biosynthetic pathway, whereas the pathway that proceeds to the left is a reductive pathway. The modern citric acid cycle may have evolved by connecting these pathways.

the metabolism of organisms that resemble earlier life-forms. Such organisms emerged before atmospheric oxygen was available and may have used sulfur as their ultimate oxidizing agent, reducing it to H_2S. Their modern-day counterparts are anaerobic autotrophs that harvest free energy by pathways that are independent of the pathways of carbon metabolism. These organisms therefore do not use the citric acid cycle to generate reduced cofactors that are subsequently oxidized by molecular oxygen. However, all organisms must synthesize small molecules that can be used to build proteins, nucleic acids, carbohydrates, and so on.

Even organisms that do not use the citric acid cycle contain genes for some citric acid cycle enzymes. For example, the cells may condense acetyl-CoA with oxaloacetate, leading to α-ketoglutarate, which is a precursor of several amino acids. They may also convert oxaloacetate to malate, proceeding to fumarate and then to succinate. Together, these two pathways resemble the citric acid cycle, with the right arm following the usual oxidative sequence of the cycle and the left arm following a reversed, reductive sequence **(Fig. 14-14)**. The reductive sequence of reactions might have evolved as a way to regenerate the cofactors reduced during other catabolic reactions (for example, the NADH produced by the glyceraldehyde-3-phosphate dehydrogenase reaction of glycolysis; see Section 13-1).

It is easy to theorize that the evolution of an enzyme to interconvert α-ketoglutarate and succinate could have created a cyclic pathway similar to the modern citric acid cycle. Interestingly, *E. coli*, which uses the citric acid cycle under aerobic growth conditions, uses an interrupted citric acid cycle like the one diagrammed in Figure 14-14 when it is growing anaerobically.

Since the final four reactions of the modern citric acid cycle are metabolically reversible, the primitive citric acid cycle might easily have accommodated one-way flux in the clockwise direction, forming an oxidative cycle. If the complete cycle proceeded in the counterclockwise direction, the result would have been a reductive biosynthetic pathway **(Fig. 14-15)**. This pathway, which would incorporate, or "fix," atmospheric CO_2 into biological molecules, may have preceded the modern CO_2-fixing pathway found in green plants and some photosynthetic bacteria (described in Section 16-3).

CONCEPT REVIEW

- Describe the sources of the acetyl groups that enter the citric acid cycle.
- List the substrates and products for each of the cycle's eight reactions.
- Which products of the citric acid cycle represent forms of energy currency for the cell?
- Which substrates and products of the citric acid cycle regulate flux through the pathway?
- Describe how primitive oxidative and reductive biosynthetic pathways might have been combined to generate a circular metabolic pathway.

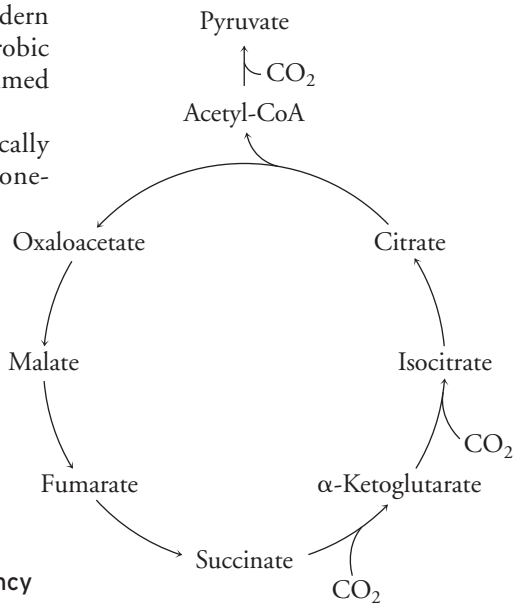


Figure 14-15 A proposed reductive biosynthetic pathway based on the citric acid cycle. This pathway might have operated to incorporate CO_2 into biological molecules.

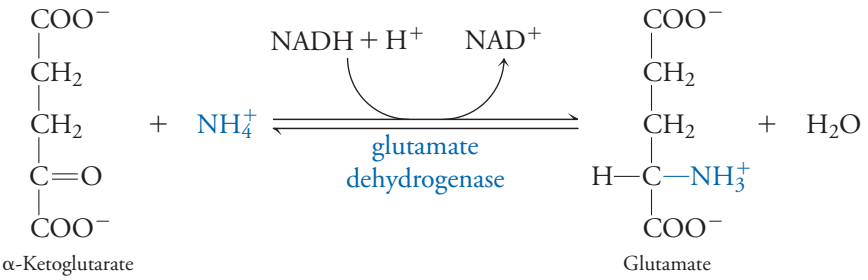
14-3 Anabolic and Catabolic Functions of the Citric Acid Cycle

KEY CONCEPTS
- The citric acid cycle supplies precursors for the synthesis of other compounds.
- Citric acid cycle intermediates can be replenished.

In mammals, six of the eight citric acid cycle intermediates (all except isocitrate and succinate) are the precursors or products of other pathways. For this reason, it is impossible to designate the citric acid cycle as a purely catabolic or anabolic pathway.

Citric acid cycle intermediates are precursors of other molecules

Intermediates of the citric acid cycle can be siphoned off to form other compounds (Fig. 14-16). For example, succinyl-CoA is used for the synthesis of heme. The five-carbon α-ketoglutarate (sometimes called 2-oxoglutarate) can undergo reductive amination by glutamate dehydrogenase to produce the amino acid glutamate:



Glutamate is a precursor of the amino acids glutamine, arginine, and proline. Glutamine in turn is a precursor for the synthesis of purine and pyrimidine nucleotides. We have already seen that oxaloacetate is a precursor of monosaccharides (Section 13-2). Consequently, *any of the citric acid cycle intermediates, which can be converted by the cycle to oxaloacetate, can ultimately serve as gluconeogenic precursors.*

Citrate produced by the condensation of acetyl-CoA with oxaloacetate can be transported out of the mitochondria to the cytosol. ATP-citrate lyase then catalyzes the reaction

$$\text{ATP} + \text{citrate} + \text{CoA} \rightarrow \text{ADP} + P_i + \text{oxaloacetate} + \text{acetyl-CoA}$$

The resulting acetyl-CoA is used for fatty acid and cholesterol synthesis, which take place in the cytosol. Note that the ATP-citrate lyase reaction undoes the work of the

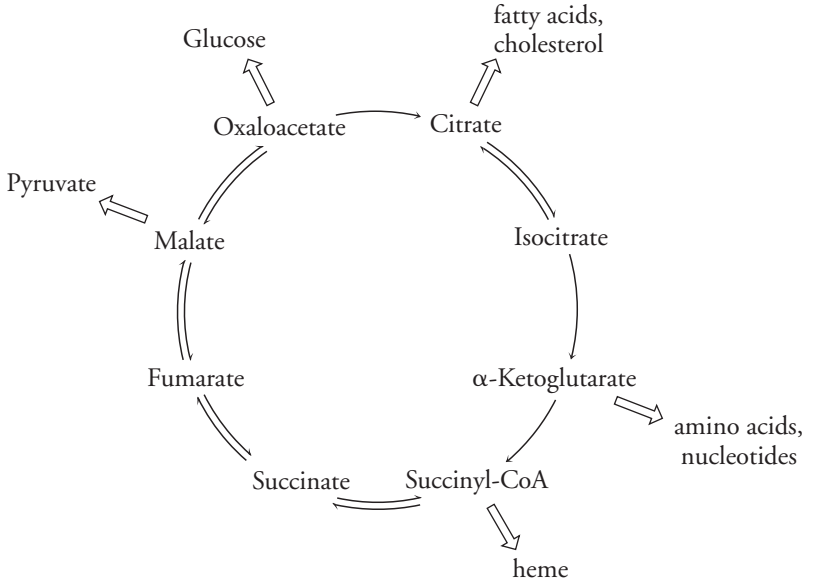
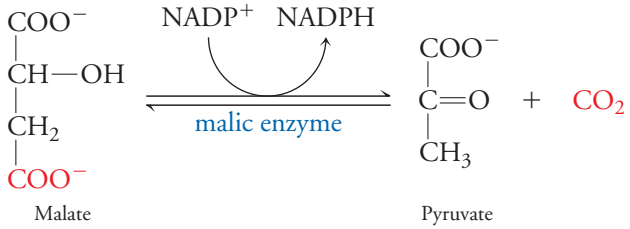


Figure 14-16 **Citric acid cycle intermediates as biosynthetic precursors.**

exergonic citrate synthase reaction. This seems wasteful, but cytosolic ATP-citrate lyase is essential because acetyl-CoA, which is produced in the mitochondria, cannot cross the mitochondrial membrane to reach the cytosol, whereas citrate can. The oxaloacetate product of the ATP-citrate lyase reaction can be converted to malate by a cytosolic malate dehydrogenase operating in reverse. Malate is then decarboxylated by the action of malic enzyme to produce pyruvate:



Pyruvate can reenter the mitochondria and be converted back to oxaloacetate to complete the cycle shown in **Figure 14-17**. In plants, isocitrate is diverted from the citric acid cycle in a biosynthetic pathway known as the **glyoxylate pathway** (Box 14-B).

Anaplerotic reactions replenish citric acid cycle intermediates

Intermediates that are diverted from the citric acid cycle for other purposes can be replenished through **anaplerotic reactions** (from the Greek *ana*, "up," and *plerotikos*, "to fill"; **Fig. 14-18**). One of the most important of these reactions is catalyzed by pyruvate carboxylase (this is also the first step of gluconeogenesis; Section 13-2):

$$\text{pyruvate} + CO_2 + \text{ATP} + H_2O \rightarrow \text{oxaloacetate} + \text{ADP} + P_i$$

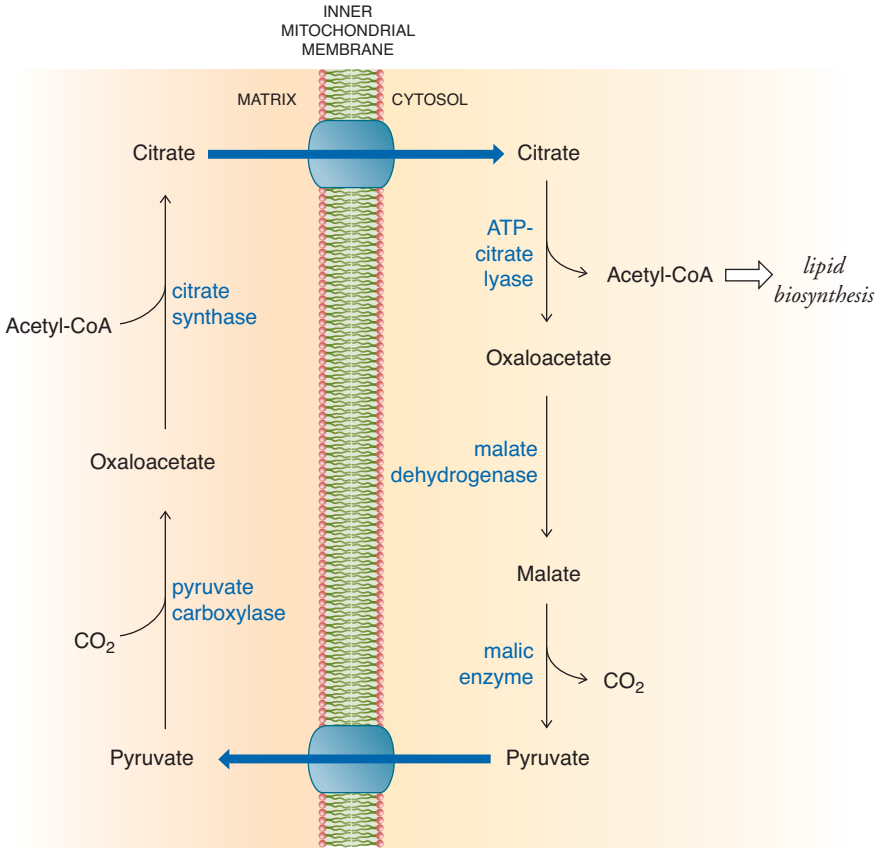


Figure 14-17 The citrate transport system. Both citrate and pyruvate cross the inner mitochondrial membrane via specific transport proteins. This system allows carbon atoms from mitochondrial acetyl-CoA to be transferred to the cytosol for the synthesis of fatty acids and cholesterol.

BOX 14-B BIOCHEMISTRY NOTE

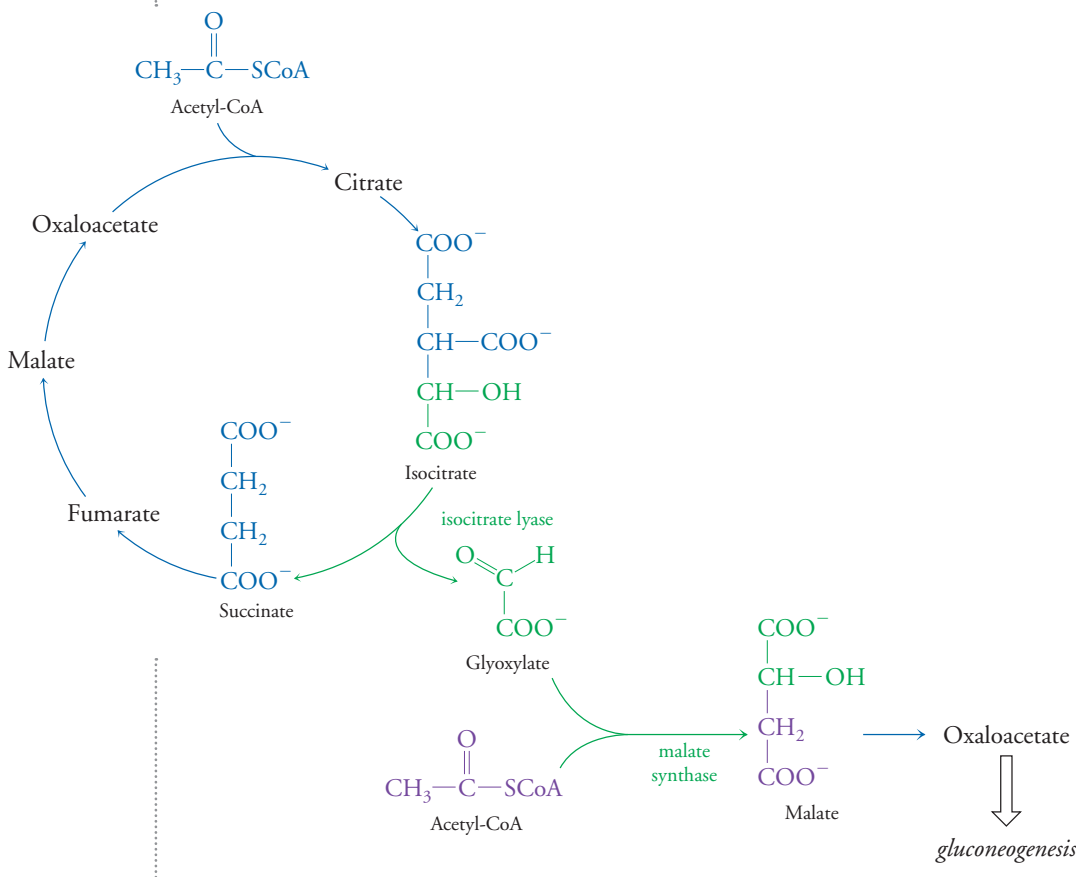
The Glyoxylate Pathway

Plants and some bacterial cells contain certain enzymes that act together with some citric acid cycle enzymes to convert acetyl-CoA to oxaloacetate, a gluconeogenic precursor. Animals lack the enzymes to do this and therefore cannot undertake the net synthesis of carbohydrates from two-carbon precursors. In plants, the glyoxylate pathway includes reactions that take place in the mitochondria and the **glyoxysome,** an organelle that, like the peroxisome, contains enzymes that carry out some essential metabolic processes.

In the glyoxysome, acetyl-CoA condenses with oxaloacetate to form citrate, which is then isomerized to isocitrate, as in the citric acid cycle. However, the next step is not the isocitrate dehydrogenase reaction but a reaction catalyzed by the glyoxysome enzyme isocitrate lyase, which converts isocitrate to succinate and the two-carbon compound glyoxylate. Succinate continues as usual through the mitochondrial citric acid cycle to regenerate oxaloacetate.

In the glyoxysome, the glyoxylate condenses with a second molecule of acetyl-CoA in a reaction catalyzed by the glyoxysome enzyme malate synthase to form the four-carbon compound malate. Malate can then be converted to oxaloacetate for gluconeogenesis. The two reactions that are unique to the glyoxylate pathway are shown in green in the figure below; reactions that are identical to those of the citric acid cycle are shown in blue.

In essence, the glyoxylate pathway bypasses the two CO_2-generating steps of the citric acid cycle (catalyzed by isocitrate dehydrogenase and α-ketoglutarate dehydrogenase) and incorporates a second acetyl unit (at the malate synthase step). The net result of the glyoxylate pathway is the production of a four-carbon compound that can be used to synthesize glucose. This pathway is highly active in germinating seeds, where stored oils (triacylglycerols) are broken down to acetyl-CoA. The glyoxylate pathway thus provides a route for synthesizing glucose from fatty acids. Because animals lack isocitrate lyase and malate synthase, they cannot undertake the net synthesis of carbohydrates from fat.



Question: Write the net equation for the glyoxylate cycle as shown here.

Acetyl-CoA activates pyruvate carboxylase, so when the activity of the citric acid cycle is low and acetyl-CoA accumulates, more oxaloacetate is produced. The concentration of oxaloacetate is normally low since the malate dehydrogenase reaction is thermodynamically unfavorable and the citrate synthase reaction is highly favorable. The replenished oxaloacetate is converted to citrate, isocitrate, α-ketoglutarate, and so on, so the concentrations of all the citric acid cycle intermediates increase and the cycle can proceed more quickly. *Since the citric acid cycle acts as a catalyst, increasing the concentrations of its components increases flux through the pathway.*

The degradation of fatty acids with an odd number of carbon atoms yields the citric acid cycle intermediate succinyl-CoA. Other anaplerotic reactions include the pathways for the degradation of some amino acids, which produce α-ketoglutarate, succinyl-CoA, fumarate, and oxaloacetate. Some of these reactions are transaminations, such as

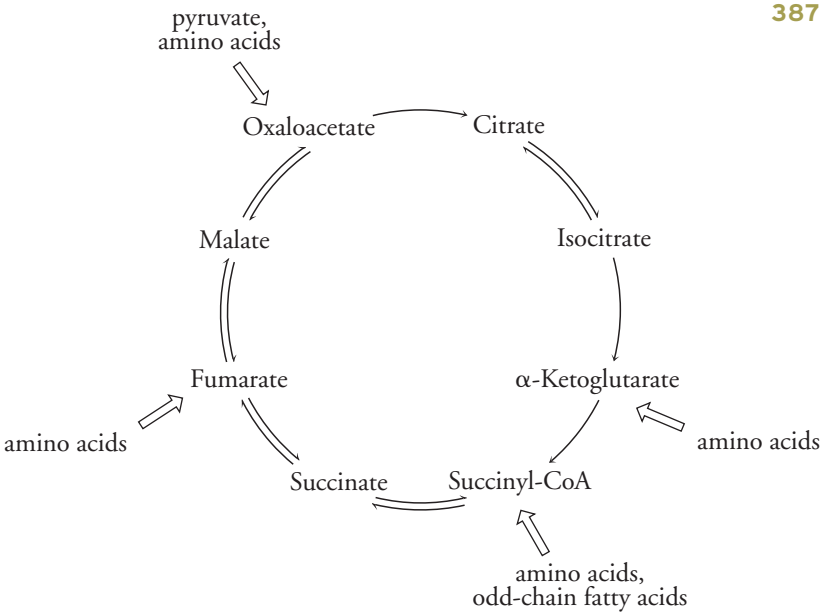
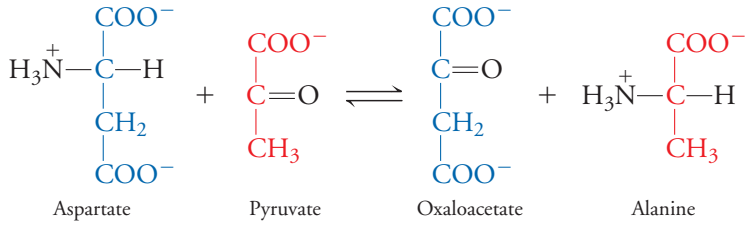
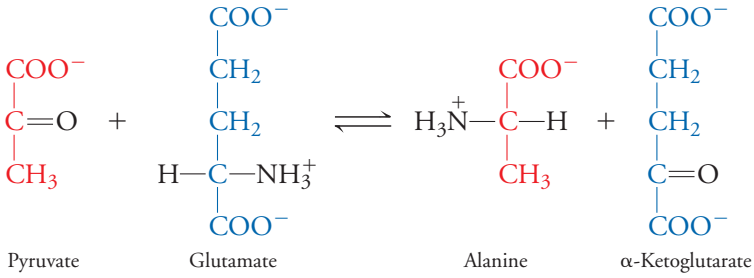


Figure 14-18 **Anaplerotic reactions of the citric acid cycle.**



Because transamination reactions have ΔG values near zero, the direction of flux into or out of the pool of citric acid cycle intermediates depends on the relative concentrations of the reactants.

In vigorously exercising muscle, the concentrations of citric acid cycle intermediates increase about three- to fourfold within a few minutes. This may help boost the energy-generating activity of the citric acid cycle, but it cannot be the sole mechanism, since flux through the citric acid cycle actually increases as much as 100-fold due to the increased activity of the three enzymes at the control points: citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase. The increase in citric acid cycle intermediates may actually be a mechanism for accommodating the large increase in pyruvate that results from rapid glycolysis at the start of exercise. Not all the pyruvate is converted to lactate (Section 13-1); some is shunted into the pool of citric acid cycle intermediates via the pyruvate carboxylase reaction. Some pyruvate also undergoes a reversible reaction catalyzed by alanine aminotransferase



The resulting α-ketoglutarate then augments the pool of citric acid cycle intermediates, thereby increasing the ability of the cycle to oxidize the extra pyruvate.

Note that any compound that enters the citric acid cycle as an intermediate is not itself oxidized; it merely boosts the catalytic activity of the cycle, whose net reaction is still the oxidation of the two carbons of acetyl-CoA.

CONCEPT REVIEW

- Describe how the citric acid cycle supplies the precursors for the synthesis of amino acids, glucose, and fatty acids.
- What does the ATP-citrate lyase reaction accomplish?
- Why is the concentration of oxaloacetate low?
- Why does synthesizing more oxaloacetate increase flux through the citric acid cycle?

SUMMARY

14-1 The Pyruvate Dehydrogenase Reaction

- In order for pyruvate, the product of glycolysis, to enter the citric acid cycle, it must undergo oxidative decarboxylation catalyzed by the multienzyme pyruvate dehydrogenase complex, which yields acetyl-CoA, CO_2, and NADH.

14-2 The Eight Reactions of the Citric Acid Cycle

- The eight reactions of the citric acid cycle function as a multistep catalyst to convert the two carbons of acetyl-CoA to 2 CO_2.
- The electrons released in the oxidative reactions of the citric acid cycle are transferred to 3 NAD^+ and to ubiquinone. The reoxidation of the reduced cofactors generates ATP by oxidative phosphorylation. In addition, succinyl-CoA synthetase yields one molecule of GTP or ATP.
- The regulated reactions of the citric acid cycle are its irreversible steps, catalyzed by citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase.
- The citric acid cycle most likely evolved from biosynthetic pathways leading to α-ketoglutarate or succinate.

14-3 Anabolic and Catabolic Functions of the Citric Acid Cycle

- Six of the eight citric acid cycle intermediates serve as precursors of other compounds, including amino acids, monosaccharides, and lipids. Anaplerotic reactions convert other compounds into citric acid cycle intermediates, thereby allowing increased flux of acetyl carbons through the pathway.

GLOSSARY TERMS

citric acid cycle
mitochondrial matrix
multienzyme complex
substrate-level phosphorylation
Pasteur effect
carcinogenesis
glyoxysome
glyoxylate pathway
anaplerotic reaction

PROBLEMS

14-1 The Pyruvate Dehydrogenase Reaction

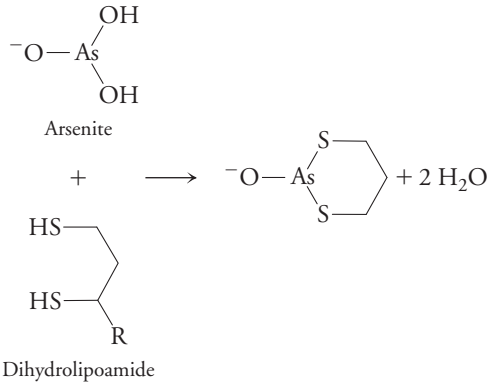
1. What are four possible transformations of pyruvate in mammalian cells?

2. Determine which one of the five steps of the pyruvate dehydrogenase complex reaction is metabolically irreversible and explain why.

3. The product of the pyruvate dehydrogenase complex, acetyl-CoA, is released in step 3 of the overall reaction. What is the purpose of steps 4 and 5?

4. Beriberi is a disease that results from a dietary lack of thiamine, the vitamin that serves as the precursor for thiamine pyrophosphate (TPP). There are two metabolites that accumulate in individuals with beriberi, especially after ingestion of glucose. Which metabolites accumulate and why?

5. Arsenite is toxic in part because it binds to sulfhydryl compounds such as lipoamide, as shown in the figure. What effect would the presence of arsenite have on the citric acid cycle?



6. Using the pyruvate dehydrogenase complex reaction as a model, reconstruct the TPP-dependent yeast pyruvate decarboxylase reaction in alcoholic fermentation (see Section 13-1).

7. How is the activity of the pyruvate dehydrogenase complex affected by **(a)** a high [NADH]/[NAD^+] ratio or **(b)** a high [acetyl-CoA]/[CoASH] ratio?

8. The activity of the pyruvate dehydrogenase complex is also controlled by phosphorylation. Pyruvate dehydrogenase kinase catalyzes the phosphorylation of a specific Ser residue on the E1 subunit of the enzyme, rendering it inactive. The pyruvate dehydrogenase phosphatase enzyme reverses the inhibition by catalyzing the removal of this phosphate group. The kinase and the phosphatase enzymes themselves are controlled by cytosolic Ca^{2+} levels. In the muscle, Ca^{2+} levels rise when the muscle contracts. Which of these two enzymes is inhibited by Ca^{2+} and which is activated by Ca^{2+}?

9. Most cases of pyruvate dehydrogenase deficiency disease that have been studied to date involve a mutation in the E1 subunit of the enzyme. The disease is extremely difficult to treat successfully, but physicians who identify patients with a pyruvate dehydrogenase deficiency will administer thiamine as a first course of treatment. Explain why.

10. A second strategy to treat a pyruvate dehydrogenase deficiency disease (see Problem 9) involves administering dichloroacetate, a compound that inhibits pyruvate dehydrogenase kinase (see Problem 8). How might this strategy be effective?

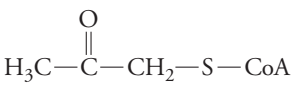
14-2 The Eight Reactions of the Citric Acid Cycle

11. Why is it advantageous for citrate, the product of Reaction 1 of the citric acid cycle, to inhibit phosphofructokinase, which catalyzes the third reaction of glycolysis (see Section 13-1)?

12. Animals that have ingested the leaves of the poisonous South African plant *Dichapetalum cymosum* exhibit a 10-fold increase in levels of cellular citrate. The plant contains fluoroacetate, which is converted to fluoroacetyl-CoA. Describe the mechanism that leads to increased levels of citrate in animals that have ingested this poisonous plant. (*Note:* Fluoroacetyl-CoA is not an inhibitor of citrate synthase.)

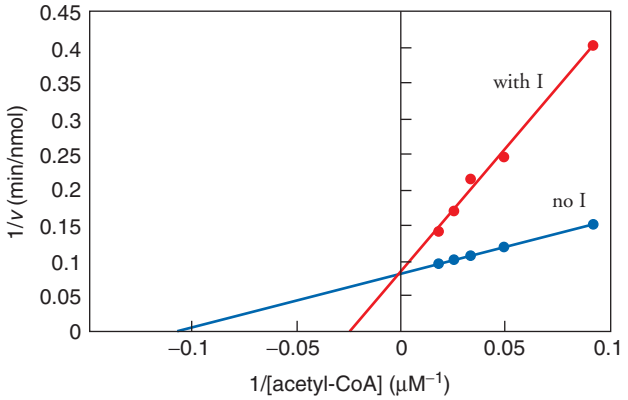
13. Site-directed mutagenesis techniques were used to synthesize a mutant citrate synthase enzyme in which the active-site histidine was converted to an alanine. Why did the mutant citrate synthase enzyme exhibit decreased catalytic activity?

14. The compound *S*-acetonyl-CoA can be synthesized from 1-bromoacetone and coenzyme A.



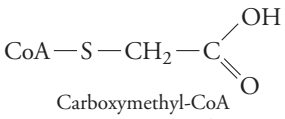
S-Acetonyl-CoA

(a) Write the reaction for the formation of *S*-acetonyl-CoA.
(b) The Lineweaver–Burk plot for the citrate synthase reaction with and without *S*-acetonyl-CoA is shown. What type of inhibitor is *S*-acetonyl-CoA? Explain.



(c) Acetyl-CoA acts as an allosteric activator of pyruvate carboxylase. *S*-acetonyl CoA does not activate pyruvate carboxylase, and it cannot compete with acetyl-CoA for binding to the enzyme. What does this tell you about the binding requirements for an allosteric activator of pyruvate carboxylase?

15. The compound carboxymethyl-CoA (shown below) is a competitive inhibitor of citrate synthase and is a proposed transition state analog. Use this information to propose a structure for the reaction intermediate derived from acetyl-CoA in the rate-limiting step of the reaction, just prior to its reaction with oxaloacetate.



Carboxymethyl-CoA
(transition state analog)

16. Citrate competes with oxaloacetate for binding to citrate synthase. Isocitrate dehydrogenase is activated by Ca^{2+} ions, which are released when muscle contracts. How do these two regulatory strategies assist the cell in making the transition from the rested state (low citric acid cycle activity) to the exercise state (high citric acid cycle activity)?

17. Administration of high concentrations of oxygen (hyperoxia) is effective in the treatment of lung injuries but at the same time can also be quite damaging.
(a) It has been shown that lung aconitase activity is dramatically decreased during hyperoxia. How would the concentration of citric acid cycle intermediates be affected?
(b) The decreased aconitase activity and decreased mitochondrial respiration in hyperoxia are accompanied by elevated levels of glycolysis and the pentose phosphate pathway. Explain why.

18. The scientists who carried out the hyperoxia experiments described in Problem 17 noted that they could mimic this effect by administering either fluoroacetate or fluorocitrate to cells in culture. Explain. (*Hint:* See Solution 12.)

19. Kinetic studies with aconitase in the 1970s revealed that *trans*-aconitate is a competitive inhibitor of the enzyme if *cis*-aconitate is used as the substrate. But if citrate is used as the substrate, *trans*-aconitate is a noncompetitive inhibitor. Propose a hypothesis that explains this observation.

20. A yeast mutant is isolated in which the gene for aconitase is nonfunctional. What are the consequences for the cell, particularly with regard to energy production?

21. The $\Delta G^{\circ\prime}$ value for the isocitrate dehydrogenase reaction is -21 kJ · mol^{-1}. What is K_{eq} for this reaction?

22. The crystal structure of isocitrate dehydrogenase shows that there is a cluster of highly conserved amino acids in the substrate binding pocket—three arginines, a tyrosine, and a lysine. Why are these residues conserved and what is a possible role for these amino acid side chains in substrate binding?

23. In bacteria, isocitrate dehydrogenase is regulated by phosphorylation of a specific Ser residue in the enzyme active site. X-ray structures of the phosphorylated and the nonphosphorylated enzyme show no significant conformational differences.

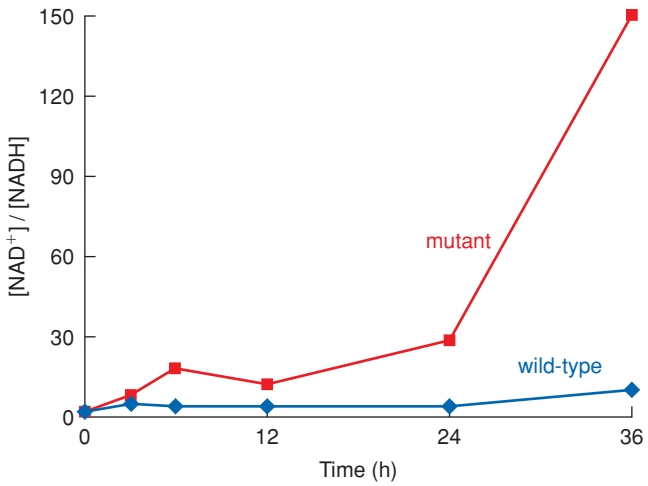
(a) How does phosphorylation regulate isocitrate dehydrogenase activity?

(b) To confirm their hypothesis, the investigators constructed a mutant enzyme in which the Ser residue was replaced with an Asp residue. The mutant was unable to bind isocitrate. Are these results consistent with the hypothesis you proposed in part (a)?

24. The expression of several enzymes changes when yeast grown on glucose are abruptly shifted to a two-carbon food source such as acetate.

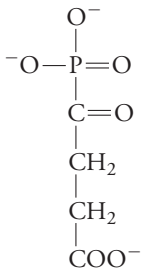
(a) Why does the level of expression of isocitrate dehydrogenase increase when the yeast are shifted from glucose to acetate?

(b) The metabolism of a yeast mutant with a nonfunctional isocitrate dehydrogenase enzyme was compared to that of a wild-type yeast. The yeast were grown on glucose and then abruptly shifted to acetate as the sole carbon source. The [NAD$^+$]/[NADH] ratio was measured over a period of 48 hours. The results are shown below. Why does the ratio increase slightly at 36 hours for the wild-type yeast? Why is there a more dramatic increase in the ratio for the mutant?



25. Using the pyruvate dehydrogenase complex reaction as a model, draw the intermediates of the α-ketoglutarate dehydrogenase reaction. Describe what happens in each of the five reaction steps.

26. Using the mechanism you drew for Problem 25, explain how succinyl phosphonate (above right) inhibits α-ketoglutarate dehydrogenase.



Succinyl phosphonate

27. Succinyl-CoA inhibits both citrate synthase and α-ketoglutarate dehydrogenase. How is succinyl-CoA able to inhibit both enzymes?

28. A patient with an α-ketoglutarate deficiency exhibits a small increase in blood pyruvate level and a large increase in blood lactate level, resulting in a [lactate]/[pyruvate] ratio that is many times greater than normal. Explain the reason for these symptoms.

29. Succinyl-CoA synthetase is also called succinate thiokinase. Why is the enzyme considered to be a kinase?

30. Succinyl-CoA synthetase is a dimer composed of an α subunit and a β subunit. A single gene codes for the α subunit protein. Two genes code for two different β subunit proteins—one subunit is specific for GDP, and one is specific for ADP.

(a) The β subunit specific for ADP is expressed in "catabolic tissues" such as brain and muscles, whereas the β subunit specific for GDP is expressed in "anabolic tissues" such as liver and kidneys. Propose a hypothesis to explain this observation.

(b) Individuals who are born with a mutation in the gene coding for the α subunit of the enzyme experience severe lactic acidosis and usually die within a few days of birth. Why is this mutation so deleterious?

(c) Individuals who are born with a mutation in the gene coding for the ADP-specific β subunit of the enzyme experience normal to moderately elevated concentrations of lactate and usually survive to their early 20s. Why is the prognosis for these patients better than for patients with a mutation in the gene for the α subunit?

31. Malonate is a competitive inhibitor of succinate dehydrogenase. What citric acid cycle intermediates accumulate if malonate is present in a preparation of isolated mitochondria?

32. Succinate dehydrogenase is not considered to be part of the glyoxylate pathway, yet it is vital to the proper functioning of the pathway. Why?

33. The $\Delta G^{\circ\prime}$ for the fumarase reaction is 23.4 kJ · mol^{-1}, but the ΔG value is close to zero. What is the ratio of fumarate to malate under cellular conditions at 37°C? Is this reaction likely to be a control point for the citric acid cycle?

34. A mutant bacterial fumarase was constructed by replacing the Glu (E) at position 315 with Gln (Q). The kinetic parameters of the mutant and wild-type enzymes were compared, and the results are shown in the table below. Explain the significance of the changes.

	Wild-type enzyme	**E315Q mutant enzyme**
V_{max} (μmol · min^{-1} · mg^{-1})	345	32
K_M (mM)	0.21	0.25
k_{cat} (s^{-1})	1150	107
k_{cat}/K_M (M^{-1} · s^{-1})	5.6×10^6	4.3×10^5

35. Reaction 8 and Reaction 1 of the citric acid cycle can be considered to be coupled because the exergonic cleavage of the thioester bond of acetyl-CoA in Reaction 1 drives the regeneration of oxaloacetate in Reaction 8.

(a) Write the equation for the overall coupled reaction and calculate its $\Delta G^{\circ\prime}$.

(b) What is the equilibrium constant for the coupled reaction? Compare this equilibrium constant with the equilibrium constant of Reaction 8 alone.

36. Malate dehydrogenase is more active in cells oxidizing glucose aerobically than in cells oxidizing glucose anaerobically. Explain why.

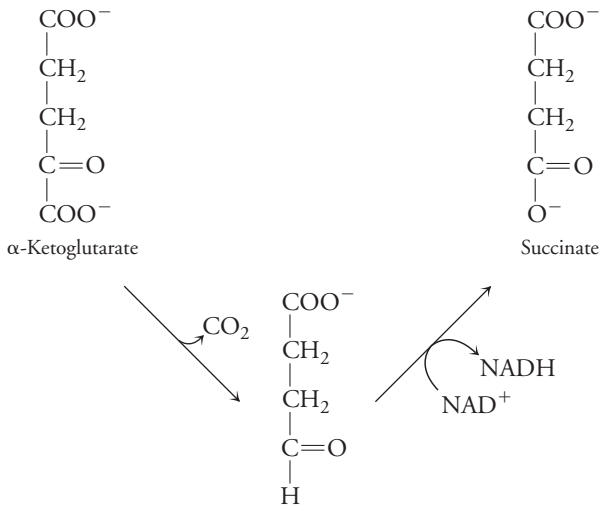
37. **(a)** Oxaloacetate labeled at C4 with ^{14}C is added to a suspension of respiring mitochondria. What is the fate of the labeled carbon?

(b) Acetyl-CoA labeled at C1 with ^{14}C is added to a suspension of respiring mitochondria. What is the fate of the labeled carbon?

38. When leavened bread is made, the bread dough is "punched" down and then put in a warm place to "rise" to increase its volume. Give a biochemical explanation for this observation.

39. Flux through the citric acid cycle is regulated by the simple mechanisms of **(a)** substrate availability, **(b)** product inhibition, and **(c)** feedback inhibition. Give examples of each.

40. Certain microorganisms with an incomplete citric acid cycle decarboxylate α-ketoglutarate to produce succinate semialdehyde. A dehydrogenase then converts succinate semialdehyde to succinate. These reactions can be combined with other standard citric acid cycle reactions to create a pathway from citrate to oxaloacetate. How does this alternative pathway compare to the standard citric acid cycle in its ability to make free energy available to the cell?



14-3 Anabolic and Catabolic Functions of the Citric Acid Cycle

41. Why is the reaction catalyzed by pyruvate carboxylase the most important anaplerotic reaction of the citric acid cycle?

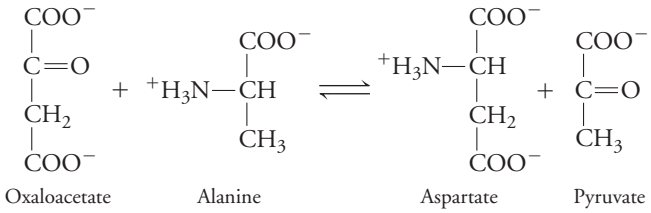
42. Why is the activation of pyruvate carboxylase by acetyl-CoA a good regulatory strategy?

43. Many amino acids are broken down to intermediates of the citric acid cycle.

(a) Why can't these amino acid "remnants" be completely oxidized to CO_2 by the citric acid cycle?

(b) Explain why amino acids that are broken down to pyruvate can be completely oxidized by the citric acid cycle.

44. Describe how the transamination reaction below could function as an anaplerotic reaction for the citric acid cycle.



45. Is net synthesis of glucose in mammals possible from the following compounds?

(a) The fatty acid palmitate (16:0), which is degraded to eight acetyl-CoA

(b) The fatty acid pentadecanoate (15:0), which is degraded to six acetyl-CoA and one propionyl-CoA

(c) Glyceraldehyde-3-phosphate

(d) Leucine, which is degraded to acetyl-CoA and acetoacetate (a compound that is metabolically equivalent to two acetyl-CoA groups)

(e) Tryptophan, which is degraded to alanine and acetoacetate

(f) Phenylalanine, which is degraded to acetoacetate and fumarate

46. Pancreatic islet cells cultured in the presence of 1–20 mM glucose showed increased activities of pyruvate carboxylase and the E1 subunit of the pyruvate dehydrogenase complex proportional to the increase in glucose concentration. Explain why.

47. A physician is attempting to diagnose a neonate with a pyruvate carboxylase deficiency. An injection of alanine normally leads to a gluconeogenic response, but in the patient no such response occurs. Explain.

48. The physician treats the patient described in Problem 47 by administering glutamine. Explain why glutamine supplements are effective in treating the disease.

49. Physicians often attempt to treat a pyruvate carboxylase deficiency by administering biotin. Explain why this strategy might be effective.

50. Patients with a pyruvate dehydrogenase deficiency and patients with a pyruvate carboxylase deficiency (see Problems 47–49) both have high blood levels of pyruvate and lactate. Explain why.

51. Metabolites in rat muscle were measured before and after exercising. After exercise, the rat muscle showed an increase in oxaloacetate concentration, a decrease in phosphoenolpyruvate concentration, and no change in pyruvate concentration. Explain.

52. Oxygen does not appear as a reactant in any of the citric acid cycle reactions, yet it is essential for the proper functioning of the cycle. Explain why.

53. The activity of isocitrate dehydrogenase in *E. coli* is regulated by the covalent attachment of a phosphate group to the enzyme. Phosphorylated isocitrate dehydrogenase is inactive. When acetate is the food source for a culture of *E. coli,* isocitrate dehydrogenase is phosphorylated.

(a) Draw a diagram showing how acetate is metabolized in *E. coli.*

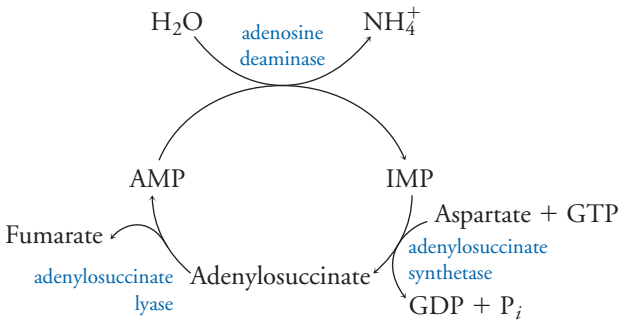
(b) When glucose is added to the culture, the phosphate group is removed from isocitrate dehydrogenase. How does flux through the metabolic pathways change in *E. coli* when glucose is the food source instead of acetate?

54. Yeast are unusual in that they are able to use ethanol as a gluconeogenic substrate. Ethanol is converted to glucose using the assistance of the glyoxylate pathway. Describe how the ethanol → glucose conversion takes place.

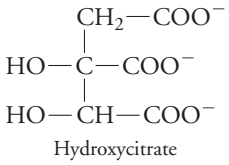
55. Animals lack a glyoxylate pathway and cannot convert fats to carbohydrates. If an animal is fed a fatty acid with all its carbons replaced by the isotope ^{14}C, some of the labeled carbons later appear in glucose. How is this possible?

56. A bacterial mutant with low levels of isocitrate dehydrogenase is able to grow normally when the culture medium is supplemented with glutamate. Explain why.

57. The purine nucleotide cycle (shown below) is an important pathway in muscle cells. The activity of the cycle increases during periods of high muscle activity. Explain how the purine nucleotide cycle contributes to the ability of the muscle cell to generate energy during intense exercise. IMP is inosine monophosphate.



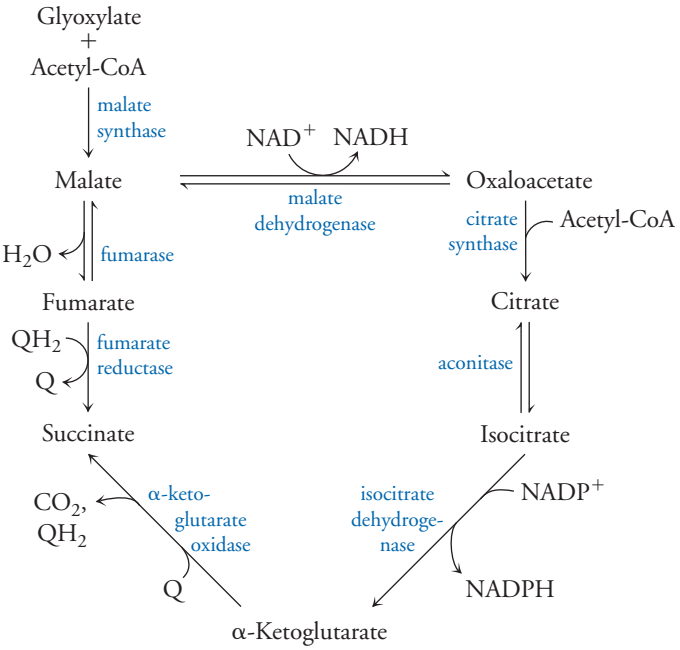
58. The plant metabolite hydroxycitrate is advertised as an agent that prevents fat buildup.



Hydroxycitrate

(a) How does this compound differ from citrate?
(b) Hydroxycitrate inhibits the activity of ATP-citrate lyase. What kind of inhibition is likely to occur?
(c) Why might inhibition of ATP-citrate lyase block the conversion of carbohydrates to fats?
(d) The synthesis of what other compounds would be inhibited by hydroxycitrate?

59. *Helicobacter pylori* is a bacterium that colonizes the upper gastrointestinal tract in humans and is the causative agent of chronic gastritis, ulcers, and possibly gastric cancer. Knowledge of the intermediary metabolism of this organism will be helpful in the development of effective drug therapies to treat these diseases. The citric acid "cycle" in *H. pylori* is a noncyclic, branched pathway that is used to produce biosynthetic intermediates instead of metabolic energy. Succinate is produced in the "reductive branch," whereas α-ketoglutarate is produced in the "oxidative branch." The two branches are linked by the α-ketoglutarate oxidase reaction. The pathway is shown in the diagram below.



(a) Compare and contrast the citric acid cycle in *H. pylori* with the citric acid cycle in mammals.
(b) The K_M values for the enzymes listed in the table below are higher than the K_M values for the corresponding enzymes in other species of bacteria. What does this tell you about the conditions under which the citric acid cycle operates in *H. pylori*?
(c) Compare the properties of *H. pylori* citrate synthase with mammalian citrate synthase.
(d) What enzymes might serve to regulate the citric acid cycle in *H. pylori*?
(e) What enzymes might be used as drug targets for persons suffering from gastritis, ulcers, or gastric cancer?

Enzyme	Substrate	Inhibitors	Activators
Citrate synthase	Acetyl-CoA, oxaloacetate	ATP	
Aconitase	Citrate		
Isocitrate dehydrogenase ($NADP^+$-dependent)	Isocitrate, $NADP^+$	Higher concentrations of $NADP^+$, isocitrate	AMP (slight)
α-ketoglutarate oxidase	α-ketoglutarate, FAD		CoASH
Malate dehydrogenase	Oxaloacetate, NADH		
Fumarase	Malate		
Fumarate reductase	Fumarate, QH_2		
Malate synthase	Glyoxylate, acetyl-CoA		

60. *H. pylori,* whose citric acid cycle has an oxidative branch and a reductive branch (see Problem 59), uses amino acids and fatty acids present in the gastrointestinal tract as a source of biosynthetic intermediates.

(a) Describe how *H. pylori* uses the acetyl-CoA derived from fatty acid breakdown to synthesize glucose and glutamate.

(b) Describe how *H. pylori* converts aspartate to glutamate.

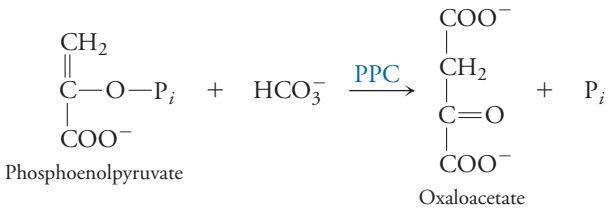
61. Yeast cells that are grown on nonfermentable substrates and then abruptly switched to glucose exhibit substrate-induced inactivation of several enzymes. Which enzymes would glucose cause to be inactivated and why?

62. Phagocytes such as macrophages and neutrophils are cellular components of the immune system that protect the host against damage caused by invading microorganisms. Phagocytes engulf and internalize a foreign microbe, forming a membrane-bound structure called a phagosome. The phagosome then fuses with the lysosome, a cellular organelle that contains a wide variety of proteolytic enzymes that destroy the pathogen if the host is fortunate. But some microbes can survive the harsh conditions of the phagolysosome. An example is *Mycobacterium tuberculosis,* which can reside in the macrophage in a dormant state for a prolonged period of time. Investigators noted that following engulfment by the macrophage, the levels of bacterial isocitrate lyase, malate synthase, citrate synthase, and malate dehydrogenase increased to levels as much as 20 times above normal.

(a) What pathway(s) does *M. tuberculosis* employ while in the phagosome and why are these pathways essential to its survival?

(b) What might be good drug targets for treating a patient infected with *M. tuberculosis*?

63. Bacteria and plants (but not animals) possess the enzyme phosphoenolpyruvate carboxylase (PPC), which catalyzes the reaction shown here.



Phosphoenolpyruvate — Oxaloacetate

(a) What is the importance of this reaction to the organism?

(b) PPC is allosterically activated by both acetyl-CoA and fructose-1,6-bisphosphate. Explain these regulatory strategies.

64. Succinic acid is an important compound used by the pharmaceutical, cosmetics, and food industries. "Green" production of succinic acid by fermenting bacteria is a more environmentally responsible way to produce the compound, which has traditionally been synthesized from petrochemicals. Investigators interested in optimizing bacterial succinic acid production noted that malate dehydrogenase activity increased under anaerobic conditions.

(a) Draw a reaction scheme outlining how succinic acid can be produced from phosphoenolpyruvate. Include the names of all reactants, products, and enzymes.

(b) Why is it essential that the production of succinic acid take place under anaerobic conditions?

65. Experiments with cancer cells grown in culture show that glutamine is consumed at a high rate and used for biosynthetic reactions, aside from protein synthesis. One possible pathway involves the conversion of glutamine to glutamate and then to α-ketoglutarate. The α-ketoglutarate can then be used to produce pyruvate for gluconeogenesis.

(a) Describe the types of reactions that convert glutamine to α-ketoglutarate.

(b) Give the sequence of enzymes that can convert α-ketoglutarate to pyruvate.

66. Many cancer cells carry out glycolysis at a high rate but convert most of the resulting pyruvate to lactate rather than to acetyl-CoA. Acetyl-CoA, however, is required for the synthesis of fatty acids, which are needed in large amounts by rapidly growing cancer cells. In these cells, the isocitrate dehydrogenase reaction apparently operates in reverse. Explain why this reaction could facilitate the conversion of amino acids such as glutamate into fatty acids.

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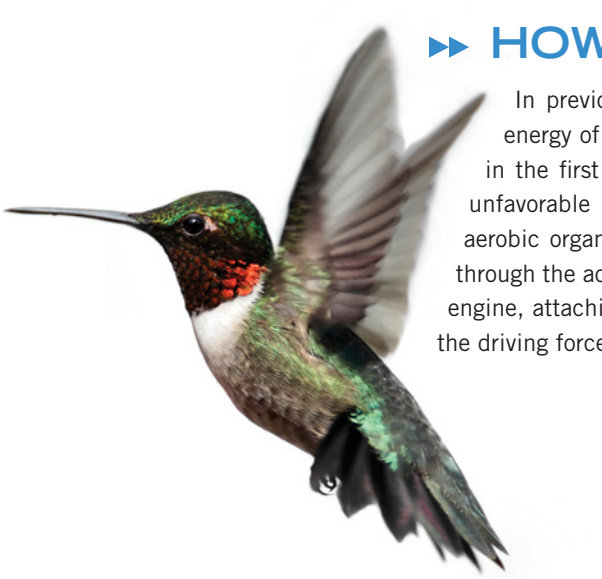
chapter 15

OXIDATIVE PHOSPHORYLATION



[Steve Byland/iStockphoto]

▸▸ HOW does ATP synthase carry out an unfavorable reaction?

In previous chapters, we have seen numerous examples of cells using the free energy of the ATP reaction to carry out cellular work. But in order to produce ATP in the first place, cells must add a third phosphate to ADP, a thermodynamically unfavorable reaction. Glycolysis and the citric acid cycle provide some ATP, but in aerobic organisms, such as the hummingbird shown here, most of the ATP is made through the action of a mitochondrial ATP synthase. This enzyme functions like a rotary engine, attaching a phosphoryl group to ADP as it spins. In this chapter, we'll examine the driving force for this unusual molecular machine.

THIS CHAPTER IN CONTEXT

Part 1 Foundations

Part 2 Molecular Structure and Function

Part 3 Metabolism

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Part 4 Genetic Information

Do You Remember?

- **Living organisms obey the laws of thermodynamics (Section 1-3).**
- **Transporters obey the laws of thermodynamics, providing a way for solutes to move down their concentration gradients or using ATP to move substances against their gradients (Section 9-1).**
- **Coenzymes such as NAD^+ and ubiquinone collect electrons from compounds that become oxidized (Section 12-2).**
- **A reaction that breaks a phosphoanhydride bond in ATP occurs with a large change in free energy (Section 12-3).**