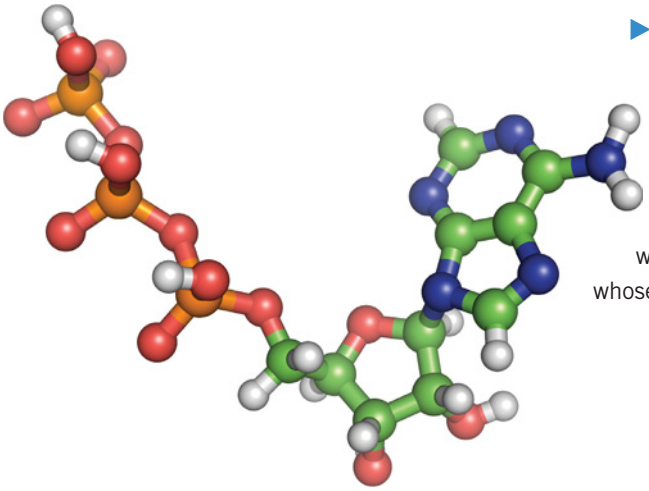
chapter 12

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.

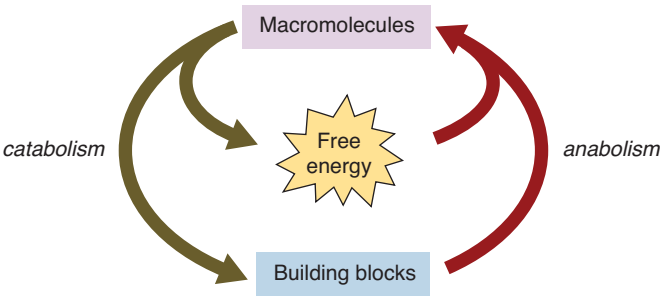


Figure 12-1 Catabolism and anabolism. Catabolic (degradative) reactions yield free energy and small molecules that can be used for anabolic (synthetic) reactions. Metabolism is the sum of all catabolic and anabolic processes.

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

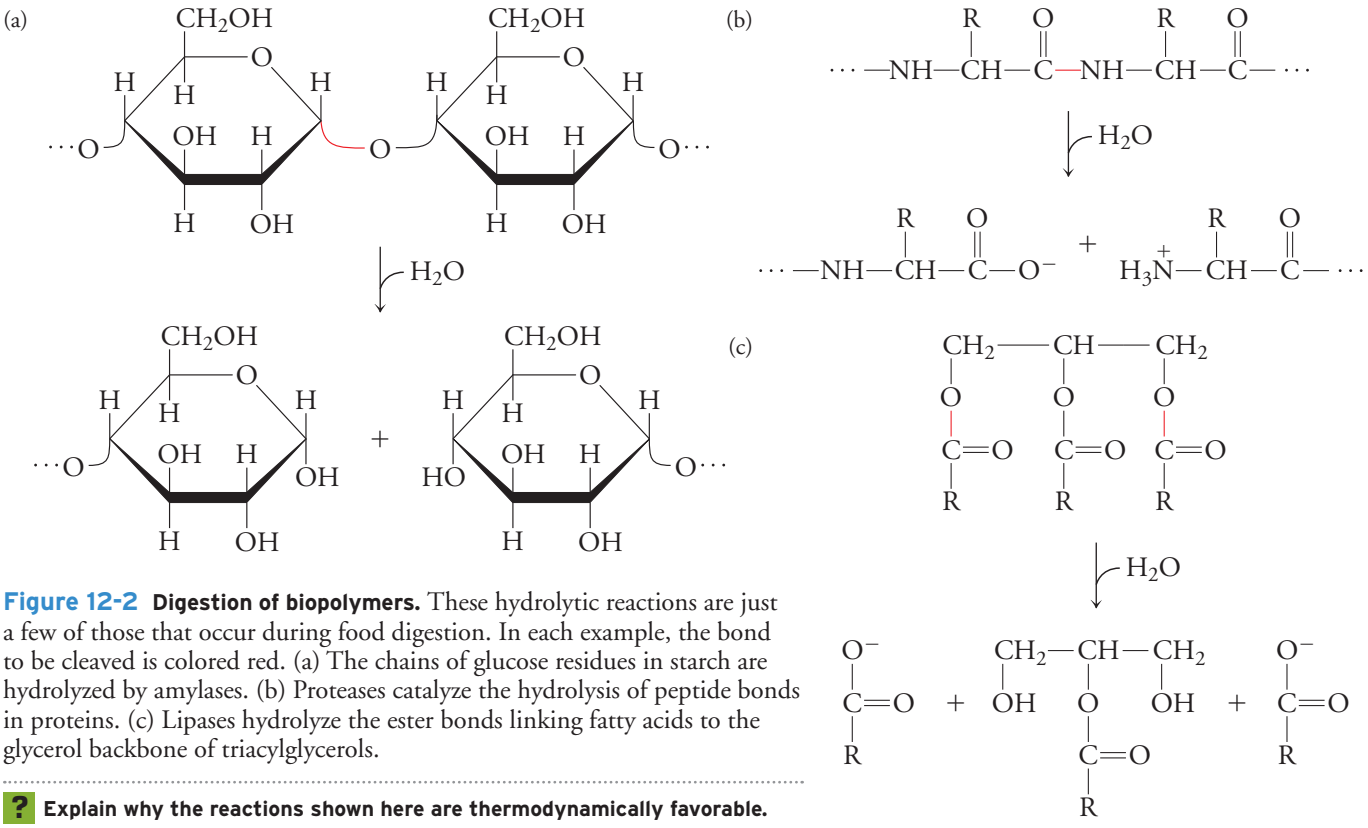
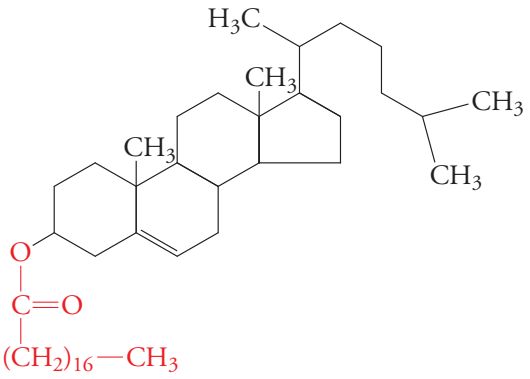


Figure 12-2 **Digestion of biopolymers.** These hydrolytic reactions are just a few of those that occur during food digestion. In each example, the bond to be cleaved is colored red. (a) The chains of glucose residues in starch are hydrolyzed by amylases. (b) Proteases catalyze the hydrolysis of peptide 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?

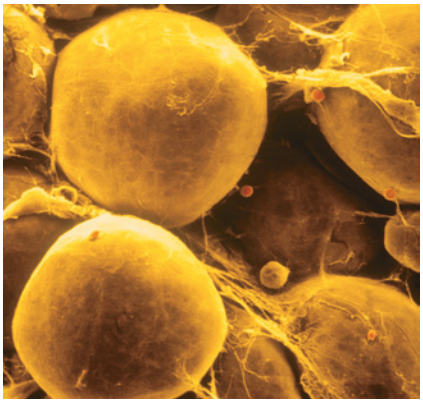


Figure 12-3 Adipocytes. These cells, which make up adipose tissue, contain a small amount of cytoplasm surrounding a large globule of triacylglycerols (fat). [© CNRI/ Phototake.]

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).

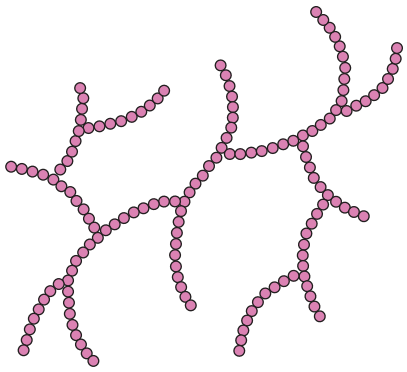
Amino acids and glucose are both required to synthesize nucleotides. Asp, Gln, and Gly supply some of the carbon and nitrogen atoms used to build the purine and pyrimidine bases (Section 18-3). The ribose-5-phosphate component of nucleotides is derived from glucose by a pathway that converts the six-carbon 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)

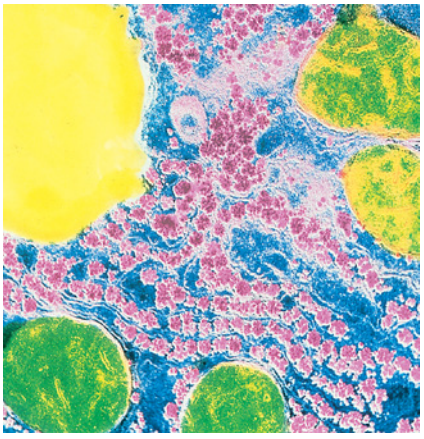
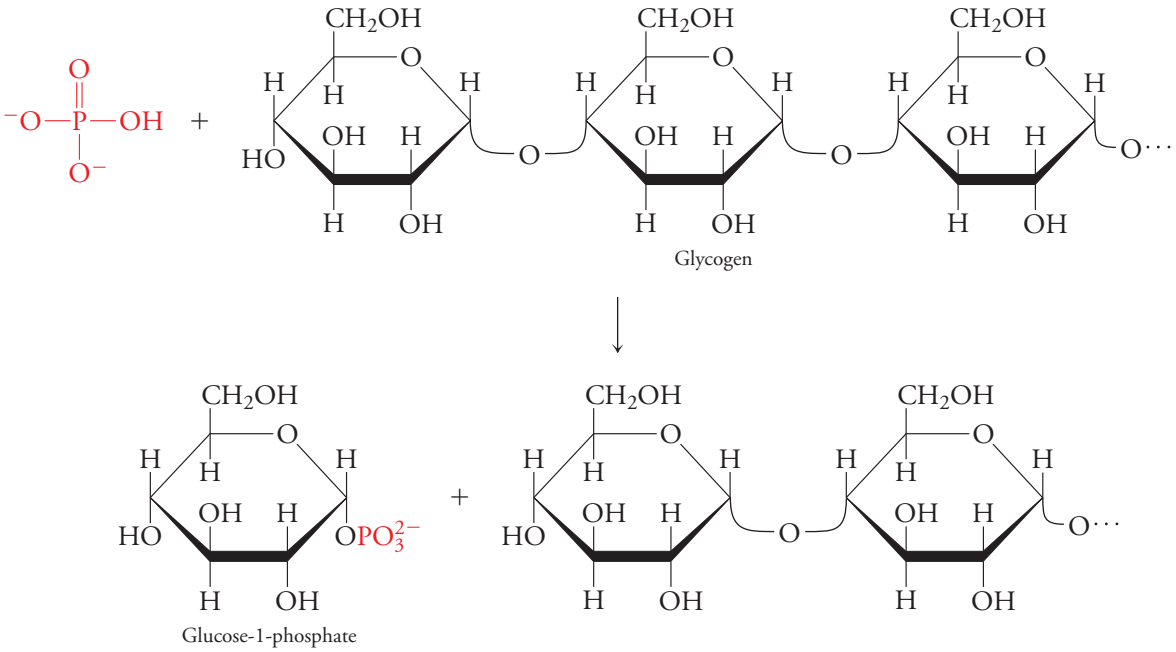


Figure 12-4 Glycogen structure. (a) Schematic diagram of a glycogen molecule. Each circle represents a glucose monomer, and branches occur every 8 to 14 residues. (b) Electron micrograph of a liver cell showing glycogen granules (colored pink). Mitochondria are green, and a fat globule is yellow. [©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

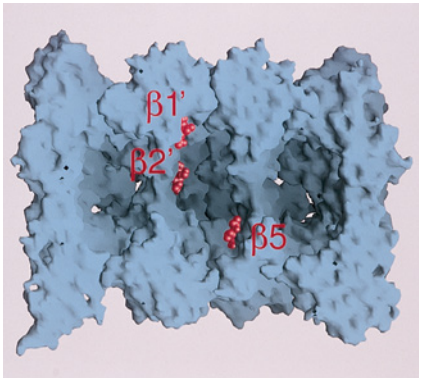


Figure 12-5 Structure of the yeast proteasome core. This cutaway view shows the inner chamber, where proteolysis occurs. Additional protein complexes (not shown) assist the entry of proteins into the proteasome. The red structures mark the locations of three protease active sites. [Courtesy Robert Huber,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

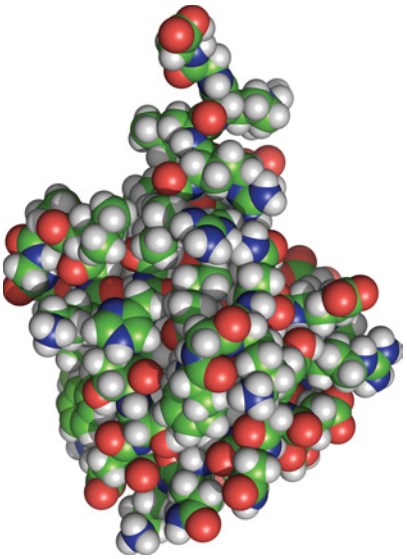


Figure 12-6 Ubiquitin. Several copies of this 76-residue protein are linked to Lys residues in proteins that are to be degraded by a proteasome. Atoms are color-coded: C green, O red, N blue, and H white. [Structure (pdb 1UBQ) determined by S. Vijay-Kumar, C. E. Bugg, and W. J. Cook.]

? 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.

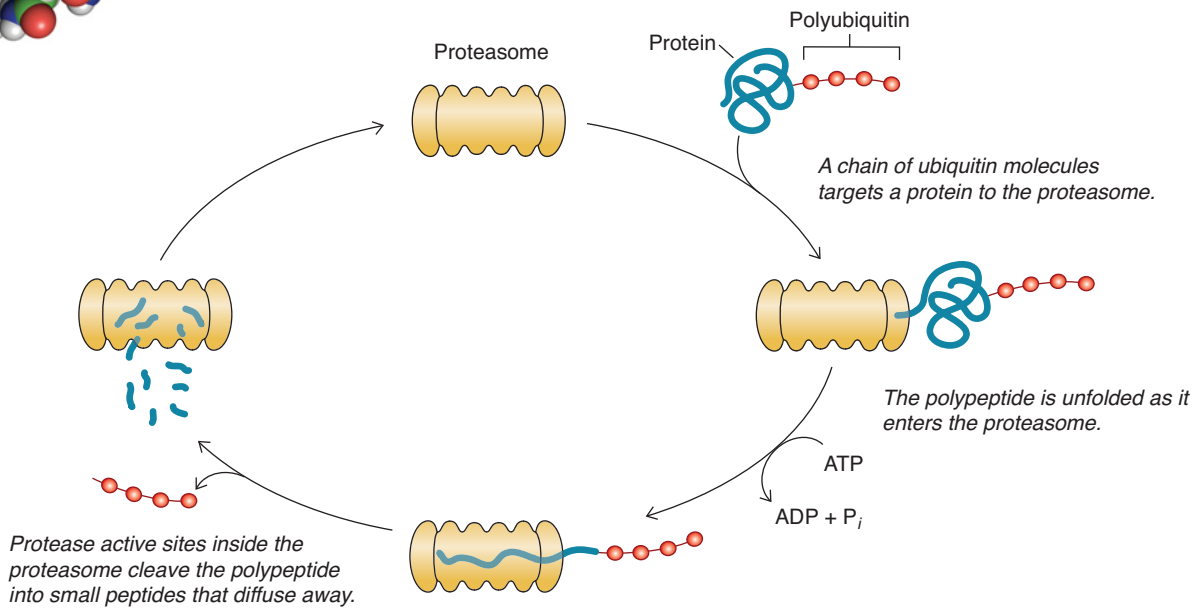


Figure 12-7 Protein degradation by the proteasome.

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.

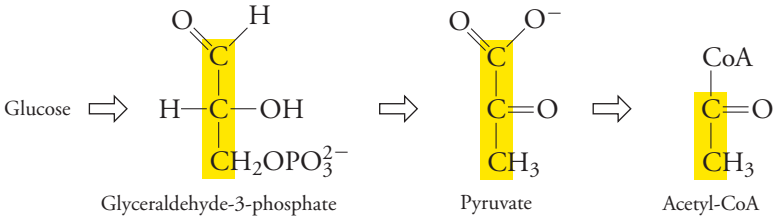


Figure 12-8 **Some intermediates resulting from glucose catabolism.**

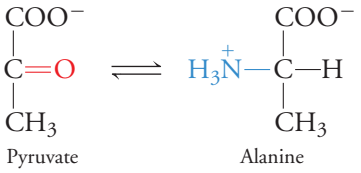
? Compare the oxidation states of the carbons in glyceraldehyde-3-phosphate and in pyruvate.

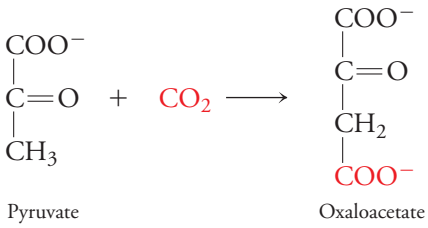
Some major metabolic pathways share a few common intermediates

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

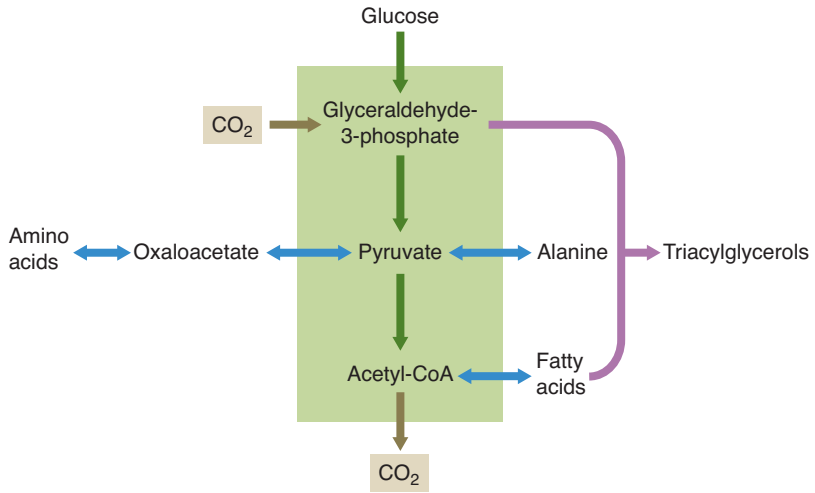


Figure 12-9 **Some of the metabolic roles of the common intermediates.**

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

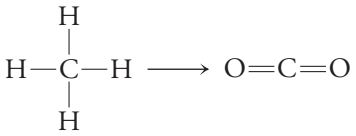
intermediates can be broken down to CO_2 by the **citric acid cycle,** a metabolic pathway essential for the catabolism of all metabolic fuels.

Many metabolic pathways include oxidation–reduction reactions

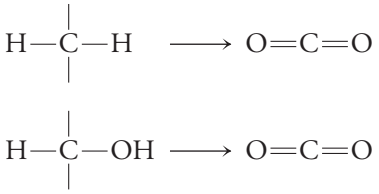
In general, *the catabolism of amino acids, monosaccharides, and fatty acids is a process of oxidizing carbon atoms, and the synthesis of these compounds involves carbon reduction.* Recall from Section 1-3 that **oxidation** is defined as the loss of electrons and **reduction** is the gain of electrons. 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.

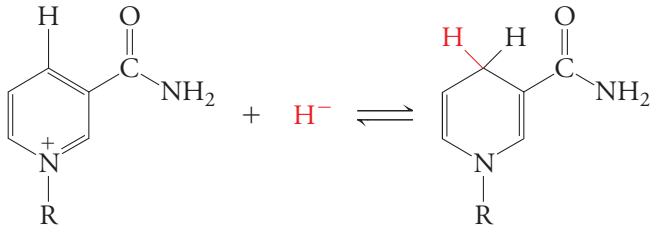
In following metabolic pathways that include oxidation–reduction reactions, we can examine the redox state of the carbon atoms, and we can also trace the path of the electrons that are transferred during the oxidation–reduction reaction. In some cases, this is straightforward, as when an oxidized metal 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,**

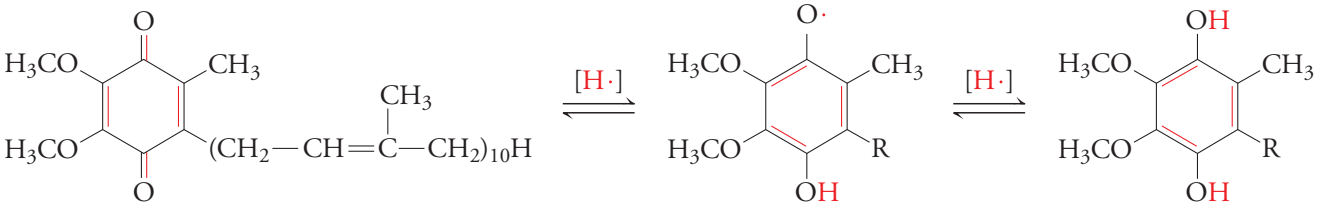
organic compounds that allow an enzyme to carry out a particular chemical reaction (Section 6-2). The redox-active portion of NAD^+ and $NADP^+$ is the nicotinamide group, which accepts a hydride ion to form NADH or NADPH.



$NAD(P)^+$ (*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.

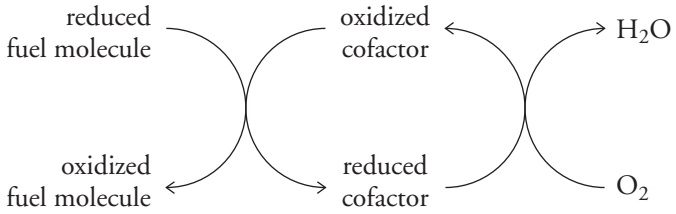
Many cellular oxidation–reduction reactions take place at membrane surfaces, for example, in the inner membranes of mitochondria and chloroplasts in eukaryotes and in the plasma membrane of prokaryotes. In these cases, a membrane-associated enzyme may transfer electrons from a substrate to a lipid-soluble 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

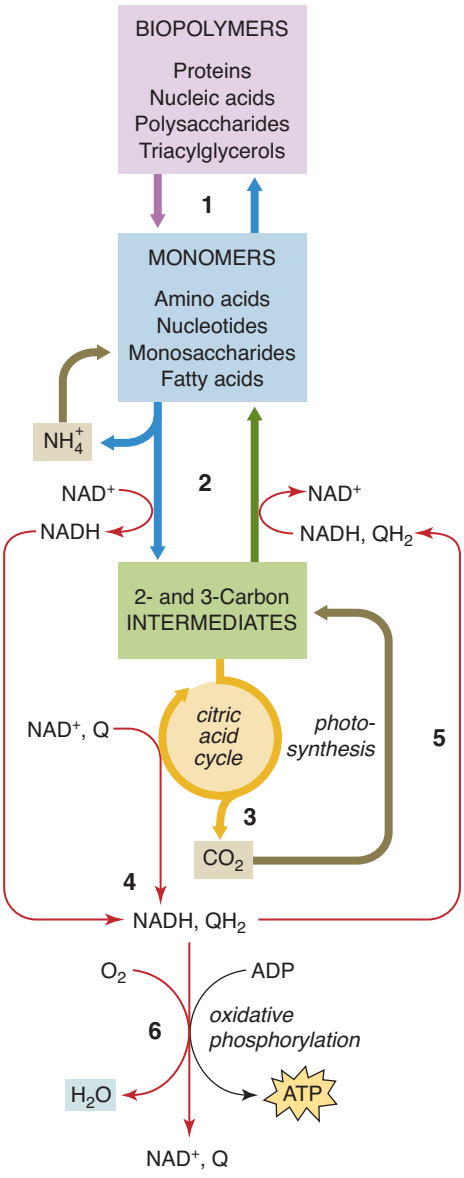


Figure 12-10 Outline of metabolism. In this composite diagram, downward arrows represent catabolic processes, and upward arrows represent anabolic processes. Red arrows indicate some major oxidation–reduction reactions. The major metabolic processes are highlighted: (1) Biological polymers (proteins, 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?

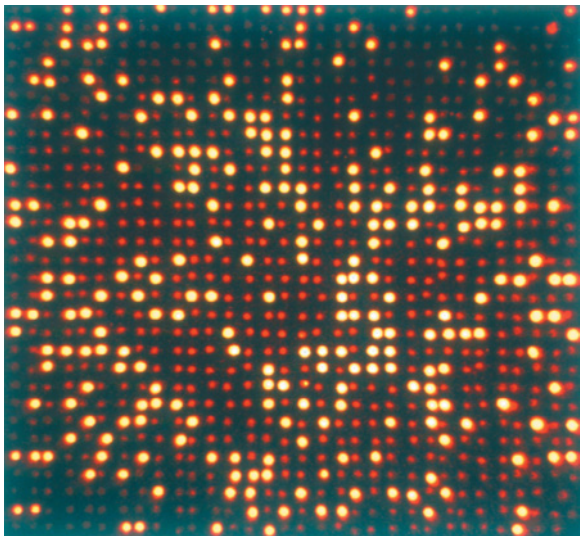
cooperativity: Thousands of different microbial species, amounting to some 100 trillion cells, can live in or on the human body. Collectively, these organisms express millions of different genes and carry out a correspondingly rich set of metabolic activities.

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.



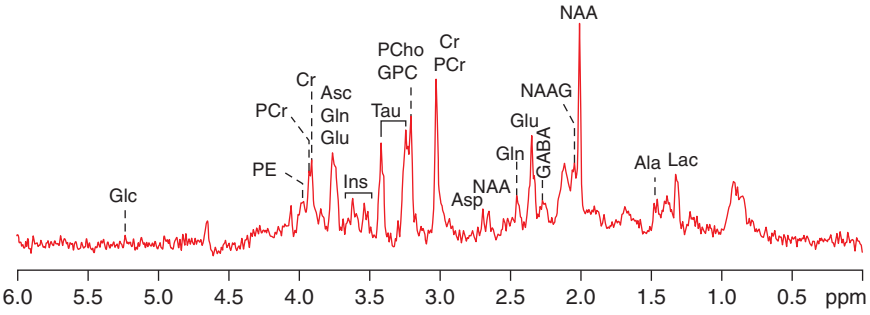
[Voker Steger/Science Photo Library/Photo Researchers.]

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,

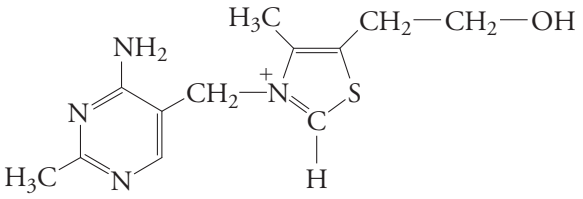
TABLE 12-2 Vitamins and Their Roles

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.

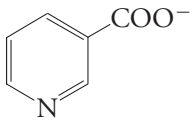
Many vitamins were discovered through studies of nutritional deficiencies. One of the earliest links between nutrition and disease was observed centuries ago in sailors suffering from scurvy, an illness caused by vitamin C deficiency (Box 5-D). A study of the disease beriberi led to the discovery of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

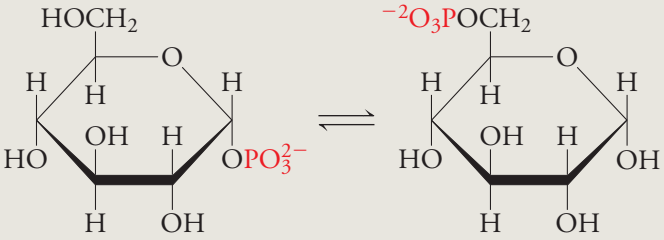
Temperature	25°C (298 K)
Pressure	1 atm
Reactant concentration	1 M
pH	7.0 ($[H^+] = 10^{-7}$ M)
Water concentration	55.5 M

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.*

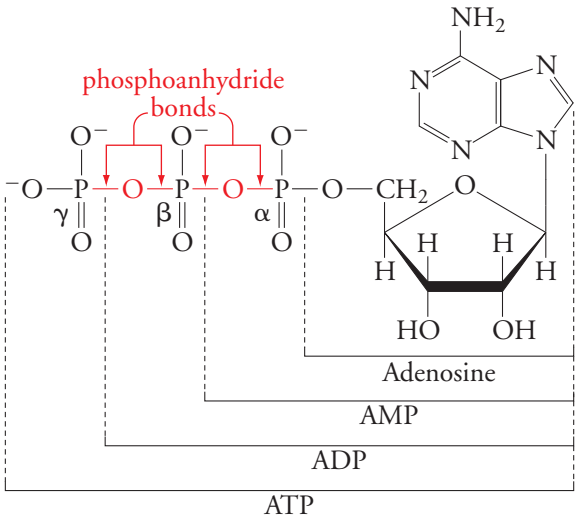


Figure 12-11 **Adenosine triphosphate.** The three phosphate groups are sometimes described by the Greek letters α, β, and γ. The linkage between the first (α) and second (β) phosphoryl groups, and between the second (β) and third (γ), is a phosphoanhydride bond. A reaction in which one or two phosphoryl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

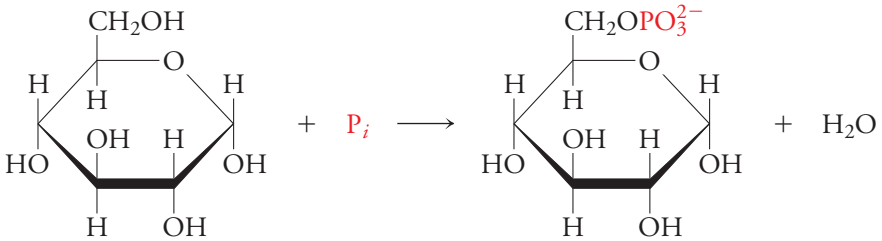
? How does hydrolysis of a phosphoanhydride bond affect the net charge of a nucleotide?

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	$-16.7\ \text{kJ} \cdot \text{mol}^{-1}$

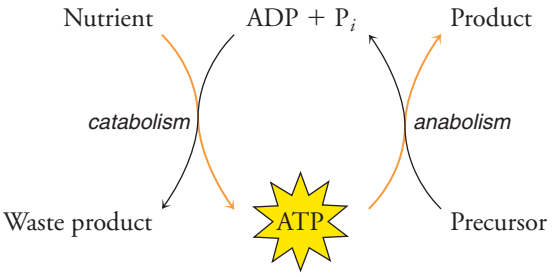
The net chemical reaction, the phosphorylation of glucose, is thermodynamically favorable ($\Delta G < 0$). *In vivo,* this reaction is catalyzed by hexokinase (introduced in Section 6-3), and a phosphoryl group is transferred from ATP directly to glucose. The ATP is not actually hydrolyzed, and there 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 $\Delta G^{\circ\prime}$.

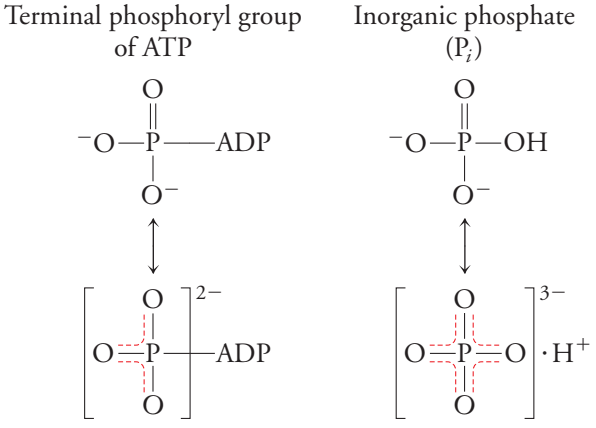
▶▶ WHAT'S so special about ATP?

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.



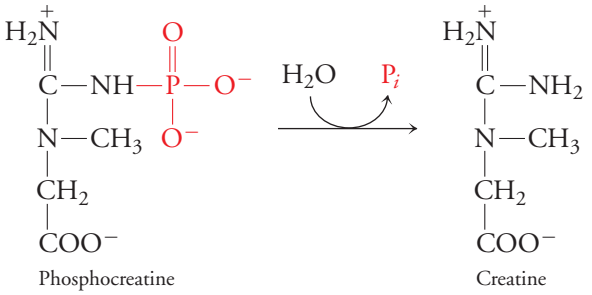
To summarize, *ATP functions as an energy currency because its reaction is highly exergonic ($\Delta G \ll 0$).* The favorable ATP reaction (ATP → ADP) can therefore pull

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

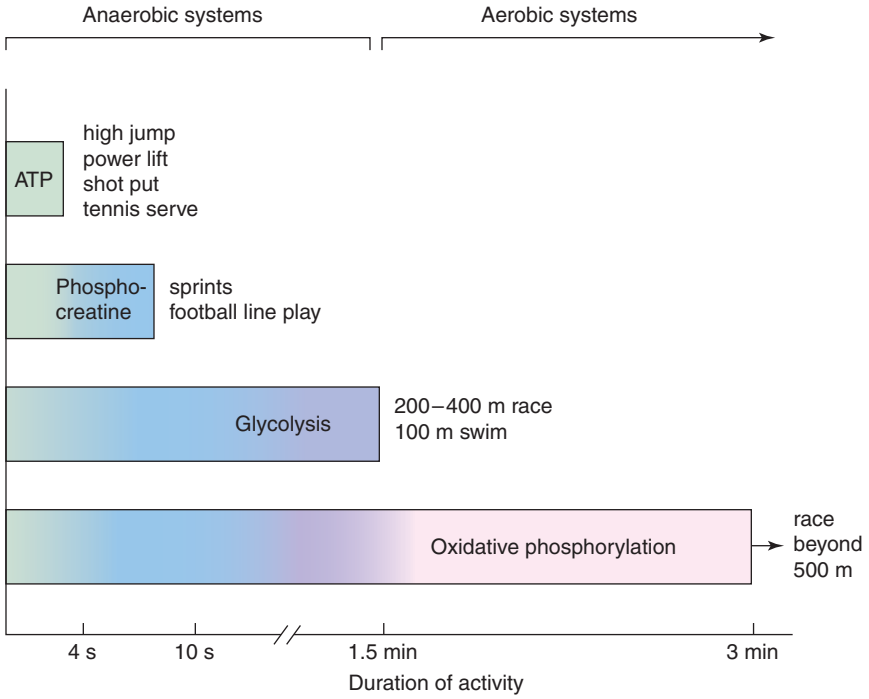
In resting muscles, when the demand for ATP is low, creatine kinase catalyzes the transfer of a phosphoryl group from ATP to creatine to produce phosphocreatine:

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

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.

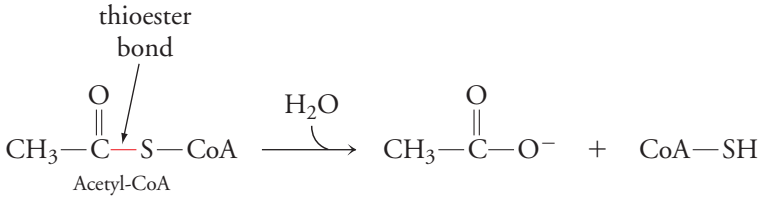
Question: Why do some athletes believe that creatine supplements boost their performance?

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.

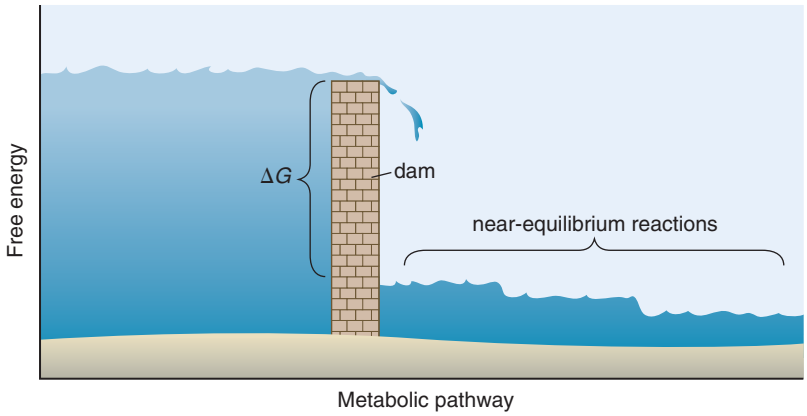
We have already seen that in oxidation–reduction reactions, cofactors such as NAD^+ and ubiquinone can collect electrons. The reduced cofactors are a form of energy currency because their subsequent reoxidation by another compound occurs with a negative change in free energy. Ultimately, the transfer 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

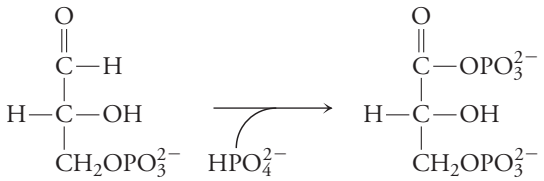
25. The common intermediates listed in the table below appear as reactants or products in several pathways. Place a checkmark in the box that indicates the appropriate pathway for each reactant.

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

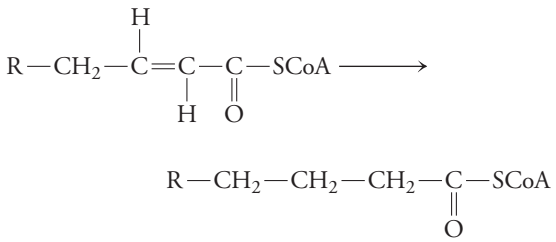
	Triacylglycerol synthesis	Photosynthesis	Transamination
Acetyl-CoA			
Glyceraldehyde-3-phosphate			
Pyruvate			

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

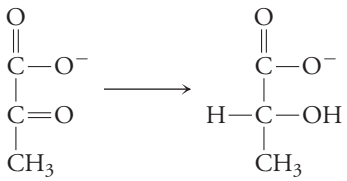
(a) A reaction from the catabolic glycolytic pathway



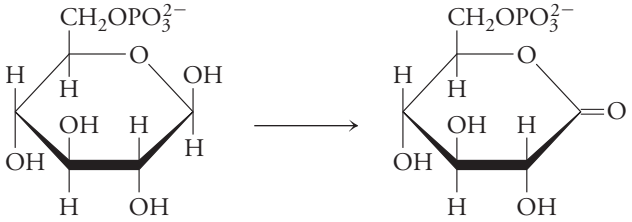
(b) A reaction from the fatty acid synthesis pathway



(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.

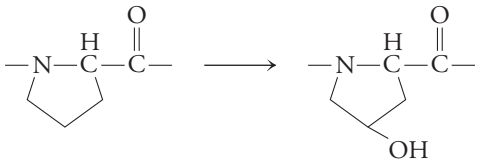
30. Hartnup disease is a hereditary disorder caused by a defective transporter for nonpolar amino acids.
(a) The symptoms of the disease (photosensitivity and neurological abnormalities) can be prevented through dietary adjustments. What sort of diet would be effective?
(b) Patients with Hartnup disease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

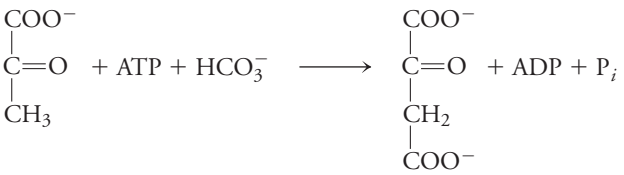
32. Would you expect vitamin A to be more easily absorbed from raw or from cooked carrots? Explain.

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

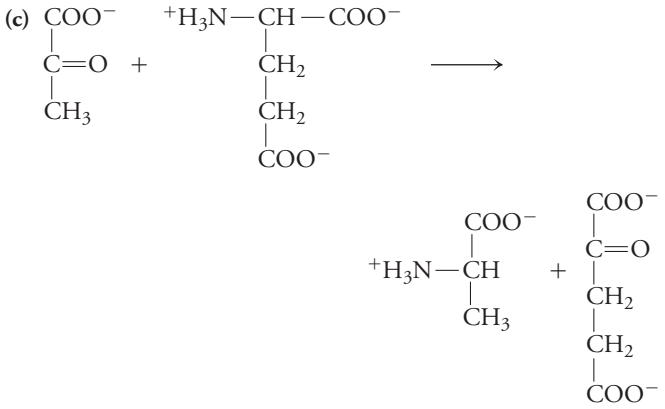
(a)



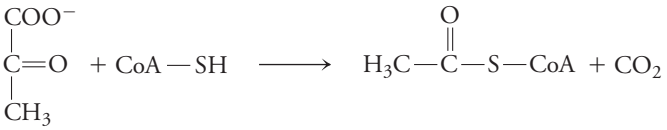
(b)



(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?

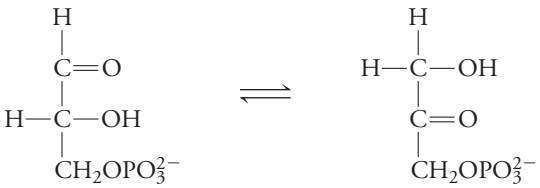
40. Calculate the ΔG value for the C $\rightleftharpoons$ D reaction described in Problem 35 when the concentrations of C and D are 0.9 mM and 0.1 mM, respectively. In which direction will the reaction proceed?

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}.



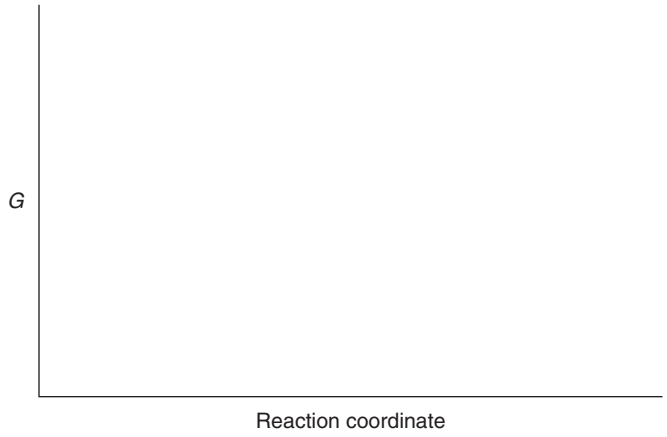
Glyceraldehyde-3-phosphate ⇌ Dihydroxyacetone phosphate

(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.

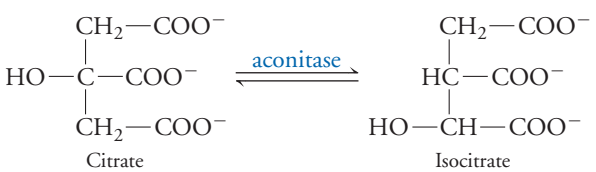
53. Calculate how many apples (see Solution 45) would be required to provide the amount of ATP calculated in Problem 52.

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?

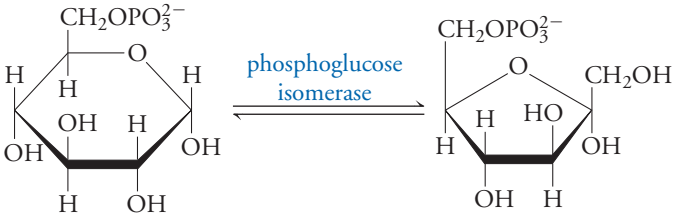
56. Which of the compounds listed in Table 12-4 could be involved in a reaction coupled to the hydrolysis of ATP to ADP + P_i?

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:

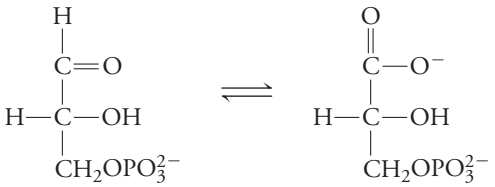
$$\text{fructose-6-phosphate} + P_i \rightleftharpoons \text{fructose-1,6-bisphosphate}$$
$$ATP + H_2O \rightleftharpoons ADP + P_i$$

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

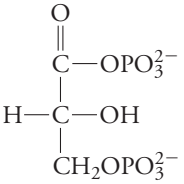
$$\text{fructose-6-phosphate} + ATP + H_2O \rightleftharpoons \text{fructose-1,6-bisphosphate} + ADP$$

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)

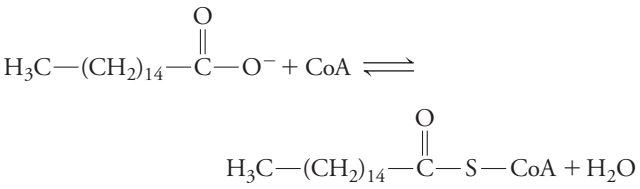
Consider these two scenarios:

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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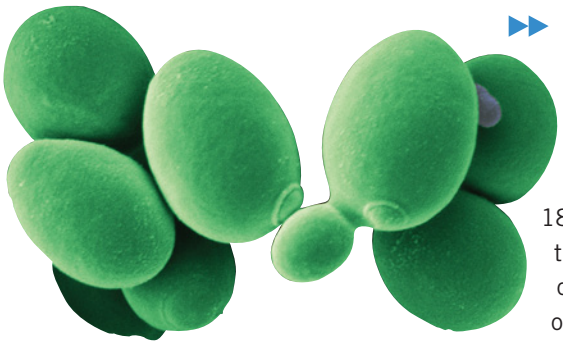
chapter 13

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

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).**