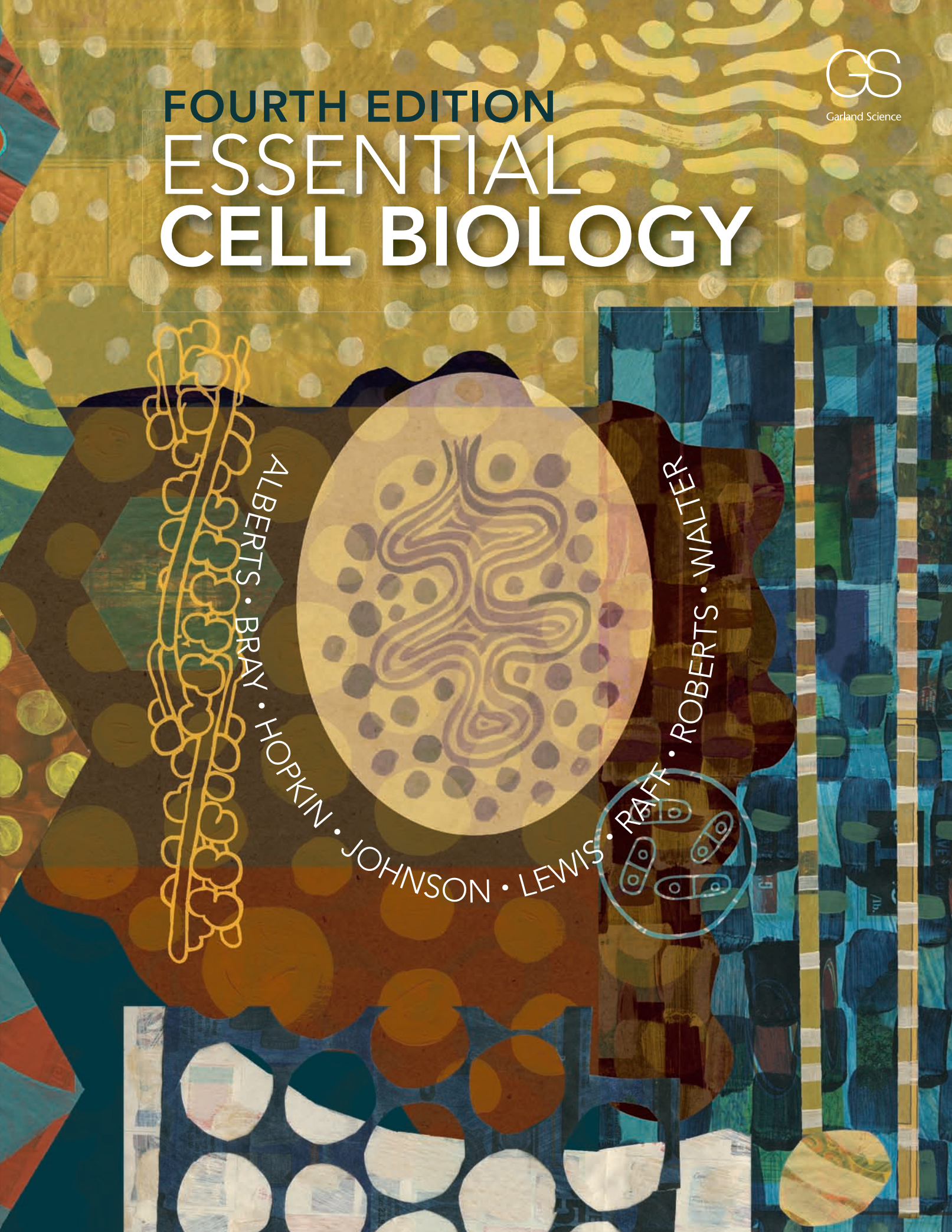
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CHAPTER **THIRTEEN** 13

How Cells Obtain Energy From Food

THE BREAKDOWN AND UTILIZATION OF SUGARS AND FATS

REGULATION OF METABOLISM

As we discussed in Chapter 3, cells require a constant supply of energy to generate and maintain the biological order that allows them to grow, divide, and carry out their day-to-day activities. This energy comes from the chemical-bond energy in food molecules, which thereby serve as fuel for cells.

Perhaps the most important fuel molecules are the sugars. Plants make their own sugars from CO_2 by photosynthesis. Animals obtain sugars—and other organic molecules that can be chemically transformed into sugars—by eating plants and other organisms. Nevertheless, the process whereby all these sugars are broken down to generate energy is very similar in both animals and plants. In both cases, the organism's cells harvest useful energy from the chemical-bond energy locked in sugars as the sugar molecule is broken down and oxidized to carbon dioxide (CO_2) and water (H_2O)—a process called **cell respiration**. The energy released during these reactions is captured in the form of "high-energy" chemical bonds—covalent bonds that release large amounts of energy when hydrolyzed—in *activated carriers* such as ATP and NADH. These carriers in turn serve as portable sources of the chemical groups and electrons needed for biosynthesis (discussed in Chapter 3).

In this chapter, we trace the major steps in the breakdown of sugars and show how ATP, NADH, and other activated carriers are produced along the way. We concentrate on the breakdown of glucose because it generates most of the energy produced in the majority of animal cells. A very similar pathway operates in plants, fungi, and many bacteria. Other molecules, such as fatty acids and proteins, can also serve as energy sources if they are funneled through appropriate enzymatic pathways. We will

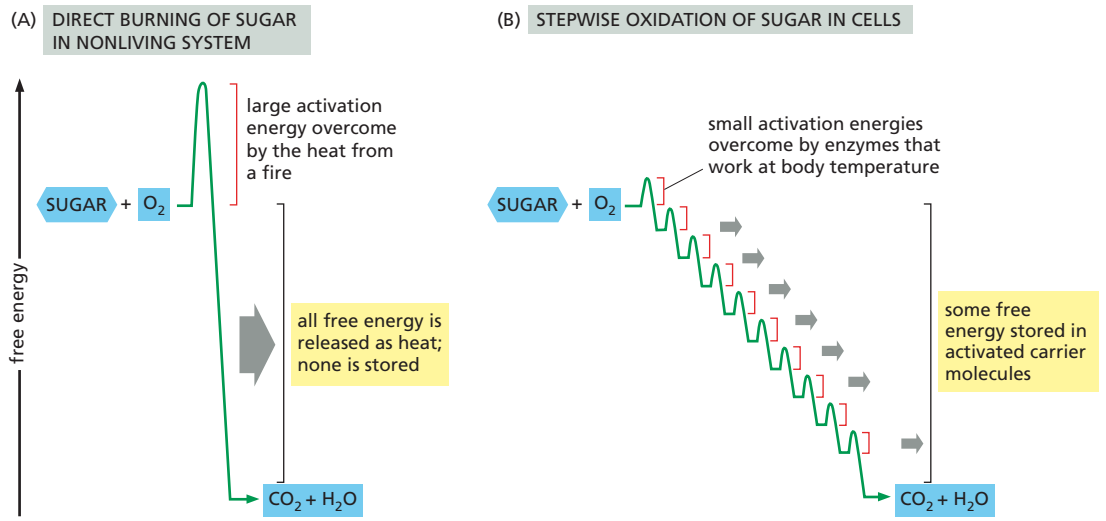


Figure 13–1 The controlled, stepwise oxidation of sugar in cells captures useful energy, unlike the simple burning of the same fuel molecule. (A) The direct burning of sugar in nonliving systems generates more energy than can be stored by any carrier molecule. This energy is thus released as heat. (B) In a cell, enzymes catalyze the breakdown of sugars via a series of small steps, in which a portion of the free energy released is captured by the formation of activated carriers—most often ATP and NADH. Each step is catalyzed by an enzyme that lowers the activation energy barrier that must be surmounted by the random collision of molecules at the temperature of cells (body temperature), so as to allow the reaction to occur. The total free energy released by the oxidative breakdown of glucose—686 kcal/mole (2880 kJ/mole)—is exactly the same in (A) and (B).

see how cells use many of the molecules generated from the breakdown of sugars and fats as starting points to make other organic molecules.

Finally, we examine how cells regulate their metabolism and how they store food molecules for their future metabolic needs. We will save our discussion of the elaborate mechanism cells use to produce the bulk of their ATP for Chapter 14.

THE BREAKDOWN AND UTILIZATION OF SUGARS AND FATS

If a fuel molecule such as glucose were oxidized to CO_2 and H_2O in a single step—by, for example, the direct application of fire—it would release an amount of energy many times larger than any carrier molecule could capture (**Figure 13–1A**). Instead, cells use enzymes to carry out the oxidation of sugars in a tightly controlled series of reactions. Thanks to the action of enzymes—which operate at temperatures typical of living things—cells degrade each glucose molecule step by step, paying out energy in small packets to activated carriers by means of coupled reactions (**Figure 13–1B**). In this way, much of the energy released by the breakdown of glucose is saved in the high-energy bonds of ATP and other activated carriers, which can then be made available to do useful work for the cell.

Animal cells make ATP in two ways. First, certain energetically favorable, enzyme-catalyzed reactions involved in the breakdown of foods are directly coupled to the energetically unfavorable reaction **ADP** + P_i → **ATP**. Thus the oxidation of food molecules can provide energy for the immediate production of ATP. Most ATP synthesis, however, requires an intermediary. In this second pathway to making ATP, the energy from other activated carriers is used to drive ATP production. This process, called *oxidative phosphorylation*, takes place on the inner mitochondrial membrane (**Figure 13–2**), and it is described in detail in Chapter 14. In this chapter, we focus on the first sequence of reactions by which food molecules are oxidized—both in the cytosol and in the mitochondrial matrix (see Figure 13–2). These reactions produce both ATP and the additional

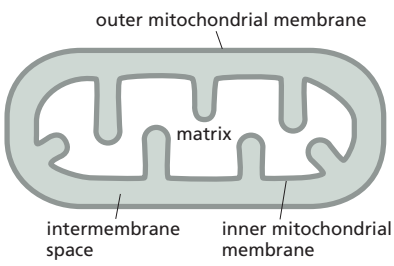


Figure 13–2 A mitochondrion has two membranes and a large internal space called the matrix. Most of the energy from food molecules is harvested in mitochondria—both in the matrix and in the inner mitochondrial membrane.

activated carriers that will subsequently help drive the production of much larger amounts of ATP by oxidative phosphorylation.

Food Molecules Are Broken Down in Three Stages

The proteins, fats, and polysaccharides that make up most of the food we eat must be broken down into smaller molecules before our cells can use them—either as a source of energy or as building blocks for making other organic molecules. This breakdown process—in which enzymes degrade complex organic molecules into simpler ones—is called **catabolism**. The process takes place in three stages, as illustrated in Figure 13–3.

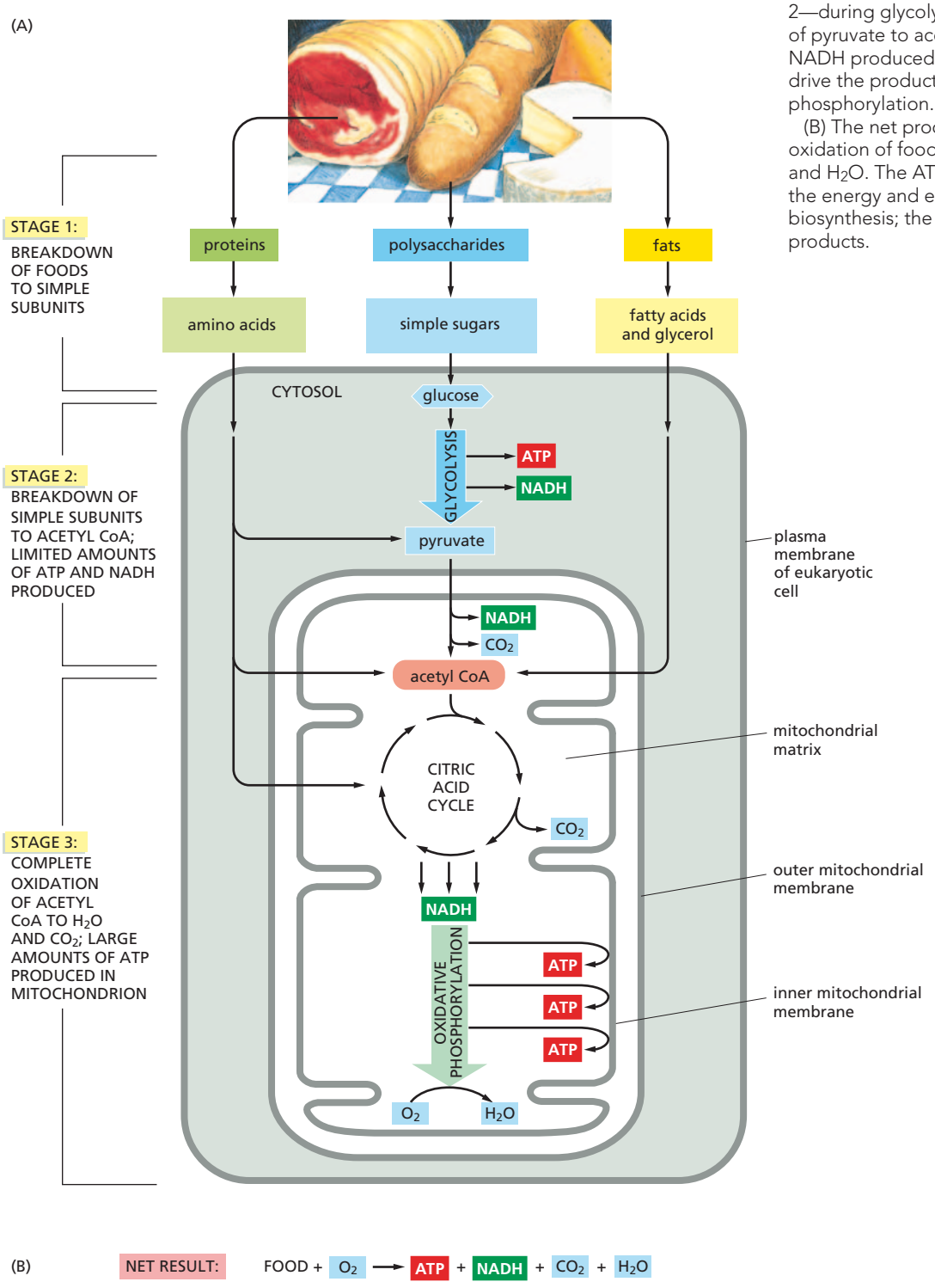


Figure 13–3 The breakdown of food molecules occurs in three stages. (A) Stage 1 mostly occurs outside cells in the mouth and the gut—although intracellular lysosomes can also digest large organic molecules. Stage 2 occurs mainly in the cytosol, except for the final step of conversion of pyruvate to acetyl groups on acetyl CoA, which occurs in the mitochondrial matrix. Stage 3 begins with the citric acid cycle in the mitochondrial matrix and concludes with oxidative phosphorylation on the mitochondrial inner membrane. The NADH generated in stage 2—during glycolysis and the conversion of pyruvate to acetyl CoA—adds to the NADH produced by the citric acid cycle to drive the production of ATP by oxidative phosphorylation.

(B) The net products of the complete oxidation of food include ATP, NADH, CO_2, and H_2O. The ATP and NADH provide the energy and electrons needed for biosynthesis; the CO_2 and H_2O are waste products.

In *stage 1* of catabolism, enzymes convert the large polymeric molecules in food into simpler monomeric subunits: proteins into amino acids, polysaccharides into sugars, and fats into fatty acids and glycerol. This stage—also called *digestion*—occurs either outside cells (in the intestine) or in specialized organelles within cells called lysosomes (discussed in Chapter 15). After digestion, the small organic molecules derived from food enter the cytosol of a cell, where their gradual oxidative breakdown begins.

In *stage 2* of catabolism, a chain of reactions called *glycolysis* splits each molecule of *glucose* into two smaller molecules of *pyruvate.* Sugars other than glucose can also be used, after first being converted into one of the intermediates in this sugar-splitting pathway. Glycolysis takes place in the cytosol and, in addition to producing pyruvate, it generates two types of activated carriers: ATP and NADH. The pyruvate is transported from the cytosol into the mitochondrion's large, internal compartment called the *matrix*. There, a giant enzyme complex converts each pyruvate molecule into CO_2 plus *acetyl CoA*, another of the activated carriers discussed in Chapter 3 (see Figure 3–36). In the same compartment, large amounts of acetyl CoA are also produced by the stepwise oxidative breakdown of fatty acids derived from fats (see Figure 13–3).

Stage 3 of catabolism takes place entirely in mitochondria. The acetyl group in acetyl CoA is transferred to an oxaloacetate molecule to form citrate, which enters a series of reactions called the *citric acid cycle.* In these reactions, the transferred acetyl group is oxidized to CO_2 with the production of large amounts of NADH. Finally, the high-energy electrons from NADH are passed along a series of enzymes within the mitochondrial inner membrane called an *electron-transport chain*, where the energy released by their transfer is used to drive oxidative phosphorylation—a process that produces ATP and consumes molecular oxygen (O_2 gas). It is in these final steps of catabolism that the majority of the energy released by oxidation is harnessed to produce most of the cell's ATP.

Through the production of ATP, the energy derived from the breakdown of sugars and fats is redistributed into packets of chemical energy in a form convenient for use in the cell. In total, nearly half of the energy that could, in theory, be derived from the breakdown of glucose or fatty acids to H_2O and CO_2 is captured and used to drive the energetically unfavorable reaction ADP + P_i → ATP. By contrast, a modern combustion engine, such as a car engine, can convert no more than 20% of the available energy in its fuel into useful work. In both cases, the remaining energy is released as heat, which in animals helps to keep the body warm.

Roughly 10^9 molecules of ATP are in solution in a typical cell at any instant. In many cells, all of this ATP is turned over (that is, consumed and replaced) every 1–2 minutes. An average person at rest will hydrolyze his or her weight in ATP molecules every 24 hours.

Glycolysis Extracts Energy from the Splitting of Sugar

The central process in stage 2 of catabolism is the oxidative breakdown of **glucose** in the sequence of reactions known as **glycolysis**. Glycolysis produces ATP without the involvement of oxygen. It occurs in the cytosol of most cells, including many anaerobic microorganisms that thrive in the absence of oxygen. Glycolysis probably evolved early in the history of life on Earth, before photosynthetic organisms introduced oxygen into the atmosphere.

The term "glycolysis" comes from the Greek *glykys*, "sweet," and *lysis*, "splitting." It is an appropriate name, as glycolysis splits a molecule of glucose, which has six carbon atoms, to form two molecules of pyruvate, each of which contains three carbon atoms. The series of chemical

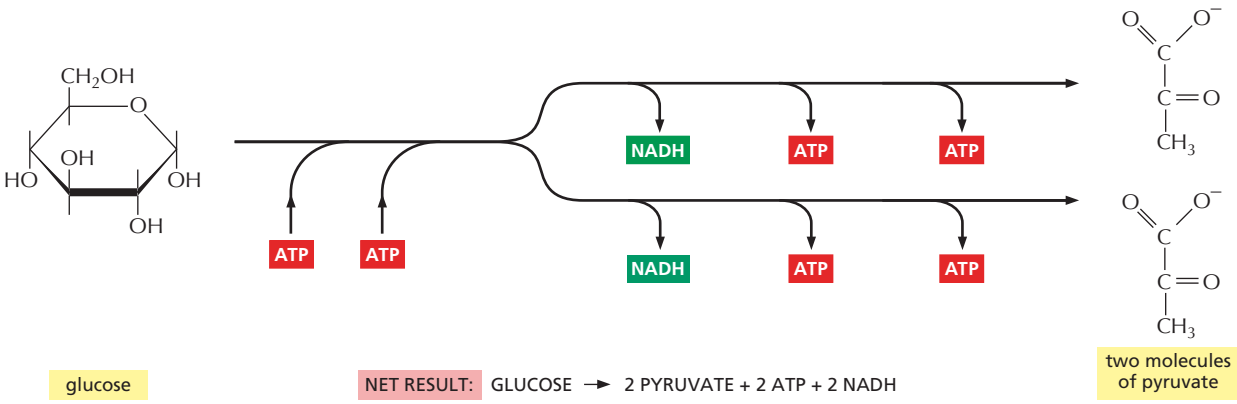


Figure 13–4 Glycolysis splits a molecule of glucose to form two molecules of pyruvate. The process requires an input of energy, in the form of ATP, at the start. This energy investment is later recouped by the production of two NADHs and four ATPs.

rearrangements that ultimately generate pyruvate release energy because the electrons in a molecule of pyruvate are, overall, at a lower energy state than those in a molecule of glucose. Nevertheless, for each molecule of glucose that enters glycolysis, two molecules of ATP are initially consumed to provide the energy needed to prepare the sugar to be split. This investment of energy is more than recouped in the later steps of glycolysis, when four molecules of ATP are produced. Energy is also captured in this "payoff phase" in the form of NADH. Thus, at the end of glycolysis, there is a net gain of two molecules of ATP and two molecules of NADH for each glucose molecule broken down (**Figure 13–4**).

Glycolysis Produces Both ATP and NADH

Piecing together the complete glycolytic pathway in the 1930s was a major triumph of biochemistry, as the pathway consists of a sequence of 10 separate reactions, each producing a different sugar intermediate and each catalyzed by a different enzyme. Like most enzymes, those that catalyze glycolysis all have names ending in *-ase*—like isomerase and dehydrogenase—which specify the type of reaction they catalyze (**Table 13–1**). The reactions of the glycolytic pathway are presented in outline in **Figure 13–5** and in detail in **Panel 13–1** (pp. 428–429).

TABLE 13–1 SOME TYPES OF ENZYMES INVOLVED IN GLYCOLYSIS

Enzyme type	General function	Role in glycolysis
Kinase	catalyzes the addition of a phosphate group to molecules	a kinase transfers a phosphate group from ATP to a substrate in steps 1 and 3; other kinases transfer a phosphate to ADP to form ATP in steps 7 and 10
Isomerase	catalyzes the rearrangement of bonds within a single molecule	isomerases in steps 2 and 5 prepare molecules for the chemical alterations to come
Dehydrogenase	catalyzes the oxidation of a molecule by removing a hydrogen atom plus an electron (a hydride ion, H^-)	the enzyme glyceraldehyde 3-phosphate dehydrogenase generates NADH in step 6
Mutase	catalyzes the shifting of a chemical group from one position to another within a molecule	the movement of a phosphate by phosphoglycerate mutase in step 8 helps prepare the substrate to transfer this group to ADP to make ATP in step 10

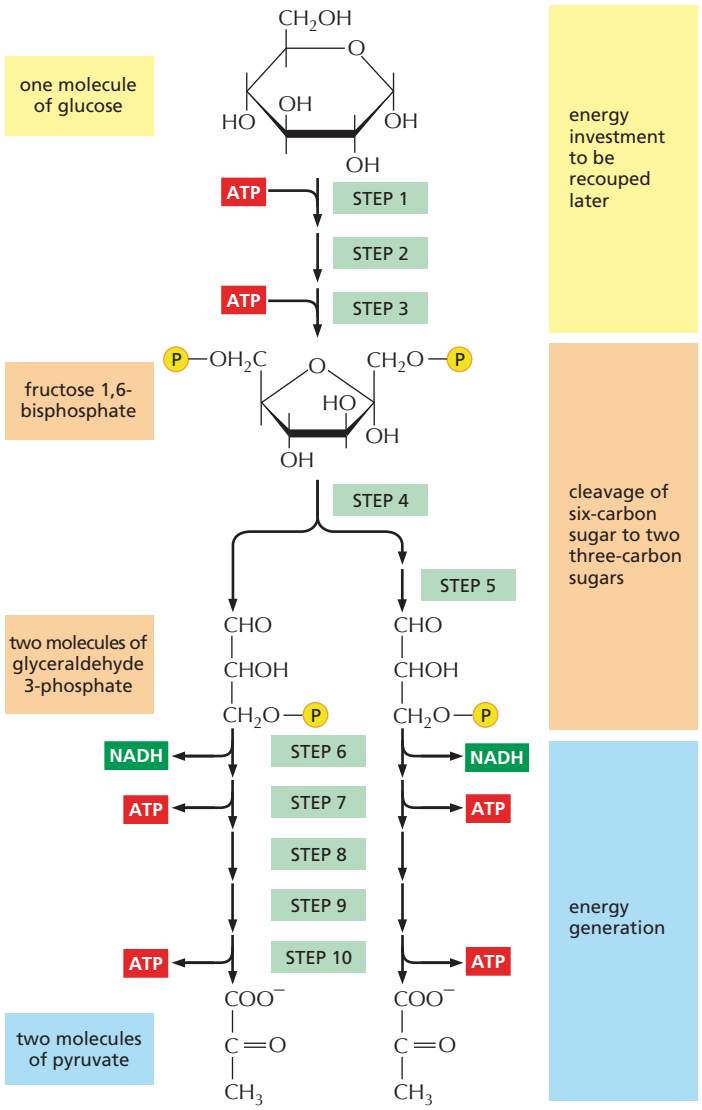


Figure 13–5 The stepwise breakdown of sugars begins with glycolysis. Each of the 10 steps of glycolysis is catalyzed by a different enzyme. Note that step 4 cleaves a six-carbon sugar into two three-carbon sugars, so that the number of molecules at every stage after this doubles. Note also that one of the products of step 4 needs to be modified (isomerized) in step 5 before it can proceed to step 6 (see Panel 13–1). As indicated, step 6 begins the energy-generation phase of glycolysis, which results in the net synthesis of ATP and NADH (see also Figure 13–4). Glycolysis is also sometimes referred to as the Embden–Meyerhof pathway, named for the chemists who first described it. All the steps of glycolysis are reviewed in **Movie 13.1**.

Much of the energy released by the breakdown of glucose is used to drive the synthesis of ATP molecules from ADP and P_i. This form of ATP synthesis, which takes place in steps 7 and 10 in glycolysis, is known as *substrate-level phosphorylation* because it occurs by the transfer of a phosphate group directly from a substrate molecule—one of the sugar intermediates—to ADP. By contrast, most phosphorylations in cells occur by the transfer of phosphate from ATP to a substrate molecule.

The remainder of the energy released during glycolysis is stored in the electrons in the **NADH** molecule produced in step 6 by an oxidation reaction. As discussed in Chapter 3, oxidation does not always involve oxygen; it occurs in any reaction in which electrons are lost from one atom and transferred to another. So, although no molecular oxygen is involved in glycolysis, oxidation does occur: in step 6, a hydrogen atom plus an electron is removed from the sugar intermediate, glyceraldehyde 3-phosphate, and transferred to **NAD^+**, producing NADH (see Panel 13–1, p. 428).

Over the course of glycolysis, two molecules of NADH are formed per molecule of glucose. In aerobic organisms, these NADH molecules donate their electrons to the electron-transport chain in the inner mitochondrial membrane, as described in detail in Chapter 14. Such electron transfers release energy as the electrons fall from a state of higher energy to a

lower one. The electrons that are passed along the electron-transport chain are ultimately passed on to O_2, forming water.

In giving up its electrons, NADH is converted back into NAD^+, which is then available to be used again for glycolysis. In the absence of oxygen, NAD^+ can be regenerated by an alternate type of energy-yielding reaction called a fermentation, as we discuss next.

Fermentations Can Produce ATP in the Absence of Oxygen

For most animal and plant cells, glycolysis is only a prelude to the third and final stage of the breakdown of food molecules, in which large amounts of ATP are generated in mitochondria by oxidative phosphorylation, a process that requires the consumption of oxygen. However, for many anaerobic microorganisms, which can grow and divide in the absence of oxygen, glycolysis is the principal source of ATP. The same is true for certain animal cells, such as skeletal muscle cells, which can continue to function at low levels of oxygen.

In these anaerobic conditions, the pyruvate and NADH made by glycolysis remain in the cytosol. The pyruvate is converted into products that are excreted from the cell: lactate in muscle cells, for example, or ethanol and CO_2 in the yeast cells used in brewing and breadmaking. The NADH gives up its electrons in the cytosol, and is converted back to the NAD^+ required to maintain the reactions of glycolysis (**Figure 13–6**). Such energy-yielding pathways that break down sugar in the absence of oxygen are called **fermentations**. Scientific studies of the commercially important fermentations carried out by yeasts laid the foundations for early biochemistry.

QUESTION 13–1

At first glance, the final steps in fermentation appear to be unnecessary: the generation of lactate or ethanol does not produce any additional energy for the cell. Explain why cells growing in the absence of oxygen could not simply discard pyruvate as a waste product. Which products derived from glucose would accumulate in cells unable to generate either lactate or ethanol by fermentation?

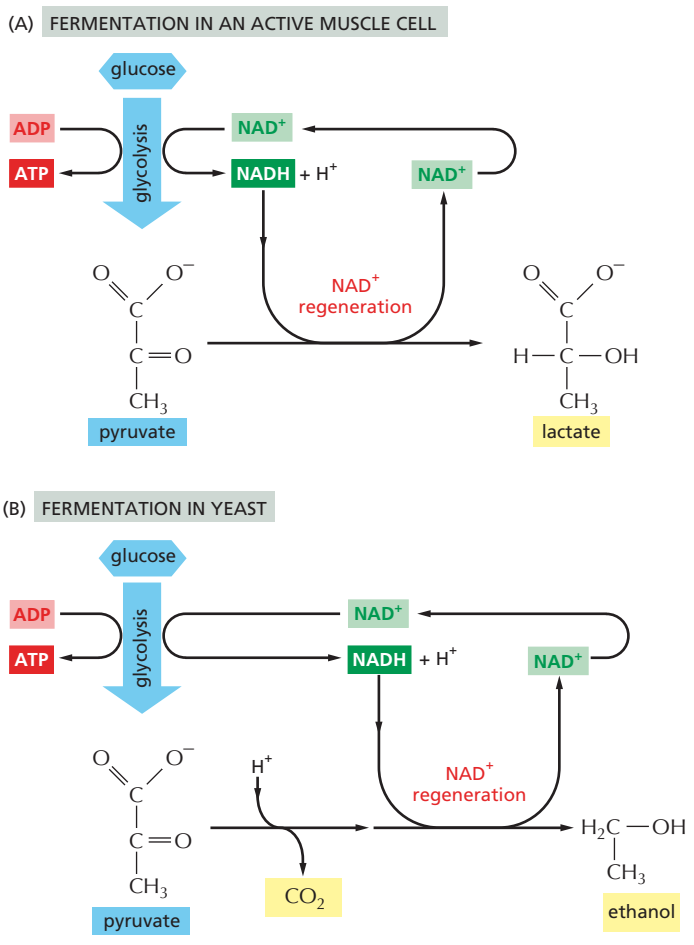


Figure 13–6 Pyruvate is broken down in the absence of oxygen by fermentation. (A) When inadequate oxygen is present, for example, in a muscle cell undergoing vigorous contraction, the pyruvate produced by glycolysis is converted to lactate in the cytosol. This reaction restores the NAD^+ consumed in step 6 of glycolysis, but the whole pathway yields much less energy overall than if the pyruvate were oxidized in mitochondria. (B) In microorganisms that can grow anaerobically, pyruvate is converted into carbon dioxide and ethanol. Again, this pathway regenerates NAD^+ from NADH, as required to enable glycolysis to continue. Both (A) and (B) are examples of fermentations. Note that in both cases, for each molecule of glucose that enters glycolysis, two molecules of pyruvate are generated (only a single pyruvate is shown here). Fermentation of these two pyruvates subsequently yields two molecules of lactate—or two molecules of CO_2 and ethanol—plus two molecules of NAD^+.

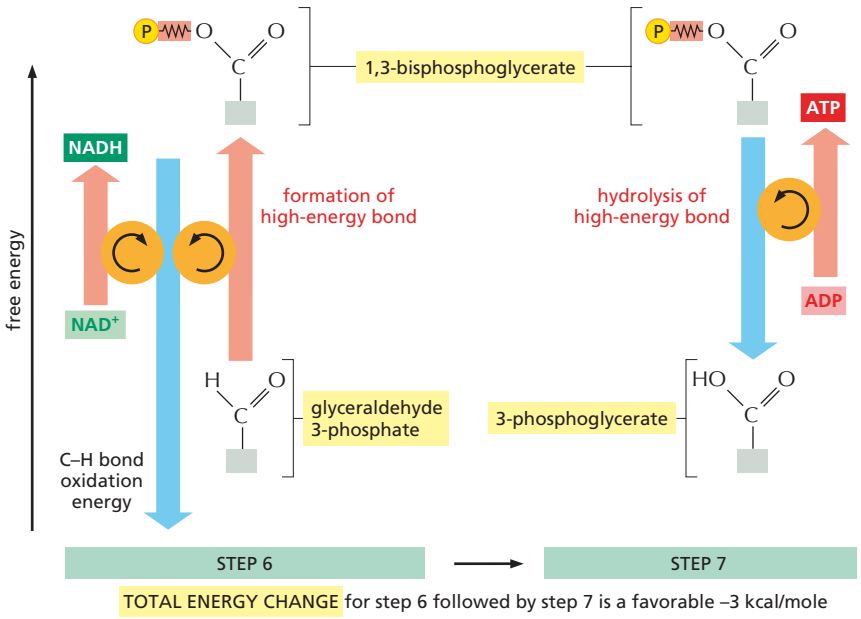


Figure 13–7 A pair of coupled reactions drives the energetically unfavorable formation of ATP in steps 6 and 7 of glycolysis. In this diagram, energetically favorable reactions are represented by *blue arrows*; energetically costly reactions by *red arrows*. In step 6, the energy released by the energetically favorable oxidation of a C–H bond in glyceraldehyde 3-phosphate (*blue arrow*) is large enough to drive two energetically costly reactions: the formation of both NADH and a high-energy phosphate bond in 1,3-bisphosphoglycerate (*red arrows*). The subsequent energetically favorable hydrolysis of that high-energy phosphate bond in step 7 then drives the formation of ATP.

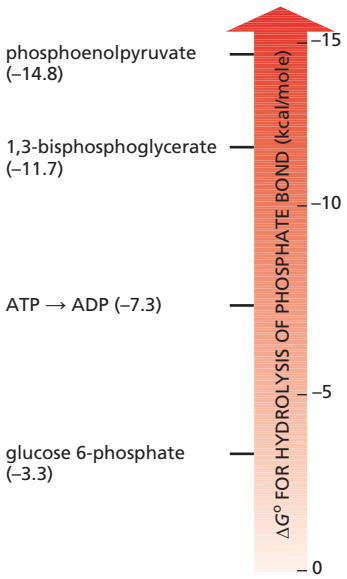


Figure 13–8 Differences in the energies of different phosphate bonds allow the formation of ATP by substrate-level phosphorylation. Examples of molecules containing different types of phosphate bonds are shown, along with the free-energy change for hydrolysis of those bonds in kcal/mole (1 kcal = 4.184 kJ). The transfer of a phosphate group from one molecule to another is energetically favorable if the standard free-energy change ($\Delta G°$) for hydrolysis of the phosphate bond is more negative for the donor molecule than for the acceptor. (The hydrolysis reactions can be thought of as the transfer of the phosphate group to water.) Thus, a phosphate group is readily transferred from 1,3-bisphosphoglycerate to ADP to form ATP. Transfer reactions involving the phosphate groups in these molecules are detailed in Panel 13–1 (pp. 428–429).

Many bacteria and archaea can also generate ATP in the absence of oxygen by *anaerobic respiration*, a process that uses a molecule other than oxygen as a final electron acceptor. Anaerobic respiration differs from fermentation in that it involves an electron-transport chain embedded in a membrane—in this case, the plasma membrane of the microbe.

Glycolytic Enzymes Couple Oxidation to Energy Storage in Activated Carriers

The "paddle wheel" analogy in Chapter 3 explained how cells harvest useful energy from the oxidation of organic molecules by coupling an energetically unfavorable reaction to an energetically favorable one (see Figure 3–30). Here, we take a closer look at a key pair of glycolytic reactions that demonstrate how enzymes—the paddle wheel in our analogy—allow coupled reactions to facilitate the transfer of chemical energy to ATP and NADH.

The reactions in question—steps 6 and 7 in Panel 13–1—convert the three-carbon sugar intermediate glyceraldehyde 3-phosphate (an aldehyde) into 3-phosphoglycerate (a carboxylic acid). This conversion, which entails the oxidation of an aldehyde group to a carboxylic acid group, occurs in two steps. The overall reaction releases enough free energy to transfer two electrons from the aldehyde to NAD^+ to form NADH and to transfer a phosphate group to a molecule of ADP to form ATP. It also releases enough heat to the environment to make the overall reaction energetically favorable: the $\Delta G°$ for step 6 followed by step 7 is –3.0 kcal/mole (**Figure 13–7**).

The energy contained in any phosphate bond can be determined by measuring the standard free-energy change ($\Delta G°$) when that bond is broken by hydrolysis. Molecules that contain phosphate bonds that have more energy than those found in ATP—including the high-energy 1,3-bisphosphoglycerate generated in step 6 of glycolysis—readily transfer their phosphate group to ADP to form ATP. **Figure 13–8** compares the high-energy phosphoanhydride bond in ATP with a few of the other phosphate bonds that are generated during glycolysis. As explained in Panel 13–1, we describe these bonds as "high energy" only in that their hydrolysis is particularly energetically favorable.

The reaction in step 6 is the only one in glycolysis that creates a high-energy phosphate linkage directly from inorganic phosphate—an example of the substrate-level phosphorylation mentioned earlier. How this high-energy linkage is generated in step 6—and then consumed in step 7 to produce ATP—is detailed in **Figure 13–9**.

QUESTION 13–2

Arsenate (AsO_4^{3-}) is chemically very similar to phosphate (PO_4^{3-}) and is used as an alternative substrate by many phosphate-requiring enzymes. In contrast to phosphate, however, an anhydride bond between arsenate and carbon is very quickly hydrolyzed nonenzymatically in water. Knowing this, suggest why arsenate is a compound of choice for murderers but not for cells. Formulate your explanation in the context of Figure 13–7.

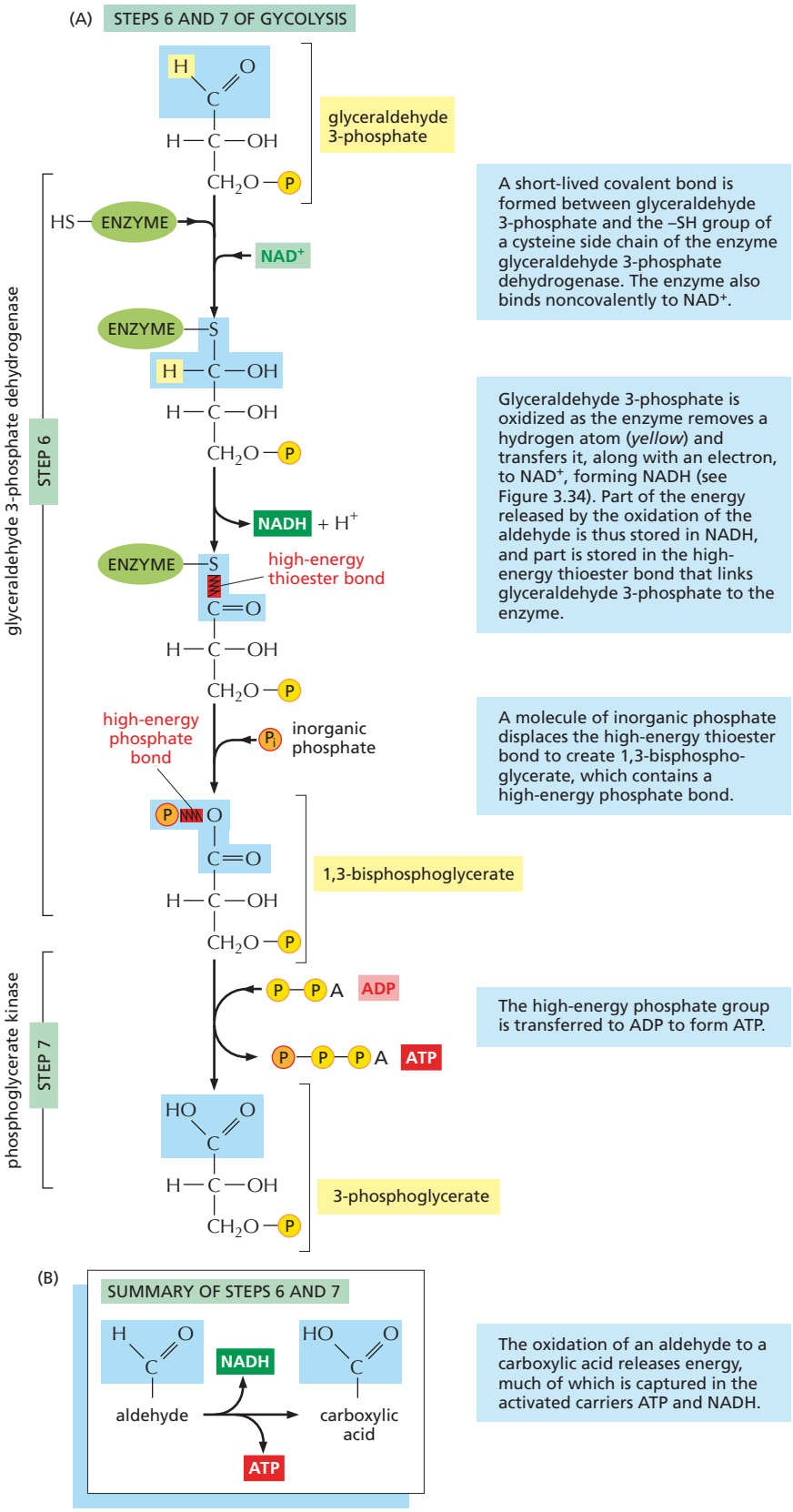
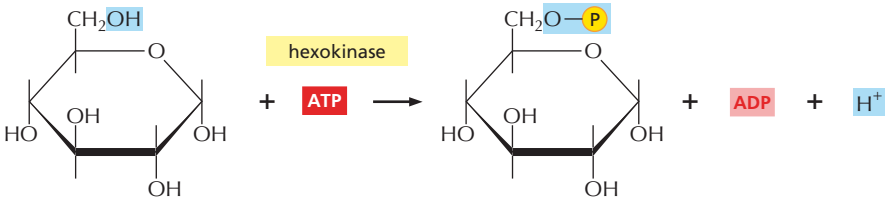


Figure 13–9 The oxidation of glyceraldehyde 3-phosphate is coupled to the formation of ATP and NADH in steps 6 and 7 of glycolysis. (A) In step 6, the enzyme glyceraldehyde 3-phosphate dehydrogenase couples the energetically favorable oxidation of an aldehyde to the energetically unfavorable formation of a high-energy phosphate bond. At the same time, it enables energy to be stored in NADH. The formation of the high-energy phosphate bond is driven by the oxidation reaction, and the enzyme thereby acts like the "paddle wheel" coupler in Figure 3–30B. In step 7, the newly formed high-energy phosphate bond in 1,3-bisphosphoglycerate is transferred to ADP, forming a molecule of ATP and leaving a free carboxylic acid group on the oxidized sugar. The part of the molecule that undergoes a change is shaded in *blue*; the rest of the molecule remains unchanged throughout all these reactions. (B) Summary of the overall chemical change produced by the reactions of steps 6 and 7.

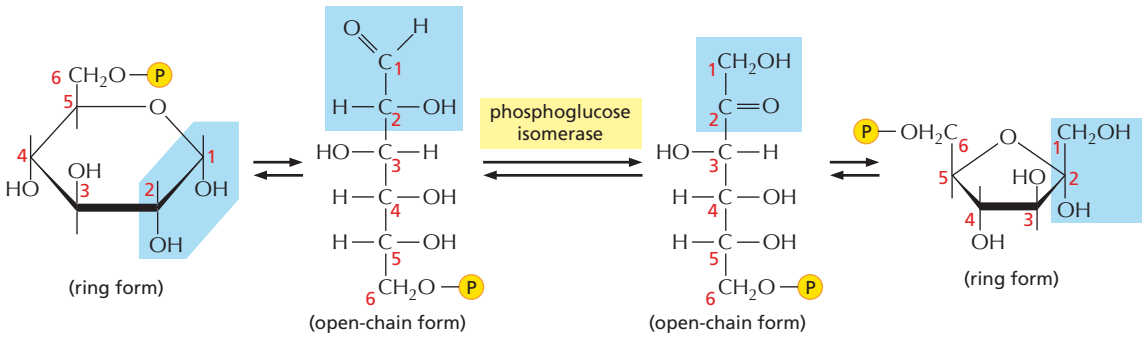
PANEL 13–1 DETAILS OF THE 10 STEPS OF GLYCOLYSIS

For each step, the part of the molecule that undergoes a change is shadowed in blue, and the name of the enzyme that catalyzes the reaction is in a yellow box. To watch a video of the reactions of glycolysis, see Movie 13.1.

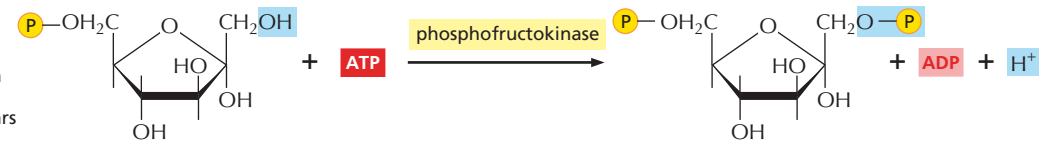
Step 1 Glucose is phosphorylated by ATP to form a sugar phosphate. The negative charge of the phosphate prevents passage of the sugar phosphate through the plasma membrane, trapping glucose inside the cell.



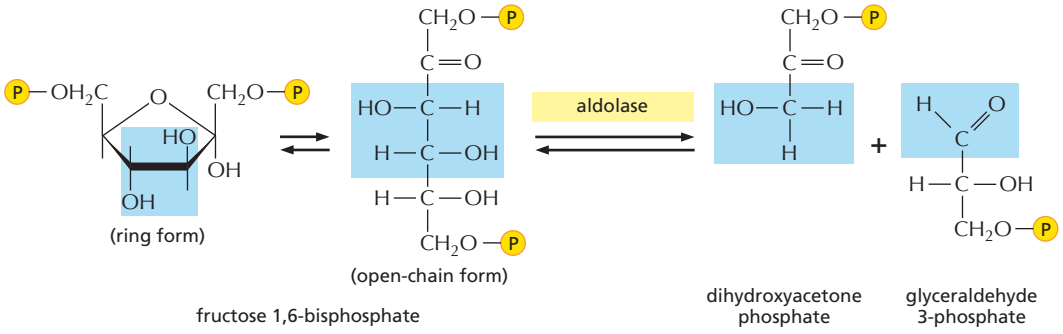
Step 2 A readily reversible rearrangement of the chemical structure (isomerization) moves the carbonyl oxygen from carbon 1 to carbon 2, forming a ketose from an aldose sugar. (See Panel 2–3, pp. 70–71.)



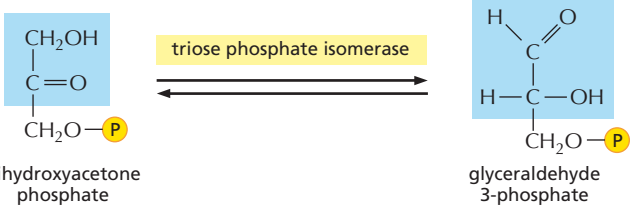
Step 3 The new hydroxyl group on carbon 1 is phosphorylated by ATP, in preparation for the formation of two three-carbon sugar phosphates. The entry of sugars into glycolysis is controlled at this step, through regulation of the enzyme *phosphofructokinase*.



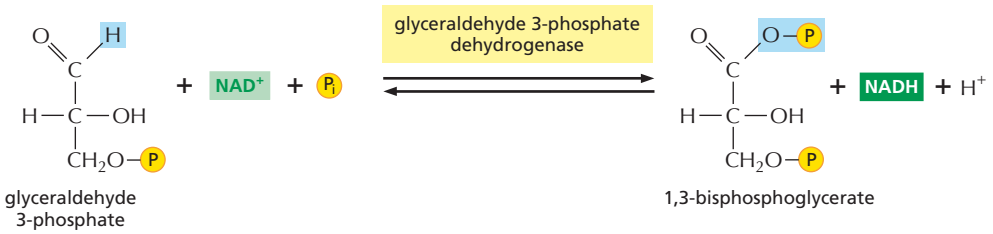
Step 4 The six-carbon sugar is cleaved to produce two three-carbon molecules. Only the glyceraldehyde 3-phosphate can proceed immediately through glycolysis.



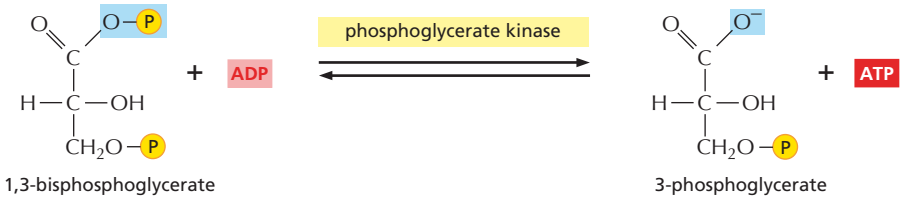
Step 5 The other product of step 4, dihydroxyacetone phosphate, is isomerized to form glyceraldehyde 3-phosphate.



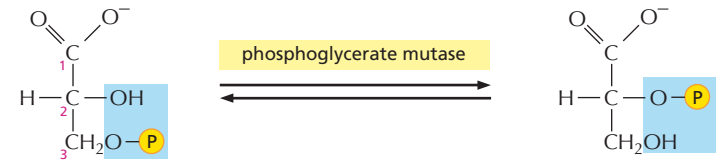
Step 6 The two molecules of glyceraldehyde 3-phosphate are oxidized. The energy-generation phase of glycolysis begins, as NADH and a new high-energy anhydride linkage to phosphate are formed (see Figure 13–5).



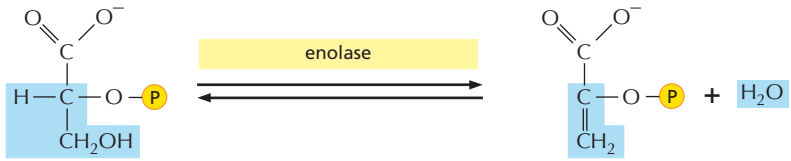
Step 7 The transfer to ADP of the high-energy phosphate group that was generated in step 6 forms ATP.



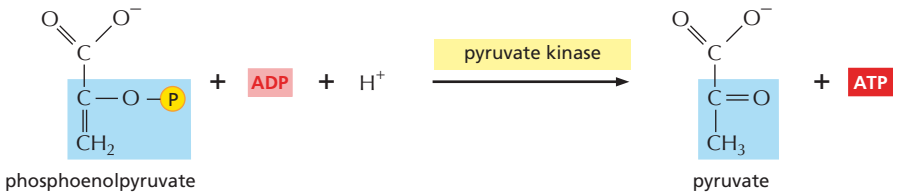
Step 8 The remaining phosphate ester linkage in 3-phosphoglycerate, which has a relatively low free energy of hydrolysis, is moved from carbon 3 to carbon 2 to form 2-phosphoglycerate.



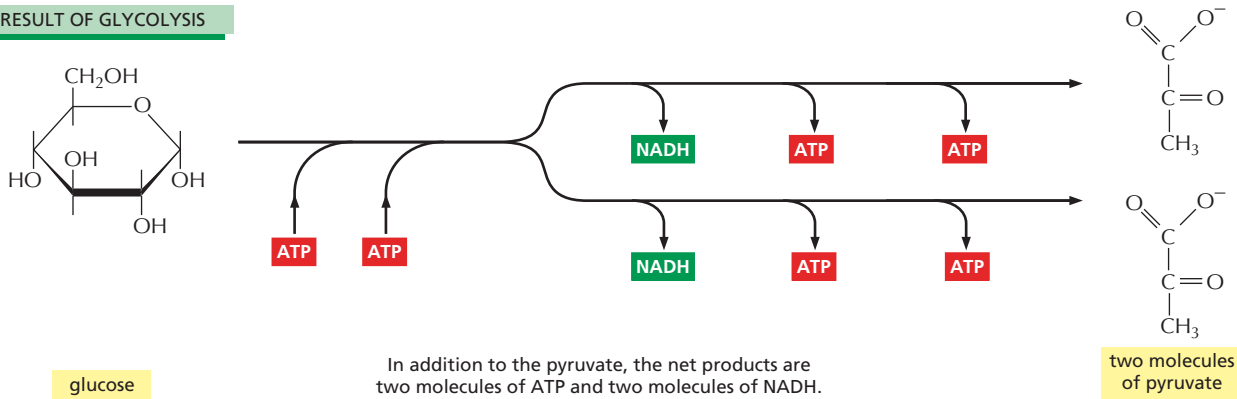
Step 9 The removal of water from 2-phosphoglycerate creates a high-energy enol phosphate linkage.



Step 10 The transfer to ADP of the high-energy phosphate group that was generated in step 9 forms ATP, completing glycolysis.



NET RESULT OF GLYCOLYSIS



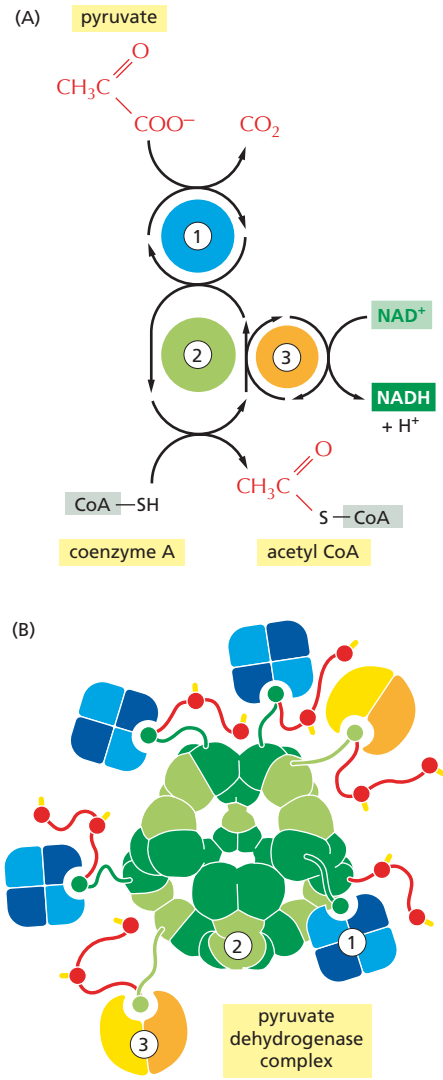


Figure 13–10 Pyruvate is converted into acetyl CoA and CO_2 by the pyruvate dehydrogenase complex in the mitochondrial matrix. (A) The pyruvate dehydrogenase complex, which contains multiple copies of three different enzymes—*pyruvate dehydrogenase* (1), *dihydrolipoyl transacetylase* (2), and *dihydrolipoyl dehydrogenase* (3)—converts pyruvate to acetyl CoA; NADH and CO_2 are also produced in this reaction. Pyruvate and its products are shown in *red lettering*. (B) In this large multienzyme complex, reaction intermediates are passed directly from one enzyme to another via flexible tethers. Only one-tenth of the subunits labeled 1 and 3, attached to the core formed by subunit 2, are shown here. To get a sense of scale, the pyruvate dehydrogenase complex is larger than a ribosome.

Several Organic Molecules Are Converted to Acetyl CoA in the Mitochondrial Matrix

In aerobic metabolism in eukaryotic cells, the **pyruvate** produced by glycolysis is actively pumped into the mitochondrial matrix (see Figure 13–3). There, it is rapidly decarboxylated by a giant complex of three enzymes, called the *pyruvate dehydrogenase complex.* The products of pyruvate decarboxylation are CO_2 (a waste product), NADH, and **acetyl CoA** (**Figure 13–10**).

In addition to sugar, which is broken down during glycolysis, **fat** is a major source of energy for most nonphotosynthetic organisms, including humans. Like the pyruvate derived from glycolysis, the fatty acids derived from fat are also converted into acetyl CoA in the mitochondrial matrix (see Figure 13–3). Fatty acids are first activated by covalent linkage to CoA and are then broken down completely by a cycle of reactions that trims two carbons at a time from their carboxyl end, generating one molecule of acetyl CoA for each turn of the cycle. Two activated carriers—NADH and another high-energy electron carrier, $FADH_2$—are also produced in this process (**Figure 13–11**).

In addition to pyruvate and fatty acids, some amino acids are transported from the cytosol into the mitochondrial matrix, where they are also converted into acetyl CoA or one of the other intermediates of the citric acid cycle (see Figure 13–3). Thus, in the eukaryotic cell, the mitochondrion is the center toward which all energy-yielding catabolic processes lead, whether they begin with sugars, fats, or proteins. In aerobic bacteria—which have no mitochondria—glycolysis and acetyl CoA production, as well as the citric acid cycle, take place in the cytosol.

Catabolism does not end with the production of acetyl CoA. In the process of converting food molecules to acetyl CoA, only a small part of their stored energy is extracted and converted into ATP, NADH, or $FADH_2$. Most of that energy is still locked up in acetyl CoA. The next stage in cell respiration is the citric acid cycle, in which the acetyl group in acetyl CoA is oxidized to CO_2 and H_2O in the mitochondrial matrix, as we now discuss.

The Citric Acid Cycle Generates NADH by Oxidizing Acetyl Groups to CO_2

The **citric acid cycle** accounts for about two-thirds of the total oxidation of carbon compounds in most cells, and its major end products are CO_2 and high-energy electrons in the form of NADH. The CO_2 is released as a waste product, while the high-energy electrons from NADH are passed to the electron-transport chain in the inner mitochondrial membrane. At the end of the chain, these electrons combine with O_2 to produce H_2O.

The citric acid cycle, which takes place in the mitochondrial matrix, does not itself use O_2. However, it requires O_2 to proceed because the

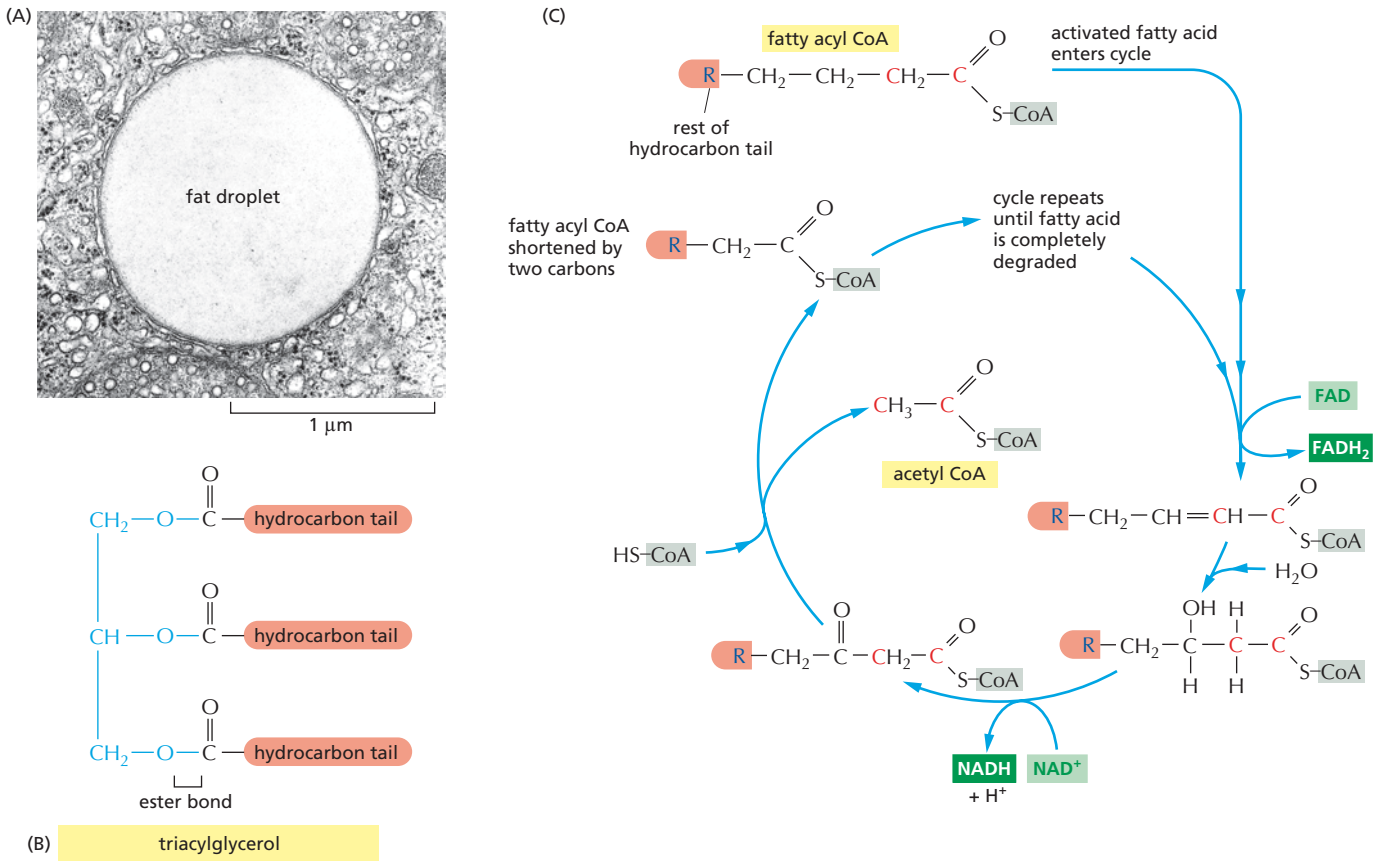


Figure 13–11 Fatty acids derived from fats are also converted to acetyl CoA in the mitochondrial matrix. (A) Fats are insoluble in water and spontaneously form large lipid droplets in specialized fat cells called adipocytes. This electron micrograph shows a lipid droplet in the cytoplasm of an adipocyte. (B) Fats are stored in the form of triacylglycerol. The glycerol portion, to which three fatty acid chains (shaded in *red*) are linked through ester bonds, is shown in *blue*. Enzymes called lipases can cleave the ester bonds that link the fatty acid chains to glycerol when fatty acids are needed for energy. (C) Fatty acids are first coupled to coenzyme A in a reaction requiring ATP (not shown). The activated fatty acid chains (fatty acyl CoA) are then oxidized in a cycle containing four enzymes. Each turn of the cycle shortens a fatty acyl CoA molecule by two carbons (*red*) and generates one molecule of acetyl CoA and one molecule each of NADH and $FADH_2$. (A, courtesy of Daniel S. Friend.)

electron-transport chain—which uses O_2 as its final acceptor—allows NADH to get rid of its electrons and thus regenerate the NAD^+ needed to keep the cycle going. Although living organisms have inhabited Earth for the past 3.5 billion years, the planet is thought to have developed an atmosphere containing O_2 gas only some 1 to 2 billion years ago (see Figure 14–45). Many of the energy-generating reactions of the citric acid cycle—also called the *tricarboxylic acid cycle* or the *Krebs cycle*—are therefore likely to be of relatively recent origin.

The citric acid cycle catalyzes the complete oxidation of the carbon atoms of the acetyl groups in acetyl CoA, converting them into CO_2. The acetyl group is not oxidized directly, however. Instead, it is transferred from acetyl CoA to a larger four-carbon molecule, oxaloacetate, to form the six-carbon tricarboxylic acid, citric acid, for which the subsequent cycle of reactions is named. The citric acid molecule (also called citrate) is then progressively oxidized, and the energy of this oxidation is harnessed to produce activated carriers in much the same manner as we described for glycolysis. The chain of eight reactions forms a cycle, because the oxaloacetate that began the process is regenerated at the end (**Figure 13–12**). The citric acid cycle is presented in detail in **Panel 13–2** (pp. 434–435), and the experiments that first revealed the cyclic nature of this series of oxidative reactions are described in **How We Know**, pp. 436–437.

QUESTION 13–3

Many catabolic and anabolic reactions are based on reactions that are similar but work in opposite directions, such as the hydrolysis and condensation reactions described in Figure 3–38. This is true for fatty acid breakdown and fatty acid synthesis. From what you know about the mechanism of fatty acid breakdown outlined in Figure 13–11, would you expect the fatty acids found in cells to most commonly have an even or an odd number of carbon atoms?

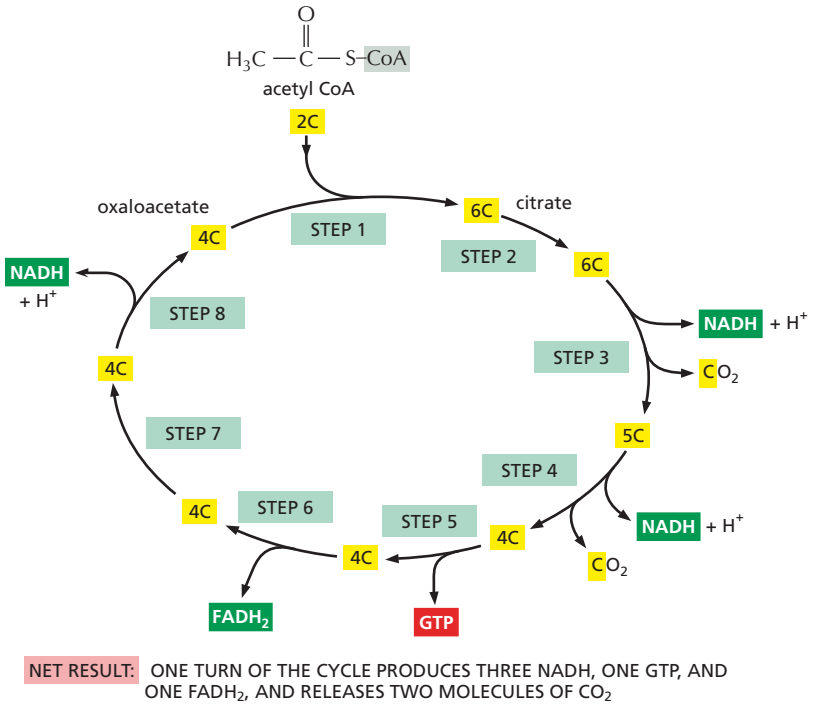


Figure 13–12 The citric acid cycle catalyzes the complete oxidation of acetyl groups derived from food. The cycle begins with the reaction of acetyl CoA (derived from pyruvate as shown in Figure 13–10) with oxaloacetate to produce citrate (citric acid). The number of carbon atoms in each intermediate is shaded in *yellow*. (See also Panel 13–2, pp. 434–435.) The steps of the citric acid cycle are reviewed in Movie 13.2.

Thus far, we have discussed only one of the three types of activated carriers that are produced by the citric acid cycle—NADH. In addition to three molecules of NADH, each turn of the cycle also produces one molecule of **$FADH_2$** (**reduced flavin adenine dinucleotide**) from FAD and one molecule of the ribonucleoside triphosphate **GTP** (**guanosine triphosphate**) from **GDP** (see Figure 13–12). The structures of these two activated carriers are illustrated in **Figure 13–13**. GTP is a close relative of ATP, and the transfer of its terminal phosphate group to ADP produces one ATP molecule in each cycle. Like NADH, $FADH_2$ is a carrier of high-energy electrons and hydrogen. As we discuss shortly, the energy stored in the readily transferred high-energy electrons of NADH and $FADH_2$ is

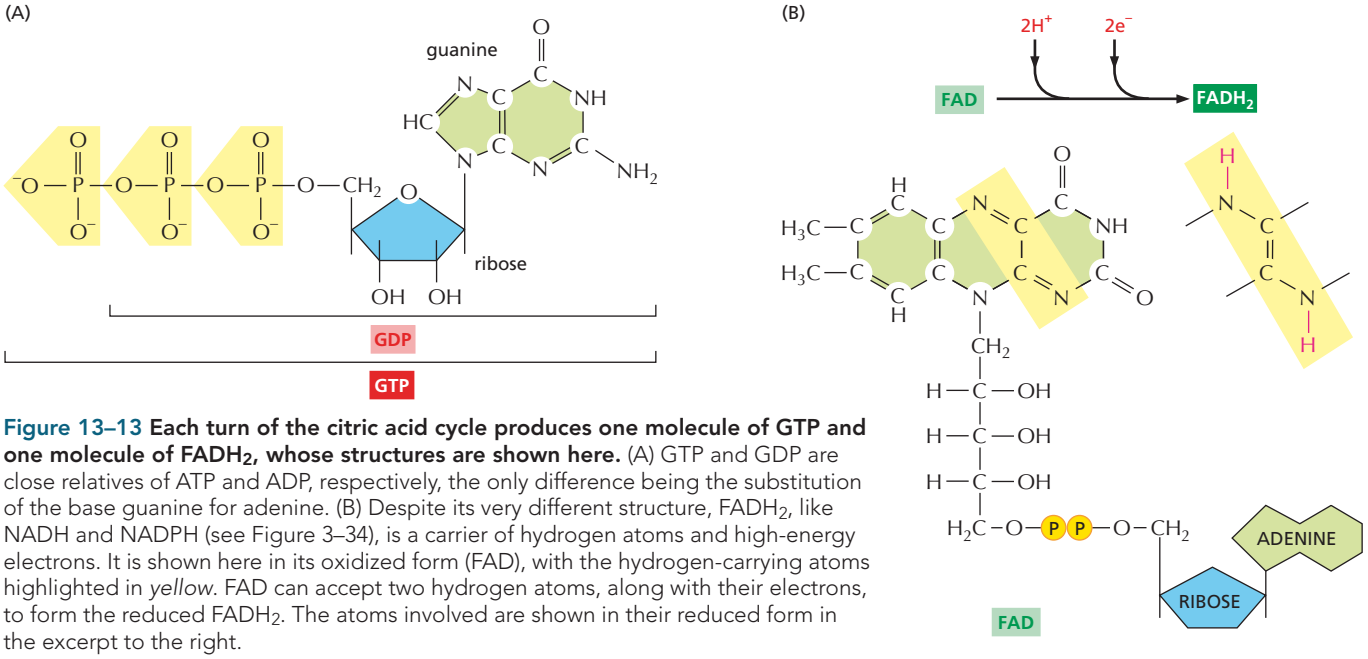


Figure 13–13 Each turn of the citric acid cycle produces one molecule of GTP and one molecule of $FADH_2$, whose structures are shown here. (A) GTP and GDP are close relatives of ATP and ADP, respectively, the only difference being the substitution of the base guanine for adenine. (B) Despite its very different structure, $FADH_2$, like NADH and NADPH (see Figure 3–34), is a carrier of hydrogen atoms and high-energy electrons. It is shown here in its oxidized form (FAD), with the hydrogen-carrying atoms highlighted in *yellow*. FAD can accept two hydrogen atoms, along with their electrons, to form the reduced $FADH_2$. The atoms involved are shown in their reduced form in the excerpt to the right.

subsequently used to produce ATP through oxidative phosphorylation on the inner mitochondrial membrane, the only step in the oxidative catabolism of foodstuffs that directly requires O_2 from the atmosphere.

A common misconception about the citric acid cycle is that the atmospheric O_2 required for the process to proceed is converted into the CO_2 that is released as a waste product. In fact, the oxygen atoms required to make CO_2 from the acetyl groups entering the citric acid cycle are supplied not by O_2 but by water. As illustrated in Panel 13–2, three molecules of water are split in each cycle, and the oxygen atoms of some of them are ultimately used to make CO_2. As we see shortly, the O_2 that we breathe is actually reduced to water by the electron-transport chain; it is not incorporated directly into the CO_2 we exhale.

Many Biosynthetic Pathways Begin with Glycolysis or the Citric Acid Cycle

Catabolic reactions, such as those of glycolysis and the citric acid cycle, produce both energy for the cell and the building blocks from which many other organic molecules are made. So far, we have emphasized energy production rather than the provision of starting materials for biosynthesis. But many of the intermediates formed in glycolysis and the citric acid cycle are siphoned off by such **anabolic pathways**, in which they are converted by series of enzyme-catalyzed reactions into amino acids, nucleotides, lipids, and other small organic molecules that the cell needs. Oxaloacetate and α-ketoglutarate from the citric acid cycle, for example, are transferred from the mitochondrial matrix back to the cytosol, where they serve as precursors for the production of many essential molecules, such as the amino acids aspartate and glutamate, respectively. An idea of the complexity of this process can be gathered from **Figure 13–14,** which illustrates some of the branches leading from the central catabolic reactions to biosyntheses.

QUESTION 13–4

Looking at the chemistry detailed in Panel 13–2 (pp. 434–435), why do you suppose it is useful to link the acetyl group first to another, larger carbon skeleton, oxaloacetate, before completely oxidizing both carbons to CO_2?

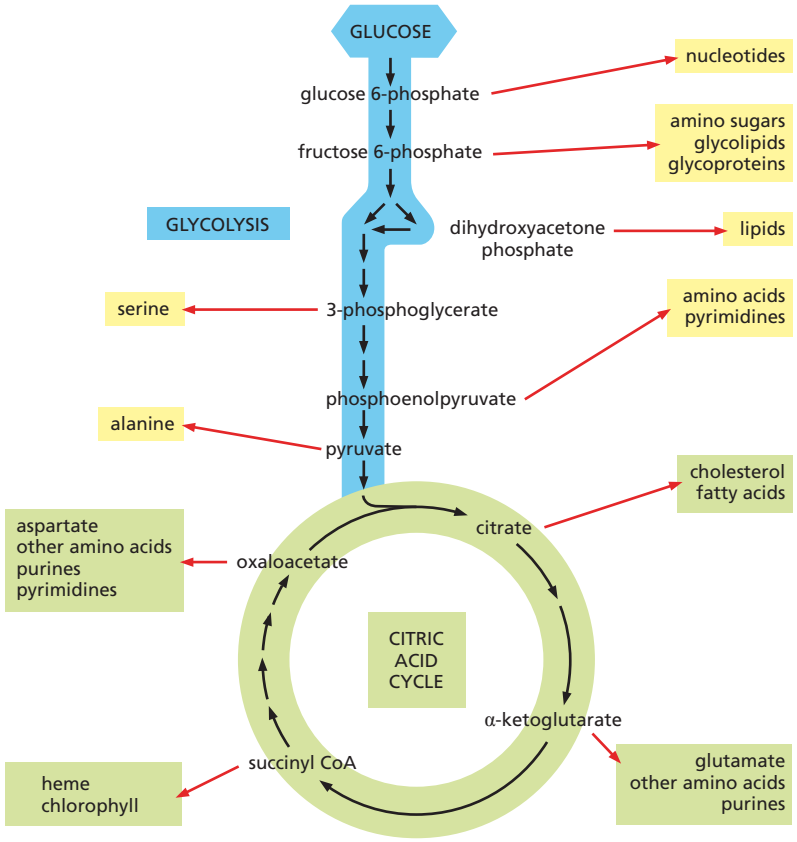
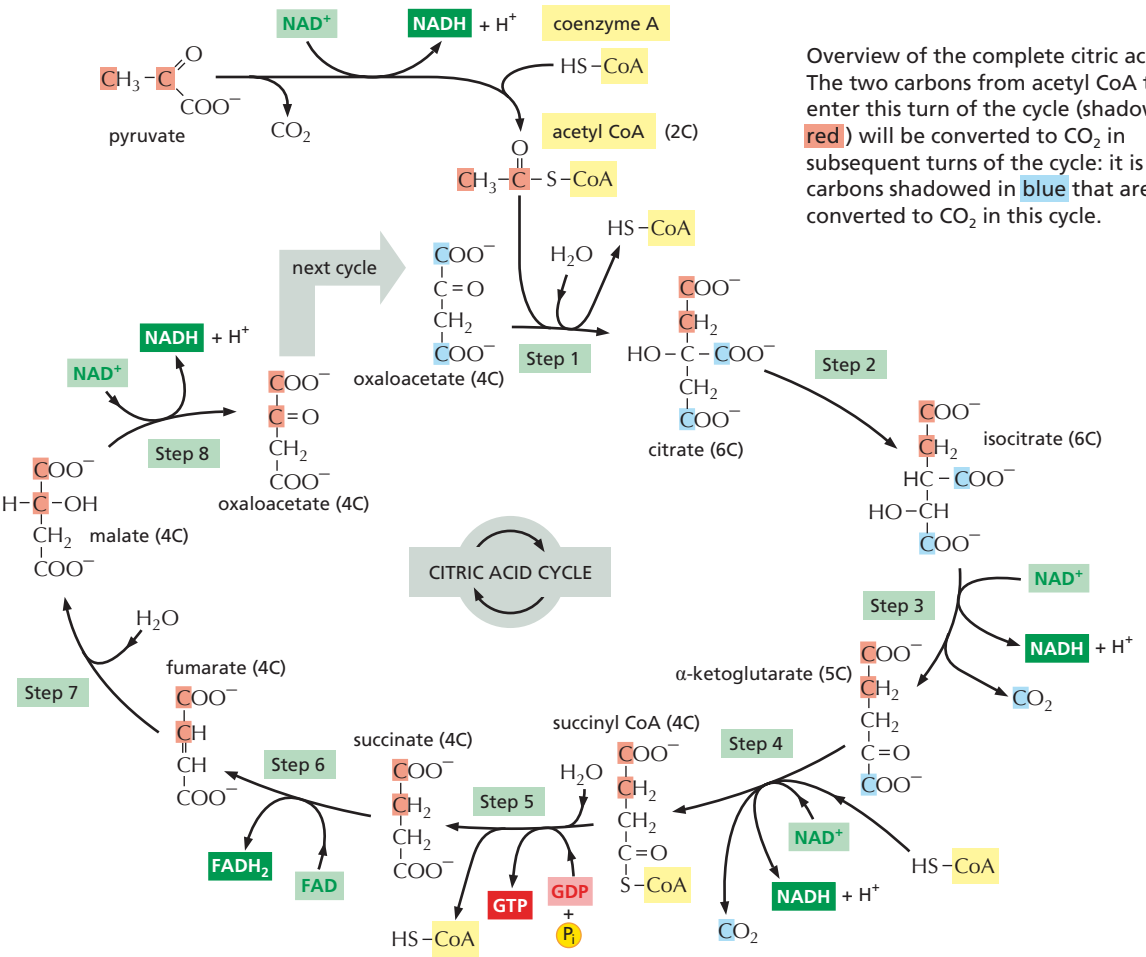


Figure 13–14 Glycolysis and the citric acid cycle provide the precursors needed for cells to synthesize many important organic molecules. The amino acids, nucleotides, lipids, sugars, and other molecules—shown here as products—in turn serve as the precursors for many of the cell's macromolecules. Each *black* arrow in this diagram denotes a single enzyme-catalyzed reaction; the *red* arrows generally represent pathways with many steps that are required to produce the indicated products.

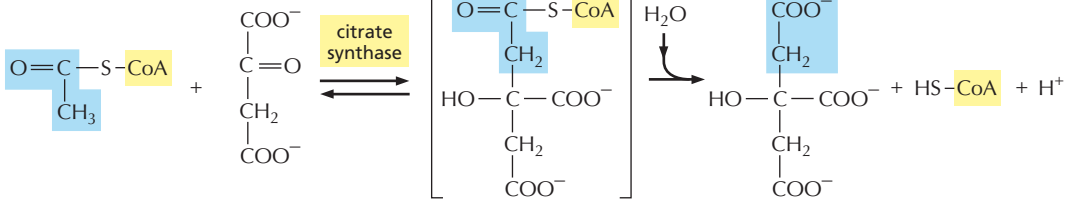
PANEL 13–2 THE COMPLETE CITRIC ACID CYCLE



Overview of the complete citric acid cycle. The two carbons from acetyl CoA that enter this turn of the cycle (shadowed in red) will be converted to CO_2 in subsequent turns of the cycle: it is the two carbons shadowed in blue that are converted to CO_2 in this cycle.

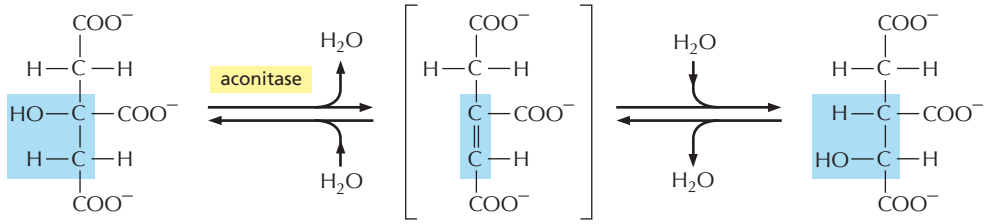
Details of these eight steps are shown below. In this part of the panel, for each step, the part of the molecule that undergoes a change is shadowed in blue, and the name of the enzyme that catalyzes the reaction is in a yellow box.
To watch a video of the reactions of the citric acid cycle, see **Movie 13.2**.

Step 1 After the enzyme removes a proton from the CH_3 group on acetyl CoA, the negatively charged CH_2^- forms a bond to a carbonyl carbon of oxaloacetate. The subsequent loss by hydrolysis of the coenzyme A (HS–CoA) drives the reaction strongly forward.



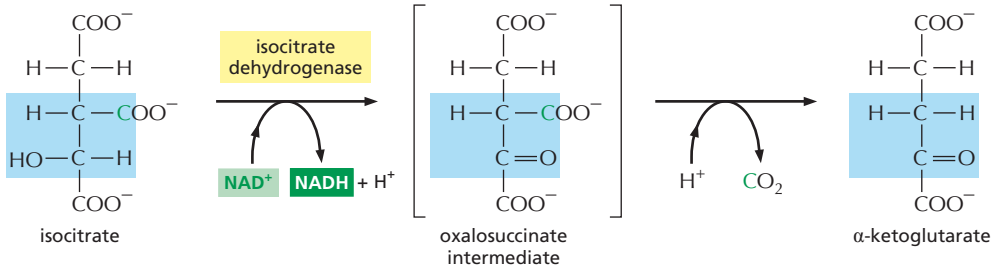
acetyl CoA — oxaloacetate — S-citryl-CoA intermediate — citrate

Step 2 An isomerization reaction, in which water is first removed and then added back, moves the hydroxyl group from one carbon atom to its neighbor.

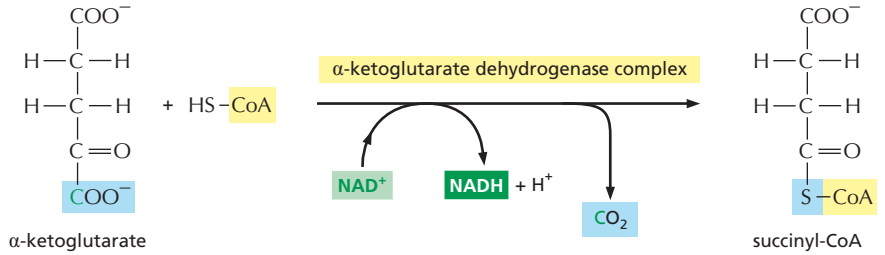


citrate — *cis*-aconitate intermediate — isocitrate

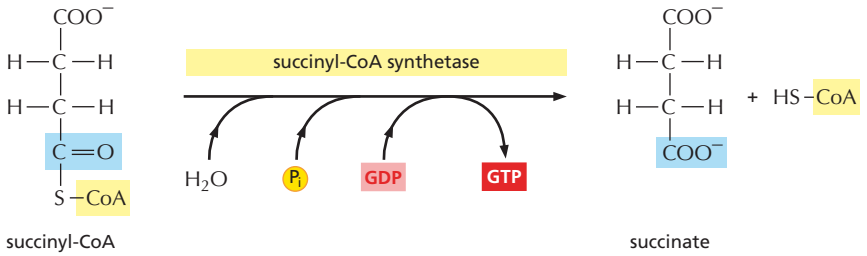
Step 3 In the first of four oxidation steps in the cycle, the carbon carrying the hydroxyl group is converted to a carbonyl group. The immediate product is unstable, losing CO_2 while still bound to the enzyme.



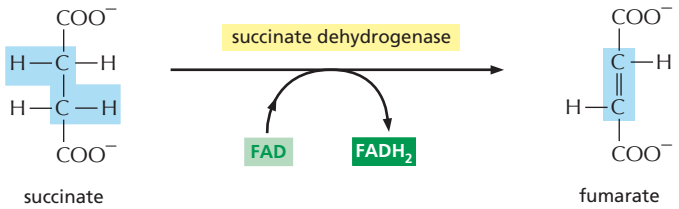
Step 4 The *α-ketoglutarate dehydrogenase complex* closely resembles the large enzyme complex that converts pyruvate to acetyl CoA, the *pyruvate dehydrogenase* complex in Figure 13–10. It likewise catalyzes an oxidation that produces NADH, CO_2, and a high-energy thioester bond to coenzyme A (CoA).



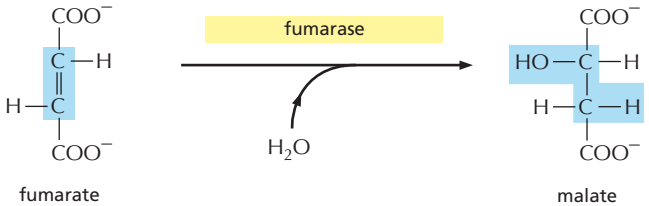
Step 5 A phosphate molecule from solution displaces the CoA, forming a high-energy phosphate linkage to succinate. This phosphate is then passed to GDP to form GTP. (In bacteria and plants, ATP is formed instead.)



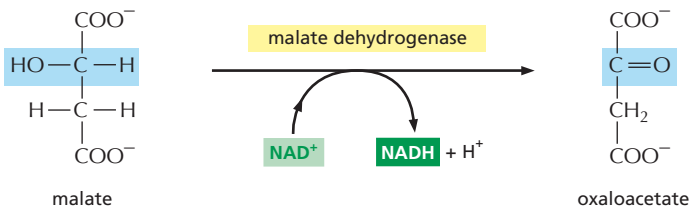
Step 6 In the third oxidation step in the cycle, FAD accepts two hydrogen atoms from succinate.



Step 7 The addition of water to fumarate places a hydroxyl group next to a carbonyl carbon.



Step 8 In the last of four oxidation steps in the cycle, the carbon carrying the hydroxyl group is converted to a carbonyl group, regenerating the oxaloacetate needed for step 1.



UNRAVELING THE CITRIC ACID CYCLE

"I have often been asked how the work on the citric acid cycle arose and developed," stated biochemist Hans Krebs in a lecture and review article in which he described his Nobel Prize-winning discovery of the cycle of reactions that lies at the center of cell metabolism. Did the concept stem from a sudden inspiration, a revelatory vision? "It was nothing of the kind," answered Krebs. Instead, his realization that these reactions occur in a cycle—rather than a set of linear pathways, as in glycolysis—arose from a "very slow evolutionary process" that occurred over a five-year period, during which Krebs coupled insight and reasoning to careful experimentation to discover one of the central pathways that underlies energy metabolism.

Minced tissues, curious catalysis

By the early 1930s, Krebs and other investigators had discovered that a select set of small organic molecules are oxidized extraordinarily rapidly in various types of tissue preparations—slices of kidney or liver, or suspensions of minced pigeon muscle. Because these reactions were seen to depend on the presence of oxygen, the researchers surmised that this set of molecules might include intermediates that are important in *cell respiration*—the consumption of O_2 and production of CO_2 that occurs when tissues break down foodstuffs.

Using the minced-tissue preparations, Krebs and others made the following observations. First, in the presence of oxygen, certain organic acids—citrate, succinate, fumarate, and malate—were readily oxidized to CO_2. These reactions depended on a continuous supply of oxygen.

Second, the oxidation of these acids occurred in two linear, sequential pathways:

citrate → α-ketoglutarate → succinate

and

succinate → fumarate → malate → oxaloacetate

Third, the addition of small amounts of several of these compounds to the minced-muscle suspensions stimulated an unusually large uptake of O_2—far greater than that needed to oxidize only the added molecules. To explain this surprising observation, Albert Szent-Györgyi (the Nobel laureate who worked out the second pathway above) suggested that a single molecule of each compound must somehow act catalytically to stimulate the oxidation of many molecules of some endogenous substance in the muscle.

At this point, most of the reactions central to the citric acid cycle were known. What was not yet clear—and caused great confusion, even to future Nobel laureates—was how these apparently linear reactions could drive such a catalytic consumption of oxygen, where each molecule of metabolite fuels the oxidation of many more molecules. To simplify the discussion of how Krebs ultimately solved this puzzle—by linking these linear reactions together into a circle—we will now refer to the molecules involved by a sequence of letters, A through H (**Figure 13–15**).

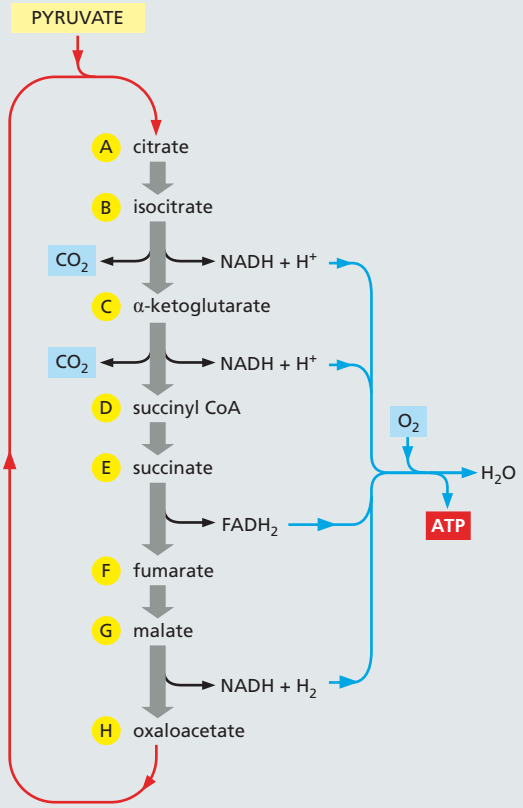


Figure 13–15 In this simplified representation of the citric acid cycle, O_2 is consumed and CO_2 is liberated as the molecular intermediates become oxidized. Krebs and others did not initially realize that these oxidation reactions occur in a cycle, as shown here.

A poison suggests a cycle

Many of the clues that Krebs used to work out the citric acid cycle came from experiments using malonate—a poisonous compound that specifically inhibits the enzyme succinate dehydrogenase, which converts E to F. Malonate closely resembles succinate (E) in its structure (**Figure 13–16**), and it serves as a competitive inhibitor of

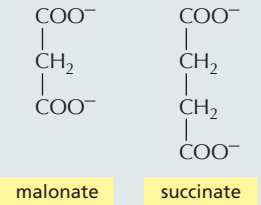


Figure 13–16 The structure of malonate closely resembles that of succinate.

the enzyme. Because the addition of malonate poisons cell respiration in tissues, Krebs concluded that succinate dehydrogenase (and the entire pathway linked to it) must play a critical role in the respiration process.

Krebs then discovered that when A, B, or C was added to malonate-poisoned tissue suspensions, E accumulated (**Figure 13–17A**). This observation reinforced the importance of succinate dehydrogenase for successful cell respiration. However, he found that E also accumulated when F, G, or H was added to malonate-poisoned muscle (**Figure 13–17B**). The latter result suggested that an additional set of reactions must exist that can convert F, G, and H molecules into E, since E was previously shown to be a precursor for F, G, and H, rather than a product of their reactions.

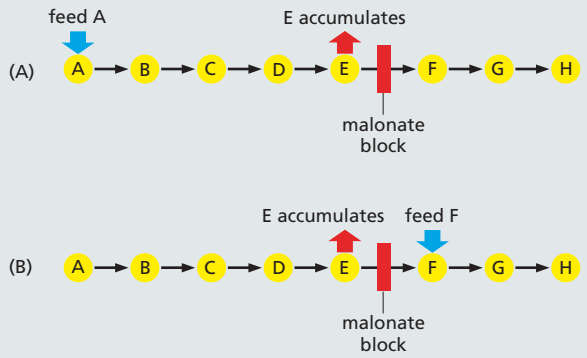


Figure 13–17 Poisoning muscle preparations with malonate provided clues to the cyclic nature of these oxidative reactions. (A) Adding A (or B or C—not shown) to malonate-poisoned muscle causes an accumulation of E. (B) Addition of F (or G or H—not shown) to a malonate-poisoned preparation also causes an accumulation of E, suggesting that enzymatic reactions can convert these molecules into E. The discovery that citrate (A) can be formed from oxaloacetate (H) and pyruvate allowed Krebs to join these two reaction pathways into a complete circle.

At about this time, Krebs also determined that when muscle suspensions were incubated with pyruvate and oxaloacetate, citrate formed: pyruvate + H → A.

This observation led Krebs to postulate that when oxygen is present, pyruvate and H condense to form A, converting the previously delineated string of linear reactions into a cyclic sequence (see Figure 13–15).

Explaining the mysterious stimulatory effects

The cycle of reactions that Krebs proposed clearly explained how the addition of small amounts of any of the intermediates A through H could cause the large increase in the uptake of O_2 that had been observed. Pyruvate is abundant in minced tissues, being readily produced by glycolysis (see Figure 13–4), using glucose derived from stored glycogen. Its oxidation requires a functioning citric acid cycle, in which each turn of the cycle results in the oxidation of one molecule of pyruvate. If the intermediates A through H are in small enough supply, the rate at which the entire cycle turns will be restricted. Adding a supply of any one of these intermediates will then have a dramatic effect on the rate at which the entire cycle operates. Thus, it is easy to see how a large number of pyruvate molecules can be oxidized, and a great deal of oxygen consumed, for every molecule of a citric acid cycle intermediate that is added (**Figure 13–18**).

Krebs went on to demonstrate that all of the individual enzymatic reactions in his postulated cycle took place in tissue preparations. Furthermore, they occured at rates high enough to account for the rate of pyruvate and oxygen consumption in these tissues. Krebs therefore concluded that this series of reactions is the major, if not the sole, pathway for the oxidation of pyruvate—at least in muscle. By fitting together pieces of information like a jigsaw puzzle, he arrived at a coherent picture of the intricate metabolic processes that underlie the oxidation—and took home a share of the 1953 Nobel Prize in Physiology or Medicine.

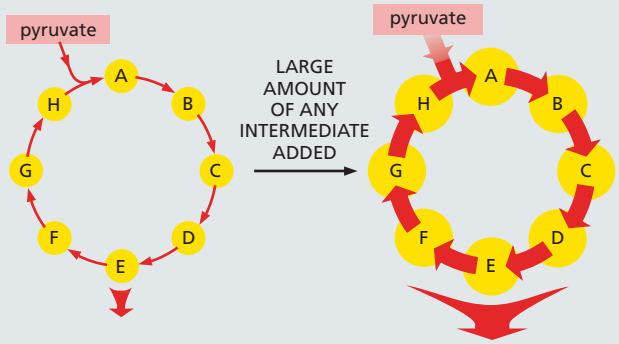


Figure 13–18 Replenishing the supply of any single intermediate has a dramatic effect on the rate at which the entire citric acid cycle operates. When the concentrations of intermediates are limiting, the cycle turns slowly and little pyruvate is used. O_2 uptake is low because only small amounts of NADH and $FADH_2$ are produced to feed oxidative phosphorylation (see Figure 13–19). But when a large amount of any one intermediate is added, the cycle turns rapidly; more of all the intermediates is made, and O_2 uptake is high.

Electron Transport Drives the Synthesis of the Majority of the ATP in Most Cells

We now return briefly to the final stage in the oxidation of food molecules: **oxidative phosphorylation**. It is in this stage that the chemical energy captured by the activated carriers produced during glycolysis and the citric acid cycle is used to generate ATP. During oxidative phosphorylation, NADH and $FADH_2$ transfer their high-energy electrons to the **electron-transport chain**—a series of electron carriers embedded in the inner mitochondrial membrane in eukaryotic cells (and in the plasma membrane of aerobic bacteria). As the electrons pass through the series of electron acceptor and donor molecules that form the chain, they fall to successively lower energy states. At specific sites in the chain, the energy released is used to drive H^+ (protons) across the inner membrane, from the mitochondrial matrix to the intermembrane space (see Figure 13–2). This movement generates a proton gradient across the inner membrane, which serves as a source of energy (like a battery) that can be tapped to drive a variety of energy-requiring reactions (discussed in Chapter 12). The most prominent of these reactions is the phosphorylation of ADP to generate ATP on the matrix side of the inner membrane (**Figure 13–19**).

At the end of the transport chain, the electrons are added to molecules of O_2 that have diffused into the mitochondrion, and the resulting reduced oxygen molecules immediately combine with protons (H^+) from the surrounding solution to produce water (see Figure 13–19). The electrons have now reached their lowest energy level, with all the available energy extracted from the food molecule being oxidized. In total, the complete oxidation of a molecule of glucose to H_2O and CO_2 can produce about 30 molecules of ATP. In contrast, only two molecules of ATP are produced per molecule of glucose by glycolysis alone.

Oxidative phosphorylation occurs in both eukaryotic cells and in aerobic bacteria. It represents a remarkable evolutionary achievement, and the ability to extract energy from food with such great efficiency has shaped the entire character of life on Earth. In the next chapter, we describe the mechanisms behind this game-changing molecular process and discuss how it likely arose.

QUESTION 13–5

What, if anything, is wrong with the following statement: "The oxygen consumed during the oxidation of glucose in animal cells is returned as part of CO_2 to the atmosphere." How could you support your answer experimentally?

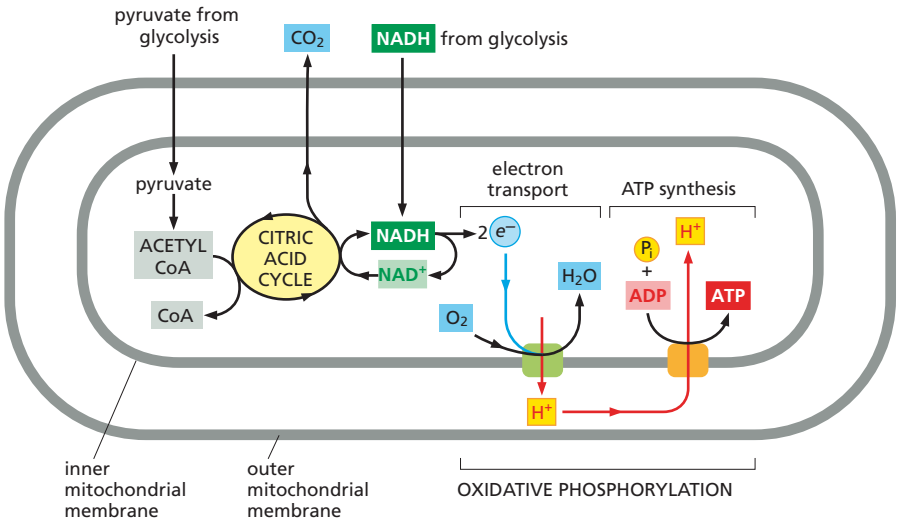


Figure 13–19 Oxidative phosphorylation completes the catabolism of food molecules and generates the bulk of the ATP made by the cell. Electron-bearing activated carriers produced by the citric acid cycle and glycolysis donate their high-energy electrons to an electron-transport chain in the inner mitochondrial membrane (or in the plasma membrane of aerobic bacteria). This electron transfer pumps protons across the inner membrane (*red arrows*). The resulting proton gradient is then used to drive the synthesis of ATP through the process of oxidative phosphorylation.

REGULATION OF METABOLISM

A cell is an intricate chemical machine, and our discussion of metabolism—with a focus on glycolysis and the citric acid cycle—has considered only a tiny fraction of the many enzymatic reactions that can take place in a cell at any time (**Figure 13–20**). For all these pathways to work together smoothly, as is required to allow the cell to survive and to respond to its environment, the choice of which pathway each metabolite will follow must be carefully regulated at every branch point.

Many sets of reactions need to be coordinated and controlled. For example, to maintain order within their cells, all organisms need to replenish their ATP pools continuously through the oxidation of sugars or fats. Yet animals have only periodic access to food, and plants need to survive without sunlight overnight, when they are unable to produce sugar through photosynthesis. Animals and plants have evolved several ways to cope with this problem. One is to synthesize food reserves in times of plenty that can be later consumed when other energy sources are scarce. Thus, depending on conditions, a cell must decide whether to route key metabolites into anabolic or catabolic pathways—in other words, whether to use them to build other molecules or burn them to provide

QUESTION 13–6

A cyclic reaction pathway requires that the starting material be regenerated and available at the end of each cycle. If compounds of the citric acid cycle are siphoned off as building blocks to make other organic molecules via a variety of metabolic reactions, why does the citric acid cycle not quickly grind to a halt?

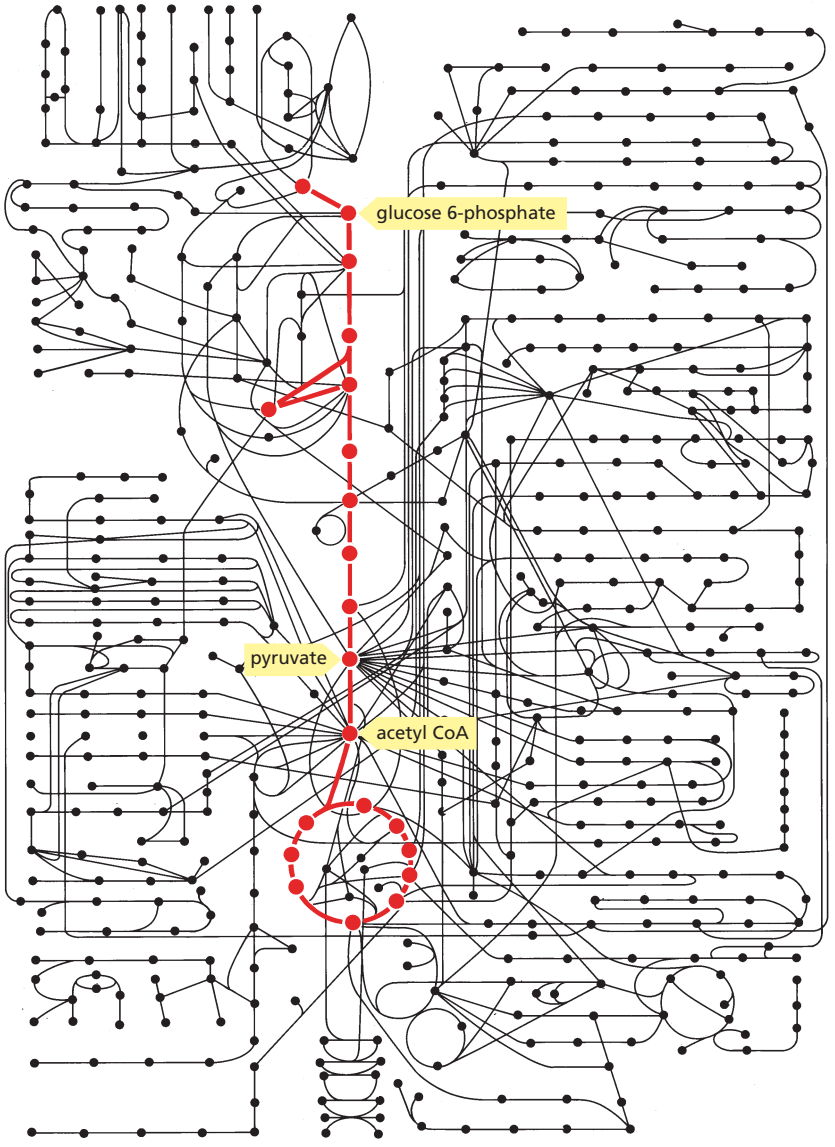


Figure 13–20 Glycolysis and the citric acid cycle constitute a small fraction of the reactions that occur in a cell. In this diagram, the filled circles represent molecules in various metabolic pathways, and the lines that connect them represent the enzymatic reactions that convert one metabolite to another. The reactions of glycolysis and the citric acid cycle are shown in *red*. Many other reactions either lead into these two central catabolic pathways—delivering small organic molecules to be oxidized for energy—or lead outward to the anabolic pathways that supply carbon compounds for biosynthesis.

immediate energy. In this section, we discuss how a cell regulates its intricate web of interconnected metabolic pathways to best serve both its immediate and long-term needs.

Catabolic and Anabolic Reactions Are Organized and Regulated

All the reactions shown in Figure 13–20 occur in a cell that is less than 0.1 mm in diameter, and each step requires a different enzyme. To add to the complexity, the same substrate is often a part of many different pathways. Pyruvate, for example, is a substrate for half a dozen or more different enzymes, each of which modifies it chemically in a different way. We have already seen that the pyruvate dehydrogenase complex converts pyruvate to acetyl CoA, and that, during fermentation, lactate dehydrogenase converts it to lactate. A third enzyme converts pyruvate to oxaloacetate, a fourth to the amino acid alanine, and so on. All these pathways compete for pyruvate molecules, and similar competitions for thousands of other small molecules go on at the same time.

To balance the activities of these interrelated reactions—and to allow organisms to adapt swiftly to changes in food availability or energy expenditure—an elaborate network of *control mechanisms* regulates and coordinates the activity of the enzymes that catalyze the myriad metabolic reactions that go on in a cell. As we discuss in Chapter 4, the activity of enzymes can be controlled by covalent modification—such as the addition or removal of a phosphate group (see Figure 4–41)—and by the binding of small regulatory molecules, often a metabolite (see pp. 150–151). Such regulation can either enhance the activity of the enzyme or inhibit it. As we see next, both types of regulation—positive and negative—control the activity of key enzymes involved in the breakdown and synthesis of glucose.

Feedback Regulation Allows Cells to Switch from Glucose Breakdown to Glucose Synthesis

Animals need an ample supply of glucose. Active muscles need glucose to power their contraction, and brain cells depend almost completely on glucose for energy. During periods of fasting or intense physical exercise, the body's glucose reserves get used up faster than they can be replenished from food. One way to increase available glucose is to synthesize it from pyruvate by a process called **gluconeogenesis**.

Gluconeogenesis is, in many ways, a reversal of glycolysis: it builds glucose from pyruvate, whereas glycolysis does the opposite. Indeed, gluconeogenesis makes use of many of the same enzymes as glycolysis; it simply runs them in reverse. For example, the isomerase that converts glucose 6-phosphate to fructose 6-phosphate in step 2 of glycolysis (see Panel 13–1, pp. 428–429) will readily catalyze the reverse reaction. There are, however, three steps in glycolysis that so strongly favor the direction of glucose breakdown that they are effectively irreversible. To get around these one-way steps, gluconeogenesis uses a special set of enzymes to catalyze a set of bypass reactions. In step 3 of glycolysis, for example, the enzyme phosphofructokinase catalyzes the phosphorylation of fructose 6-phosphate to produce the intermediate fructose 1, 6-bisphosphate. In gluconeogenesis, the enzyme fructose 1,6-bisphosphatase removes a phosphate from this intermediate to produce fructose 6-phosphate (**Figure 13–21**).

How does a cell decide whether to synthesize glucose or to degrade it? Part of the decision centers on the reactions shown in Figure 13–21. The activity of the enzyme phosphofructokinase is allosterically regulated by

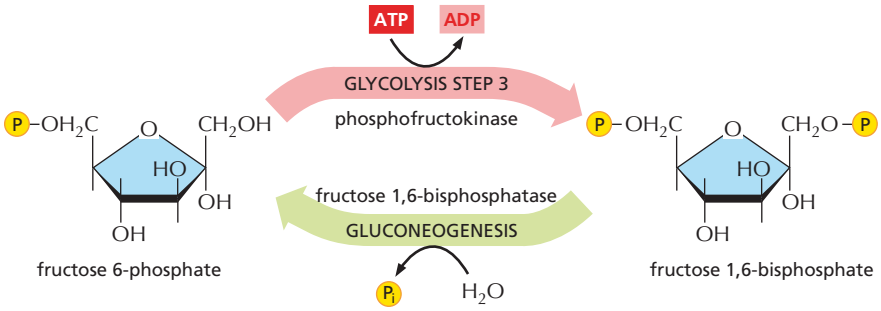


Figure 13–21 Gluconeogenesis uses specific enzymes to bypass those steps in glycolysis that are essentially irreversible. The enzyme phosphofructokinase catalyzes the phosphorylation of fructose 6-phosphate to form fructose 1, 6-bisphosphate in step 3 of glycolysis. This reaction is so energetically favorable that the enzyme will not work in reverse. To produce fructose 6-phosphate in gluconeogenesis, the enzyme fructose 1,6-bisphosphatase removes the phosphate from fructose 1,6-bisphosphate. Coordinated feedback regulation of these two enzymes helps control the flow of metabolites toward glucose synthesis or glucose breakdown.

the binding of a variety of metabolites, which provide both positive and negative *feedback regulation*. The enzyme is activated by byproducts of ATP hydrolysis—including ADP, AMP, and inorganic phosphate—and it is inhibited by ATP. Thus, when ATP is depleted and its metabolic byproducts accumulate, phosphofructokinase is turned on and glycolysis proceeds to generate ATP; when ATP is abundant, the enzyme is turned off and glycolysis shuts down. The enzyme that catalyzes the reverse reaction, fructose 1, 6–bisphosphatase (see Figure 13–21), is regulated by the same molecules but in the opposite direction. Thus this enzyme is activated when phosphofructokinase is turned off, allowing gluconeogenesis to proceed. Many such coordinated regulatory mechanisms enable a cell to respond rapidly to changing conditions and to adjust its metabolism accordingly.

Some of the biosynthetic bypass reactions required for gluconeogenesis are energetically costly. Production of a single molecule of glucose by gluconeogenesis consumes four molecules of ATP and two molecules of GTP. Thus a cell must tightly regulate the balance between glycolysis and gluconeogenesis. If both processes were to proceed simultaneously, they would shuttle metabolites back and forth in a futile cycle that would consume large amounts of energy and generate heat for no purpose.

Cells Store Food Molecules in Special Reservoirs to Prepare for Periods of Need

As we have seen, gluconeogenesis is a costly process, requiring substantial amounts of energy from the hydrolysis of ATP and GTP. During periods when food is scarce, this expensive way of producing glucose is suppressed if alternatives are available. Thus fasting cells can mobilize glucose that has been stored in the form of **glycogen**, a branched polymer of glucose (**Figure 13–22A** and see Panel 2–3, pp. 70–71). This large polysaccharide is stored as small granules in the cytoplasm of many animal cells, but mainly in liver and muscle cells (**Figure 13–22B**). The synthesis and degradation of glycogen occur by separate metabolic pathways, which can be rapidly and coordinately regulated according to need. When more ATP is needed than can be generated from food molecules taken in from the bloodstream, cells break down glycogen in a reaction that is catalyzed by the enzyme *glycogen phosphorylase*. This enzyme produces *glucose 1-phosphate*, which is then converted to the glucose 6-phosphate that feeds into the glycolytic pathway (**Figure 13–22C**).

The glycogen synthetic and degradative pathways are coordinated by feedback regulation. Enzymes in each pathway are allosterically regulated by glucose 6-phosphate, but in opposite directions: *glycogen synthetase* in the synthetic pathway is activated by glucose 6-phosphate, whereas glycogen phosphorylase, which breaks down glycogen (see Figure 13–22C), is inhibited by glucose 6-phosphate, as well as by ATP. This regulation

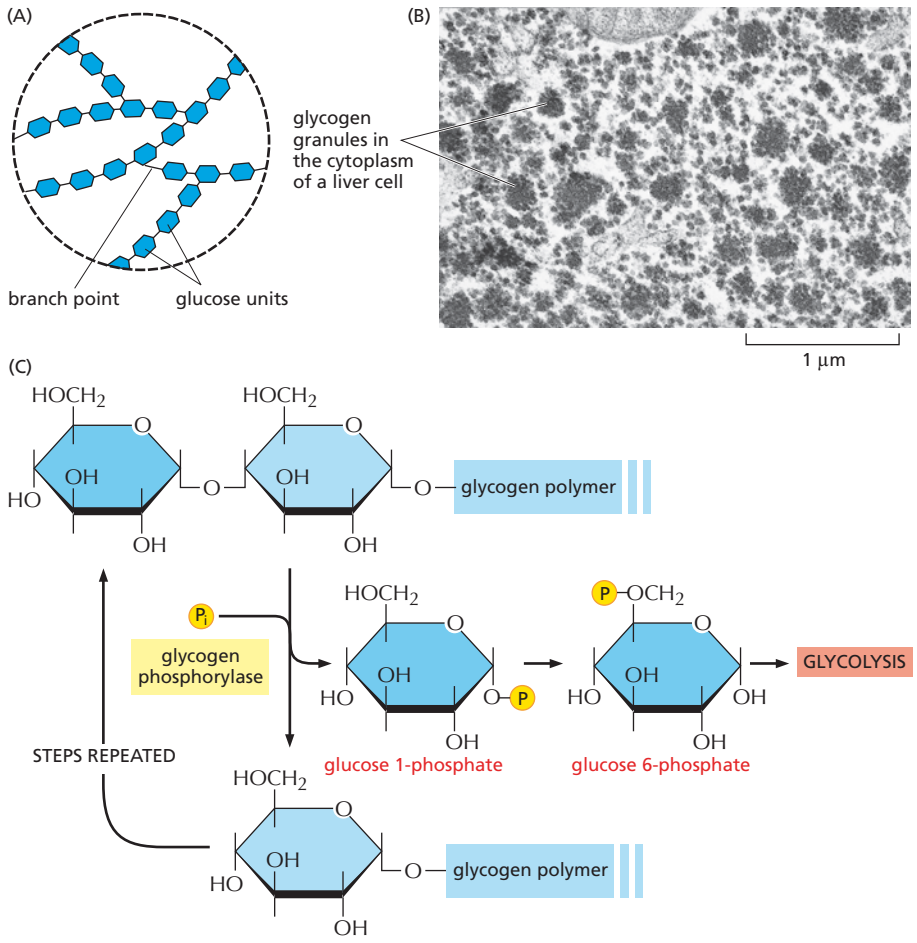


Figure 13–22 Animal cells store glucose in the form of glycogen to provide energy in times of need. (A) The structure of glycogen (starch in plants is a very similar branched polymer of glucose but has many fewer branch points). (B) An electron micrograph showing glycogen granules in the cytoplasm of a liver cell; each granule contains both glycogen and the enzymes required for glycogen synthesis and breakdown. (C) The enzyme glycogen phosphorylase breaks down glycogen when cells need more glucose. (B, courtesy of Robert Fletterick and Daniel S. Friend.)

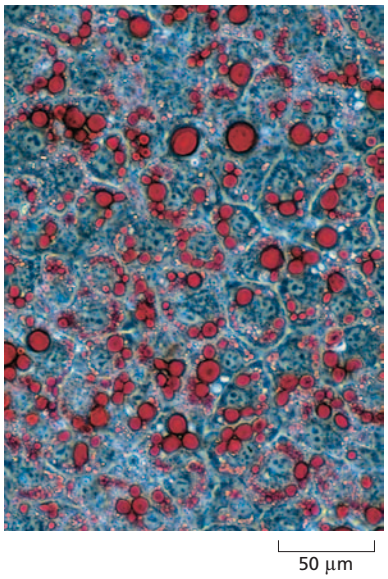


Figure 13–23 Fats are stored in the form of fat droplets in animal cells. The fat droplets (stained *red*) shown here are in the cytoplasm of developing adipocytes. (Courtesy of Peter Tontonoz and Ronald M. Evans.)

helps to prevent glycogen breakdown when ATP is plentiful and to favor glycogen synthesis when glucose 6-phosphate concentration is high. The balance between glycogen synthesis and breakdown is further regulated by intracellular signaling pathways that are controlled by the hormones insulin, adrenaline, and glucagon (see Table 16–1, p. 529 and Figure 16–25, p. 546).

Quantitatively, fat is a far more important storage material than glycogen, in part because the oxidation of a gram of fat releases about twice as much energy as the oxidation of a gram of glycogen. Moreover, glycogen binds a great deal of water, producing a sixfold difference in the actual mass of glycogen required to store the same amount of energy as fat. An average adult human stores enough glycogen for only about a day of normal activity, but enough fat to last nearly a month. If our main fuel reserves had to be carried as glycogen instead of fat, body weight would need to be increased by an average of about 60 pounds (nearly 30 kilograms).

Most of our fat is stored as droplets of water-insoluble triacylglycerols in specialized fat cells called *adipocytes* (**Figure 13–23** and see Figure 13–11 A and B). In response to hormonal signals, fatty acids can be released from these depots into the bloodstream for other cells to use as required. Such a need arises after a period of not eating. Even a normal overnight fast results in the mobilization of fat: in the morning, most of the acetyl CoA that enters the citric acid cycle is derived from fatty acids rather than from glucose. After a meal, however, most of the acetyl CoA entering the citric acid cycle comes from glucose derived from food, and any excess

Figure 13–24 Some plant seeds serve as important foods for humans. Corn, nuts, and peas all contain rich stores of starch and fats, which provide the plant embryo in the seed with energy and building blocks for biosynthesis. (Courtesy of the John Innes Foundation.)

glucose is used to make glycogen or fat. (Although animal cells can readily convert sugars to fats, they cannot convert fatty acids to sugars.)

The food reserves in both animals and plants form a vital part of the human diet. Plants convert some of the sugars they make through photosynthesis during daylight into fats and into **starch**, a branched polymer of glucose very similar to animal glycogen. The fats in plants are triacylglycerols, as they are in animals, and they differ only in the types of fatty acids that predominate (see Figures 2–19 and 2–20).

The embryo inside a plant seed must live on stored food reserves for a long time, until the seed germinates to produce a plant with leaves that can harvest the energy in sunlight. The embryo uses these food stores as sources of energy and of small molecules to build cell walls and to synthesize many other biological molecules as it develops. For this reason, plant seeds often contain especially large amounts of fats and starch—which make them a major food source for animals, including ourselves (**Figure 13–24**). Germinating seeds convert the stored fat and starch into glucose as needed.

In plant cells, fats and starch are both stored in chloroplasts—specialized organelles that carry out photosynthesis (**Figure 13–25**). These energy-rich molecules serve as food reservoirs that are mobilized by the cell to produce ATP in mitochondria during periods of darkness. In the next chapter, we take a closer look at chloroplasts and mitochondria, and review the elaborate mechanisms by which they harvest energy from sunlight and from food.

QUESTION 13–7

After looking at the structures of sugars and fatty acids (discussed in Chapter 2), give an intuitive explanation as to why oxidation of a sugar yields only about half as much energy as the oxidation of an equivalent dry weight of a fatty acid.

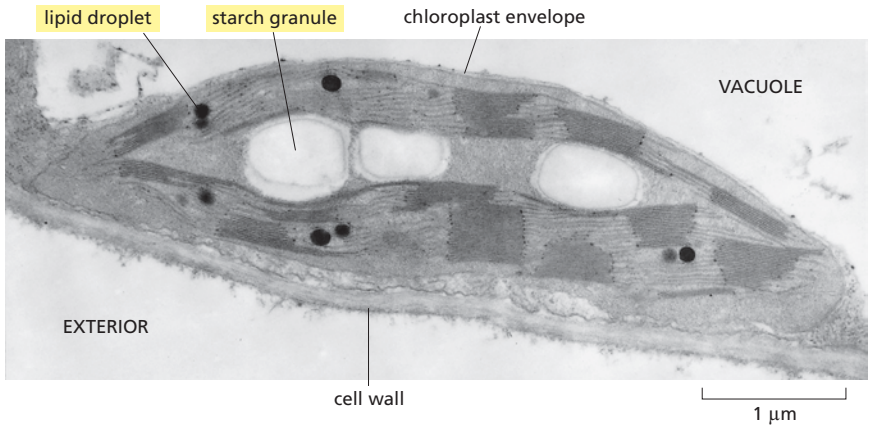


Figure 13–25 Plant cells store both starch and fats in their chloroplasts. An electron micrograph of a single chloroplast in a plant cell shows the starch granules and lipid droplets (fats) that have been synthesized in the organelle. (Courtesy of K. Plaskitt.)

ESSENTIAL CONCEPTS

- Food molecules are broken down in successive steps, in which energy is captured in the form of activated carriers such as ATP and NADH.
- In plants and animals, these catabolic reactions occur in different cell compartments: glycolysis in the cytosol, the citric acid cycle in the mitochondrial matrix, and oxidative phosphorylation on the inner mitochondrial membrane.
- During glycolysis, the six-carbon sugar glucose is split to form two molecules of the three-carbon sugar pyruvate, producing small amounts of ATP and NADH.
- In the presence of oxygen, eukaryotic cells convert pyruvate into acetyl CoA plus CO_2 in the mitochondrial matrix. The citric acid cycle then converts the acetyl group in acetyl CoA to CO_2 and H_2O, capturing much of the energy released as high-energy electrons in the activated carriers NADH and $FADH_2$.
- Fatty acids produced from the digestion of fats are also imported into mitochondria and converted to acetyl CoA molecules, which are then further oxidized through the citric acid cycle.
- In the mitochondrial matrix, NADH and $FADH_2$ pass their high-energy electrons to an electron-transport chain in the inner mitochondrial membrane, where a series of electron transfers is used to drive the formation of ATP. Most of the energy captured during the breakdown of food molecules is harvested during this process of oxidative phosphorylation (described in detail in Chapter 14).
- Many intermediates of glycolysis and the citric acid cycle are starting points for the anabolic pathways that lead to the synthesis of proteins, nucleic acids, and the many other organic molecules of the cell.
- The thousands of different reactions carried out simultaneously by a cell are regulated and coordinated by positive and negative feedback, enabling the cell to adapt to changing conditions; for example, such feedback allows a cell to switch from glucose breakdown to glucose synthesis when food is scarce.
- Cells store food molecules in special reserves. Glucose subunits are stored as glycogen in animal cells and as starch in plant cells; both animal and plant cells store fatty acids as fats. The food reserves stored by plants are major sources of food for animals, including humans.

KEY TERMS

acetyl CoA
ADP, ATP
anabolic pathways
catabolism
cell respiration
citric acid cycle
electron-transport chain
FAD, $FADH_2$
fat
fermentation
GDP, GTP
gluconeogenesis
glucose
glycogen
glycolysis
NAD^+, NADH
oxidative phosphorylation
pyruvate
starch

QUESTIONS

QUESTION 13–8

The oxidation of sugar molecules by the cell takes place according to the general reaction $C_6H_{12}O_6$ (glucose) + $6O_2 \rightarrow 6CO_2 + 6H_2O$ + energy. Which of the following statements are correct? Explain your answers.

A. All of the energy produced is in the form of heat.

B. None of the produced energy is in the form of heat.

C. The energy is produced by a process that involves the oxidation of carbon atoms.

D. The reaction supplies the cell with essential water.

E. In cells, the reaction takes place in more than one step.

F. Many steps in the oxidation of sugar molecules involve reaction with oxygen gas.

G. Some organisms carry out the reverse reaction.

H. Some cells that grow in the absence of O_2 produce CO_2.

QUESTION 13–9

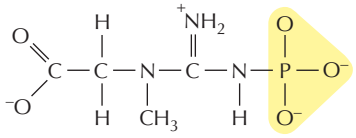
An exceedingly sensitive instrument (yet to be devised) shows that one of the carbon atoms in Charles Darwin's last breath is resident in your bloodstream, where it forms part of a hemoglobin molecule. Suggest how this carbon atom might have traveled from Darwin to you, and list some of the molecules it could have entered en route.

QUESTION 13–10

Yeast cells can grow both in the presence of O_2 (aerobically) and in its absence (anaerobically). Under which of the two conditions could you expect the cells to grow better? Explain your answer.

QUESTION 13–11

During movement, muscle cells require large amounts of ATP to fuel their contractile apparatus. These cells contain high levels of creatine phosphate (**Figure Q13–11**), which has a standard free-energy change ($\Delta G°$) for hydrolysis of its phosphate bond of –10.3 kcal/mole. Why is this a useful compound to store energy? Justify your answer with the information shown in Figure 13–8.



creatine phosphate

Figure Q13–11

QUESTION 13–12

Identical pathways that make up the complicated sequence of reactions of glycolysis, shown in Panel 13–1 (pp. 428–429), are found in most living cells, from bacteria to humans. One could envision, however, countless alternative chemical reaction mechanisms that would allow the oxidation of sugar molecules and that could, in principle, have evolved to take the place of glycolysis. Discuss this fact in the context of evolution.

QUESTION 13–13

An animal cell, roughly cubical in shape with side length of 10 μm, uses 10^9 ATP molecules every minute. Assume that the cell replaces this ATP by the oxidation of glucose according to the overall reaction $6O_2 + C_6H_{12}O_6 \rightarrow 6CO_2 + 6H_2O$ and that complete oxidation of each glucose molecule produces 30 ATP molecules. How much oxygen does the cell consume every minute? How long will it take before the cell has used up an amount of oxygen gas equal to its own volume? (Recall that one mole of a gas has a volume of 22.4 liter.)

QUESTION 13–14

Under the conditions existing in the cell, the free energies of the first few reactions in glycolysis (in Panel 13–1, pp. 428–429) are:

step 1 ΔG = –8.0 kcal/mole

step 2 ΔG = –0.6 kcal/mole

step 3 ΔG = –5.3 kcal/mole

step 4 ΔG = –0.3 kcal/mole

Are these reactions energetically favorable? Using these values, draw to scale an energy diagram (A) for the overall reaction and (B) for the pathway composed of the four individual reactions.

QUESTION 13–15

The chemistry of most metabolic reactions was deciphered by synthesizing metabolites containing atoms that are different isotopes from those occurring naturally. The products of reactions starting with isotopically labeled metabolites can be analyzed to determine precisely which atoms in the products are derived from which atoms in the starting material. The methods of detection exploit, for example, the fact that different isotopes have different masses that can be distinguished using biophysical techniques such as mass spectrometry. Moreover, some isotopes are radioactive and can therefore be readily recognized with electronic counters or photographic film that becomes exposed by radiation.

A. Assume that pyruvate containing radioactive ^{14}C in its carboxyl group is added to a cell extract that can support oxidative phosphorylation. Which of the molecules produced should contain the vast majority of the ^{14}C that was added?

B. Assume that oxaloacetate containing radioactive ^{14}C in its keto group (refer to Panel 13–2, pp. 434–435) is added to the extract. Where should the ^{14}C atom be located after precisely one turn of the cycle?

QUESTION 13–16

In cells that can grow both aerobically and anaerobically, fermentation is inhibited in the presence of O_2. Suggest a reason for this observation.