

9

How Cells Harvest Energy

Concept Outline

9.1 Cells harvest the energy in chemical bonds.

Using Chemical Energy to Drive Metabolism. The energy in C—H, C—O, and other chemical bonds can be captured and used to fuel the synthesis of ATP.

9.2 Cellular respiration oxidizes food molecules.

An Overview of Glucose Catabolism. The chemical energy in sugar is harvested by both substrate-level phosphorylation and by aerobic respiration.

Stage One: Glycolysis. The 10 reactions of glycolysis capture energy from glucose by reshuffling the bonds.

Stage Two: The Oxidation of Pyruvate. Pyruvate, the product of glycolysis, is oxidized to acetyl-CoA.

Stage Three: The Krebs Cycle. In a series of reactions, electrons are stripped from acetyl-CoA.

Harvesting Energy by Extracting Electrons. The respiration of glucose is a series of oxidation-reduction reactions which involve stripping electrons from glucose and using the energy of these electrons to power the synthesis of ATP.

Stage Four: The Electron Transport Chain. The electrons harvested from glucose pass through a chain of membrane proteins that use the energy to pump protons, driving the synthesis of ATP.

Summarizing Aerobic Respiration. The oxidation of glucose by aerobic respiration in eukaryotes produces up to three dozen ATP molecules, over half the energy in the chemical bonds of glucose.

Regulating Aerobic Respiration. High levels of ATP tend to shut down cellular respiration by feedback-inhibiting key reactions.

9.3 Catabolism of proteins and fats can yield considerable energy.

Glucose Is Not the Only Source of Energy. Proteins and fats are dismantled and the products fed into cellular respiration.

9.4 Cells can metabolize food without oxygen.

Fermentation. Fermentation allows continued metabolism in the absence of oxygen by donating the electrons harvested in glycolysis to organic molecules.



FIGURE 9.1
Harvesting chemical energy. Organisms such as these harvest mice depend on the energy stored in the chemical bonds of the food they eat to power their life processes.

Life is driven by energy. All the activities organisms carry out—the swimming of bacteria, the purring of a cat, your reading of these words—use energy. In this chapter, we will discuss the processes all cells use to derive chemical energy from organic molecules and to convert that energy to ATP. We will consider photosynthesis, which uses light energy rather than chemical energy, in detail in chapter 10. We examine the conversion of chemical energy to ATP first because all organisms, both photosynthesizers and the organisms that feed on them (like the field mice in figure 9.1), are capable of harvesting energy from chemical bonds. As you will see, though, this process and photosynthesis have much in common.

9.1 Cells harvest the energy in chemical bonds.

Using Chemical Energy to Drive Metabolism

Plants, algae, and some bacteria harvest the energy of sunlight through photosynthesis, converting radiant energy into chemical energy. These organisms, along with a few others that use chemical energy in a similar way, are called **autotrophs** (“self-feeders”). All other organisms live on the energy autotrophs produce and are called **heterotrophs** (“fed by others”). At least 95% of the kinds of organisms on earth—all animals and fungi, and most protists and bacteria—are heterotrophs.

Where is the chemical energy in food, and how do heterotrophs harvest it to carry out the many tasks of living (figure 9.2)? Most foods contain a variety of carbohydrates, proteins, and fats, all rich in energy-laden chemical bonds. Carbohydrates and fats, for example, possess many carbon-hydrogen (C—H), as well as carbon-oxygen (C—O) bonds. The job of extracting energy from this complex organic mixture is tackled in stages. First, enzymes break the large molecules down into smaller ones, a process called **digestion**. Then, other enzymes dismantle these fragments a little at a time, harvesting energy from C—H and other chemical bonds at each stage. This process is called **catabolism**.

While you obtain energy from many of the constituents of food, it is traditional to focus first on the catabolism of carbohydrates. We will follow the six-carbon sugar, glucose ($C_6H_{12}O_6$), as its chemical bonds are progressively harvested for energy. Later, we will come back and examine the catabolism of proteins and fats.

Cellular Respiration

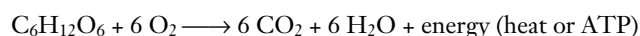
The energy in a chemical bond can be visualized as potential energy borne by the electrons that make up the covalent bond. Cells harvest this energy by putting the electrons to work, often to produce ATP, the energy currency of the cell. Afterward, the energy-depleted electron (associated with a proton as a hydrogen atom) is donated to some other molecule. When oxygen gas (O_2) accepts the hydrogen atom, water forms, and the process is called **aerobic respiration**. When an inorganic molecule other than oxygen accepts the hydrogen, the process is called **anaerobic respiration**. When an organic molecule accepts the hydrogen atom, the process is called **fermentation**.

Chemically, there is little difference between the catabolism of carbohydrates in a cell and the burning of wood in a



FIGURE 9.2
Start every day with a good breakfast. The carbohydrates, proteins, and fats in this fish contain energy that the bear’s cells can use to power their daily activities.

fireplace. In both instances, the reactants are carbohydrates and oxygen, and the products are carbon dioxide, water, and energy:



The change in free energy in this reaction is -720 kilocalories (-3012 kilojoules) per mole of glucose under the conditions found within a cell (the traditional value of -686 kilocalories, or -2870 kJ, per mole refers to standard conditions—room temperature, one atmosphere of pressure, etc.). This change in free energy results largely from the breaking of the six C—H bonds in the glucose molecule. The negative sign indicates that the products possess *less* free energy than the reactants. The same amount of energy is released whether glucose is catabolized or burned, but when it is burned most of the energy is released as heat. This heat cannot be used to perform work in cells. The key to a cell’s ability to harvest useful energy from the catabolism of food molecules such as glucose is its conversion of a portion of the energy into a more useful form. Cells do this by using some of the energy to drive the production of ATP, a molecule that can power cellular activities.

The ATP Molecule

Adenosine triphosphate (ATP) is the energy currency of the cell, the molecule that transfers the energy captured during respiration to the many sites that use energy in the cell. How is ATP able to transfer energy so readily? Recall from chapter 8 that ATP is composed of a sugar (ribose) bound to an organic base (adenine) and a chain of three phosphate groups. As shown in figure 9.3, each phosphate group is negatively charged. Because like charges repel each other, the linked phosphate groups push against the bond that holds them together. Like a cocked mousetrap, the linked phosphates store the energy of their electrostatic repulsion. Transferring a phosphate group to another molecule relaxes the electrostatic spring of ATP, at the same time cocking the spring of the molecule that is phosphorylated. This molecule can then use the energy to undergo some change that requires work.

How Cells Use ATP

Cells use ATP to do most of those activities that require work. One of the most obvious is movement. Some bacteria swim about, propelling themselves through the water by rapidly spinning a long, tail-like flagellum, much as a ship moves by spinning a propeller. During your development as an embryo, many of your cells moved about, crawling over one another to reach new positions. Movement also occurs within cells. Tiny fibers within muscle cells pull against one another when muscles contract. Mitochondria pass a meter or more along the narrow nerve cells that connect your feet with your spine. Chromosomes are pulled by microtubules during cell division. All of these movements by cells require the expenditure of ATP energy.

A second major way cells use ATP is to *drive endergonic reactions*. Many of the synthetic activities of the cell are endergonic, because building molecules takes energy. The chemical bonds of the products of these reactions contain more energy, or are more organized, than the reactants. The reaction can't proceed until that extra energy is supplied to the reaction. It is ATP that provides this needed energy.

How ATP Drives Endergonic Reactions

How does ATP drive an endergonic reaction? The enzyme that catalyzes the endergonic reaction has *two* binding sites on its surface, one for the reactant and another for ATP. The ATP site splits the ATP molecule, liberating over 7 kcal (30 kJ) of chemical energy. This energy pushes the reactant at the second site “uphill,” driving the endergonic reaction. (In a similar way, you can make water in a swimming pool leap straight up in the air, despite the fact that gravity prevents water from rising spontaneously—just jump in the pool! The energy you add going in more than compensates for the force of gravity holding the water back.)

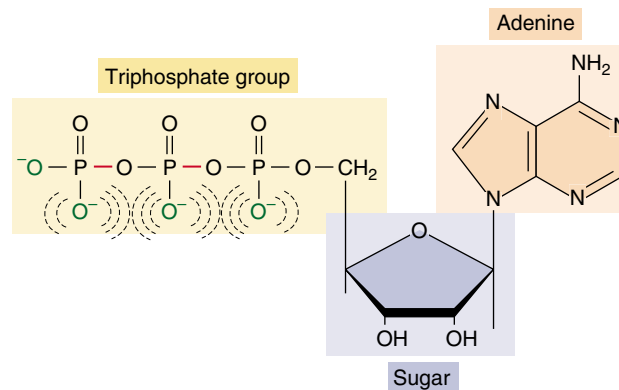


FIGURE 9.3
Structure of the ATP molecule. ATP is composed of an organic base and a chain of phosphates attached to opposite ends of a five-carbon sugar. Notice that the charged regions of the phosphate chain are close to one another. These like charges tend to repel one another, giving the bonds that hold them together a particularly high energy transfer potential.

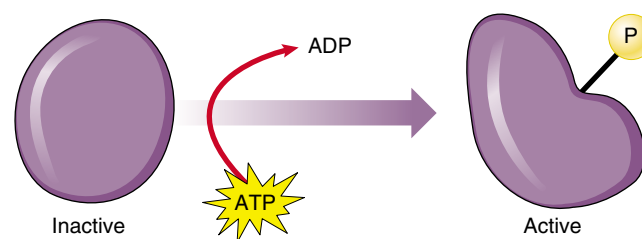


FIGURE 9.4
How ATP drives an endergonic reaction. In many cases, a phosphate group split from ATP activates a protein, catalyzing an endergonic process.

When the splitting of ATP molecules drives an energy-requiring reaction in a cell, the two parts of the reaction—ATP-splitting and endergonic—take place in concert. In some cases, the two parts both occur on the surface of the same enzyme; they are physically linked, or “coupled,” like two legs walking. In other cases, a high-energy phosphate from ATP attaches to the protein catalyzing the endergonic process, activating it (figure 9.4). Coupling energy-requiring reactions to the splitting of ATP in this way is one of the key tools cells use to manage energy.

The catabolism of glucose into carbon dioxide and water in living organisms releases about 720 kcal (3012 kJ) of energy per mole of glucose. This energy is captured in ATP, which stores the energy by linking charged phosphate groups near one another. When the phosphate bonds in ATP are hydrolyzed, energy is released and available to do work.

9.2 Cellular respiration oxidizes food molecules.

An Overview of Glucose Catabolism

Cells are able to make ATP from the catabolism of organic molecules in two different ways.

- 1. Substrate-level phosphorylation.** In the first, called **substrate-level phosphorylation**, ATP is formed by transferring a phosphate group directly to ADP from a phosphate-bearing intermediate (figure 9.5). During glycolysis, discussed below, the chemical bonds of glucose are shifted around in reactions that provide the energy required to form ATP.
- 2. Aerobic respiration.** In the second, called **aerobic respiration**, ATP forms as electrons are harvested, transferred along the electron transport chain, and eventually donated to oxygen gas. Eukaryotes produce the majority of their ATP from glucose in this way.

In most organisms, these two processes are combined. To harvest energy to make ATP from the sugar glucose in the presence of oxygen, the cell carries out a complex series of enzyme-catalyzed reactions that occur in four stages: the first stage captures energy by substrate-level phosphorylation through glycolysis, the following three stages carry out aerobic respiration by oxidizing the end product of glycolysis.

Glycolysis

Stage One: Glycolysis. The first stage of extracting energy from glucose is a 10-reaction biochemical pathway called glycolysis that produces ATP by substrate-level phosphorylation. The enzymes that catalyze the glycolytic reactions are in the cytoplasm of the cell, not bound to any membrane or organelle. Two ATP molecules are used up early in the pathway, and four ATP molecules are formed by substrate-level phosphorylation. This yields a net of two ATP molecules for each molecule of glucose catabolized. In addition, four electrons are harvested as NADH that can be used to form ATP by aerobic respiration. Still, the total yield of ATP is small. When the glycolytic process is completed, the two molecules of pyruvate that are formed still contain most of the energy the original glucose molecule held.

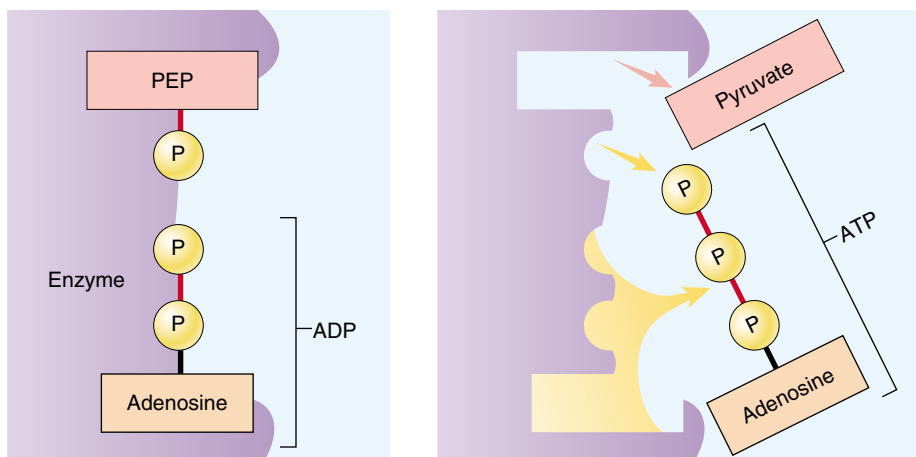


FIGURE 9.5
Substrate-level phosphorylation. Some molecules, such as phosphoenolpyruvate (PEP), possess a high-energy phosphate bond similar to the bonds in ATP. When PEP's phosphate group is transferred enzymatically to ADP, the energy in the bond is conserved and ATP is created.

Aerobic Respiration

Stage Two: Pyruvate Oxidation. In the second stage, pyruvate, the end product from glycolysis, is converted into carbon dioxide and a two-carbon molecule called acetyl-CoA. For each molecule of pyruvate converted, one molecule of NAD^+ is reduced to NADH.

Stage Three: The Krebs Cycle. The third stage introduces this acetyl-CoA into a cycle of nine reactions called the Krebs cycle, named after the British biochemist, Sir Hans Krebs, who discovered it. (The Krebs cycle is also called the citric acid cycle, for the citric acid, or citrate, formed in its first step, and less commonly, the tricarboxylic acid cycle, because citrate has three carboxyl groups.) In the Krebs cycle, two more ATP molecules are extracted by substrate-level phosphorylation, and a large number of electrons are removed by the reduction of NAD^+ to NADH.

Stage Four: Electron Transport Chain. In the fourth stage, the energetic electrons carried by NADH are employed to drive the synthesis of a large amount of ATP by the electron transport chain.

Pyruvate oxidation, the reactions of the Krebs cycle, and ATP production by electron transport chains occur within many forms of bacteria and inside the mitochondria of all eukaryotes. Recall from chapter 5 that mitochondria are thought to have evolved from bacteria. Although plants and algae can produce ATP by photosynthesis, they also produce ATP by aerobic respiration, just as animals and other nonphotosynthetic eukaryotes do. Figure 9.6 provides an overview of aerobic respiration.

Anaerobic Respiration

In the presence of oxygen, cells can respire aerobically, using oxygen to accept the electrons harvested from food molecules. In the absence of oxygen to accept the electrons, some organisms can still respire anaerobically, using inorganic molecules to accept the electrons. For example, many bacteria use sulfur, nitrate, or other inorganic compounds as the electron acceptor in place of oxygen.

Methanogens. Among the heterotrophs that practice anaerobic respiration are primitive archaeobacteria such as the thermophiles discussed in chapter 4. Some of these, called methanogens, use CO_2 as the electron acceptor, reducing CO_2 to CH_4 (methane) with the hydrogens derived from organic molecules produced by other organisms.

Sulfur Bacteria. Evidence of a second anaerobic respiratory process among primitive bacteria is seen in a group of rocks about 2.7 billion years old, known as the Woman River iron formation. Organic material in these rocks is enriched for the light isotope of sulfur, ^{32}S , rela-

tive to the heavier isotope ^{34}S . No known geochemical process produces such enrichment, but biological sulfur reduction does, in a process still carried out today by certain primitive bacteria. In this sulfate respiration, the bacteria derive energy from the reduction of inorganic sulfates (SO_4) to H_2S . The hydrogen atoms are obtained from organic molecules other organisms produce. These bacteria thus do the same thing methanogens do, but they use SO_4 as the oxidizing (that is, electron-accepting) agent in place of CO_2 .

The sulfate reducers set the stage for the evolution of photosynthesis, creating an environment rich in H_2S . As discussed in chapter 8, the first form of photosynthesis obtained hydrogens from H_2S using the energy of sunlight.

In aerobic respiration, the cell harvests energy from glucose molecules in a sequence of four major pathways: glycolysis, pyruvate oxidation, the Krebs cycle, and the electron transport chain. Oxygen is the final electron acceptor. Anaerobic respiration donates the harvested electrons to other inorganic compounds.

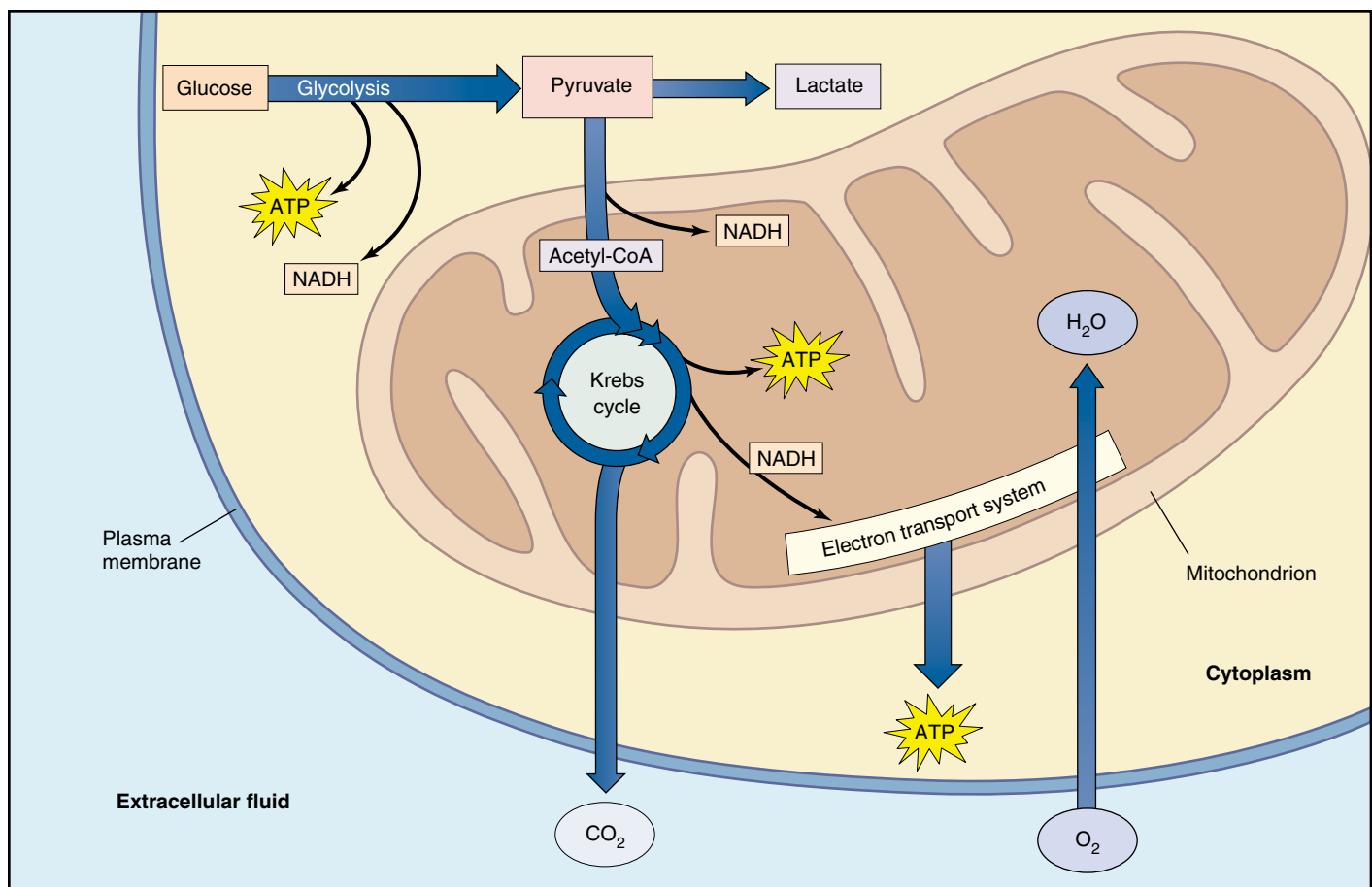


FIGURE 9.6
An overview of aerobic respiration.

Stage One: Glycolysis

The metabolism of primitive organisms focused on glucose. Glucose molecules can be dismantled in many ways, but primitive organisms evolved a glucose-catabolizing process that releases enough free energy to drive the synthesis of ATP in coupled reactions. This process, called glycolysis, occurs in the cytoplasm and involves a sequence of 10 reactions that convert glucose into 2 three-carbon molecules of pyruvate (figure 9.7). For each molecule of glucose that passes through this transformation, the cell nets two ATP molecules by substrate-level phosphorylation.

Priming

The first half of glycolysis consists of five sequential reactions that convert one molecule of glucose into two molecules of the three-carbon compound, glyceraldehyde 3-phosphate (G3P). These reactions demand the expenditure of ATP, so they are an energy-requiring process.

Step A: Glucose priming. Three reactions “prime” glucose by changing it into a compound that can be cleaved readily into 2 three-carbon phosphorylated molecules. Two of these reactions require the cleavage of ATP, so this step requires the cell to use two ATP molecules.

Step B: Cleavage and rearrangement. In the first of the remaining pair of reactions, the six-carbon product of step A is split into 2 three-carbon molecules. One is G3P, and the other is then converted to G3P by the second reaction (figure 9.8).

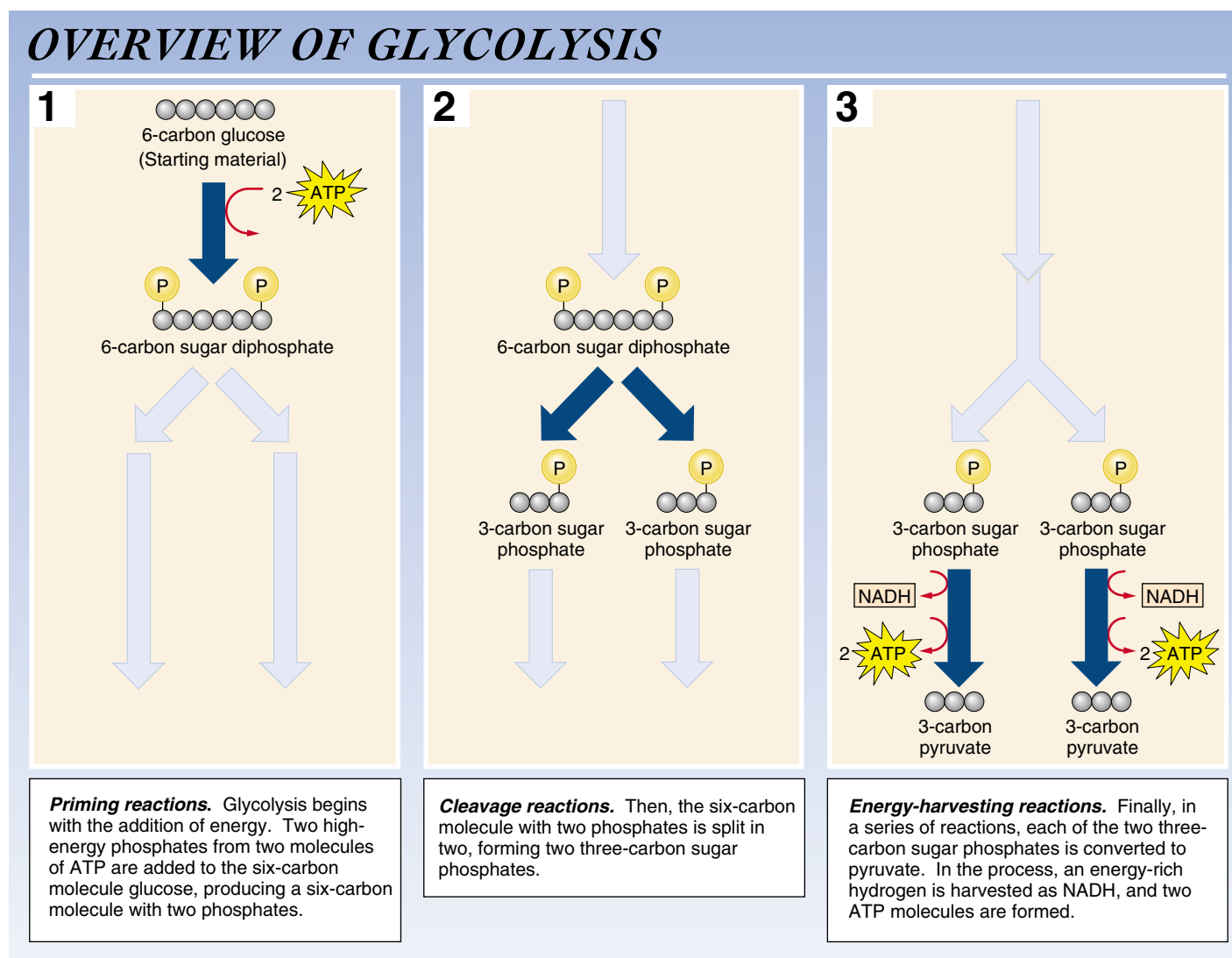


FIGURE 9.7
How glycolysis works.

1. Phosphorylation of glucose by ATP.

2–3. Rearrangement, followed by a second ATP phosphorylation.

4–5. The six-carbon molecule is split into two three-carbon molecules—one G3P, another that is converted into G3P in another reaction.

6. Oxidation followed by phosphorylation produces two NADH molecules and two molecules of BPG, each with one high-energy phosphate bond.

7. Removal of high-energy phosphate by two ADP molecules produces two ATP molecules and leaves two 3PG molecules.

8–9. Removal of water yields two PEP molecules, each with a high-energy phosphate bond.

10. Removal of high-energy phosphate by two ADP molecules produces two ATP molecules and two pyruvate molecules.

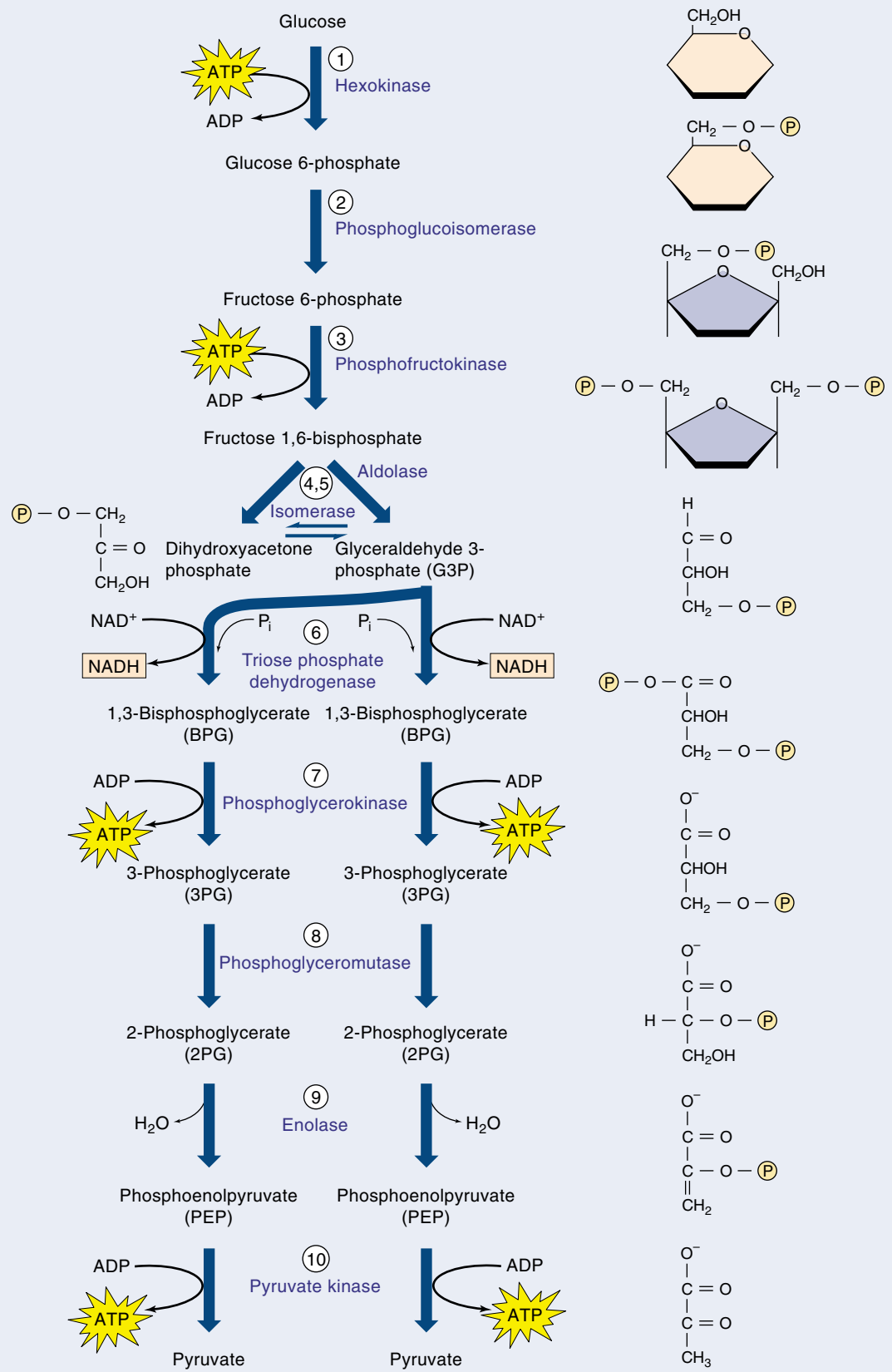


FIGURE 9.8 The glycolytic pathway. The first five reactions convert a molecule of glucose into two molecules of G3P. The second five reactions convert G3P into pyruvate.

Substrate-Level Phosphorylation

In the second half of glycolysis, five more reactions convert G3P into pyruvate in an energy-yielding process that generates ATP. Overall, then, glycolysis is a series of 10 enzyme-catalyzed reactions in which some ATP is invested in order to produce more.

Step C: Oxidation. Two electrons and one proton are transferred from G3P to NAD^+ , forming NADH. Note that NAD^+ is an ion, and that both electrons in the new covalent bond come from G3P.

Step D: ATP generation. Four reactions convert G3P into another three-carbon molecule, pyruvate. This process generates two ATP molecules (see figure 9.5).

Because each glucose molecule is split into two G3P molecules, the overall reaction sequence yields two molecules of ATP, as well as two molecules of NADH and two of pyruvate:

$$\begin{array}{r} 4 \text{ ATP (2 ATP for each of the 2 G3P molecules in step D)} \\ -2 \text{ ATP (used in the two reactions in step A)} \\ \hline 2 \text{ ATP} \end{array}$$

Under the nonstandard conditions within a cell, each ATP molecule produced represents the capture of about 12 kcal (50 kJ) of energy per mole of glucose, rather than the 7.3 traditionally quoted for standard conditions. This means glycolysis harvests about 24 kcal/mole (100 kJ/mole). This is not a great deal of energy. The total energy content of the chemical bonds of glucose is 686 kcal (2870 kJ) per mole, so glycolysis harvests only 3.5% of the chemical energy of glucose.

Although far from ideal in terms of the amount of energy it releases, glycolysis does generate ATP. For more than a billion years during the anaerobic first stages of life on earth, it was the primary way heterotrophic organisms generated ATP from organic molecules. Like many biochemical pathways, glycolysis is believed to have evolved backward, with the last steps in the process being the most ancient. Thus, the second half of glycolysis, the ATP-yielding breakdown of G3P, may have been the original process early heterotrophs used to generate ATP. The synthesis of G3P from glucose would have appeared later, perhaps when alternative sources of G3P were depleted.

All Cells Use Glycolysis

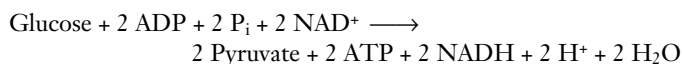
The glycolytic reaction sequence is thought to have been among the earliest of all biochemical processes to evolve. It uses no molecular oxygen and occurs readily in an anaerobic environment. All of its reactions occur free in the cytoplasm; none is associated with any organelle or membrane structure. Every living creature is capable of carrying out

glycolysis. Most present-day organisms, however, can extract considerably more energy from glucose through aerobic respiration.

Why does glycolysis take place even now, since its energy yield in the absence of oxygen is comparatively so paltry? The answer is that evolution is an incremental process: change occurs by improving on past successes. In catabolic metabolism, glycolysis satisfied the one essential evolutionary criterion: it was an improvement. Cells that could not carry out glycolysis were at a competitive disadvantage, and only cells capable of glycolysis survived the early competition of life. Later improvements in catabolic metabolism built on this success. Glycolysis was not discarded during the course of evolution; rather, it served as the starting point for the further extraction of chemical energy. Metabolism evolved as one layer of reactions added to another, just as successive layers of paint cover the walls of an old building. Nearly every present-day organism carries out glycolysis as a metabolic memory of its evolutionary past.

Closing the Metabolic Circle: The Regeneration of NAD^+

Inspect for a moment the net reaction of the glycolytic sequence:



You can see that three changes occur in glycolysis: (1) glucose is converted into two molecules of pyruvate; (2) two molecules of ADP are converted into ATP via substrate level phosphorylation; and (3) two molecules of NAD^+ are reduced to NADH.

The Need to Recycle NADH

As long as food molecules that can be converted into glucose are available, a cell can continually churn out ATP to drive its activities. In doing so, however, it accumulates NADH and depletes the pool of NAD^+ molecules. A cell does not contain a large amount of NAD^+ , and for glycolysis to continue, NADH must be recycled into NAD^+ . Some other molecule than NAD^+ must ultimately accept the hydrogen atom taken from G3P and be reduced. Two molecules can carry out this key task (figure 9.9):

1. Aerobic respiration. Oxygen is an excellent electron acceptor. Through a series of electron transfers, the hydrogen atom taken from G3P can be donated to oxygen, forming water. This is what happens in the cells of eukaryotes in the presence of oxygen. Because air is rich in oxygen, this process is also referred to as aerobic metabolism.

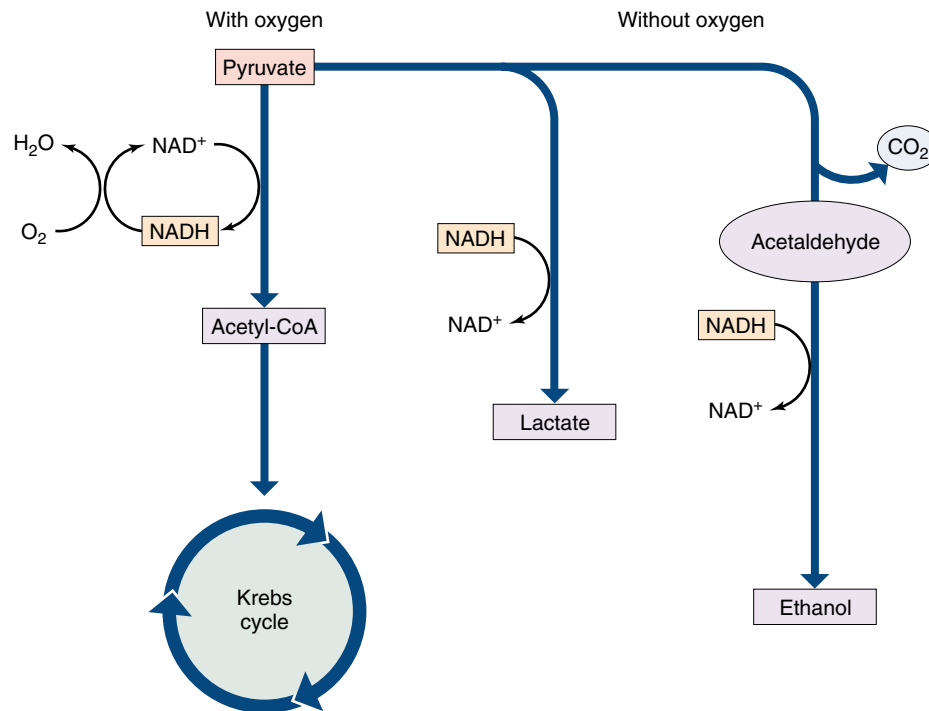


FIGURE 9.9

What happens to pyruvate, the product of glycolysis? In the presence of oxygen, pyruvate is oxidized to acetyl-CoA, which enters the Krebs cycle. In the absence of oxygen, pyruvate is instead reduced, accepting the electrons extracted during glycolysis and carried by NADH. When pyruvate is reduced directly, as in muscle cells, the product is lactate. When CO₂ is first removed from pyruvate and the product, acetaldehyde, is then reduced, as in yeast cells, the product is ethanol.

2. Fermentation. When oxygen is unavailable, an organic molecule can accept the hydrogen atom instead. Such fermentation plays an important role in the metabolism of most organisms (figure 9.10), even those capable of aerobic respiration.

The fate of the pyruvate that is produced by glycolysis depends upon which of these two processes takes place. The aerobic respiration path starts with the oxidation of pyruvate to a molecule called acetyl-CoA, which is then further oxidized in a series of reactions called the Krebs cycle. The fermentation path, by contrast, involves the reduction of all or part of pyruvate. We will start by examining aerobic respiration, then look briefly at fermentation.

Glycolysis generates a small amount of ATP by reshuffling the bonds of glucose molecules. In glycolysis, two molecules of NAD⁺ are reduced to NADH. NAD⁺ must be regenerated for glycolysis to continue unabated.



FIGURE 9.10

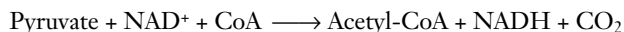
Fermentation. The conversion of pyruvate to ethanol takes place naturally in grapes left to ferment on vines, as well as in fermentation vats of crushed grapes. Yeasts carry out the process, but when their conversion increases the ethanol concentration to about 12%, the toxic effects of the alcohol kill the yeast cells. What is left is wine.

Stage Two: The Oxidation of Pyruvate

In the presence of oxygen, the oxidation of glucose that begins in glycolysis continues where glycolysis leaves off—with pyruvate. In eukaryotic organisms, the extraction of additional energy from pyruvate takes place exclusively inside mitochondria. The cell harvests pyruvate's considerable energy in two steps: first, by oxidizing pyruvate to form acetyl-CoA, and then by oxidizing acetyl-CoA in the Krebs cycle.

Producing Acetyl-CoA

Pyruvate is oxidized in a single “decarboxylation” reaction that cleaves off one of pyruvate's three carbons. This carbon then departs as CO_2 (figure 9.11, *top*). This reaction produces a two-carbon fragment called an acetyl group, as well as a pair of electrons and their associated hydrogen, which reduce NAD^+ to NADH. The reaction is complex, involving three intermediate stages, and is catalyzed within mitochondria by a *multienzyme complex*. As chapter 8 noted, such a complex organizes a series of enzymatic steps so that the chemical intermediates do not diffuse away or undergo other reactions. Within the complex, component polypeptides pass the substrates from one enzyme to the next, without releasing them. *Pyruvate dehydrogenase*, the complex of enzymes that removes CO_2 from pyruvate, is one of the largest enzymes known: it contains 60 subunits! In the course of the reaction, the acetyl group removed from pyruvate combines with a cofactor called coenzyme A (CoA), forming a compound known as **acetyl-CoA**:



This reaction produces a molecule of NADH, which is later used to produce ATP. Of far greater significance than the reduction of NAD^+ to NADH, however, is the production of acetyl-CoA (figure 9.11, *bottom*). Acetyl-CoA is important because so many different metabolic processes generate it. Not only does the oxidation of pyruvate, an intermediate in carbohydrate catabolism, produce it, but the metabolic breakdown of proteins, fats, and other lipids also generate acetyl-CoA. Indeed, almost all molecules catabolized for energy are converted into acetyl-CoA. Acetyl-CoA is then channeled into fat synthesis or into ATP production, depending on the organism's energy requirements. Acetyl-CoA is a key point of focus for the many catabolic processes of the eukaryotic cell.

Using Acetyl-CoA

Although the cell forms acetyl-CoA in many ways, only a limited number of processes use acetyl-CoA. Most of it is either directed toward energy storage (lipid synthesis, for example) or oxidized in the Krebs cycle to produce ATP. Which of these two options is taken depends on the level of ATP in the cell. When ATP levels are high, the oxida-

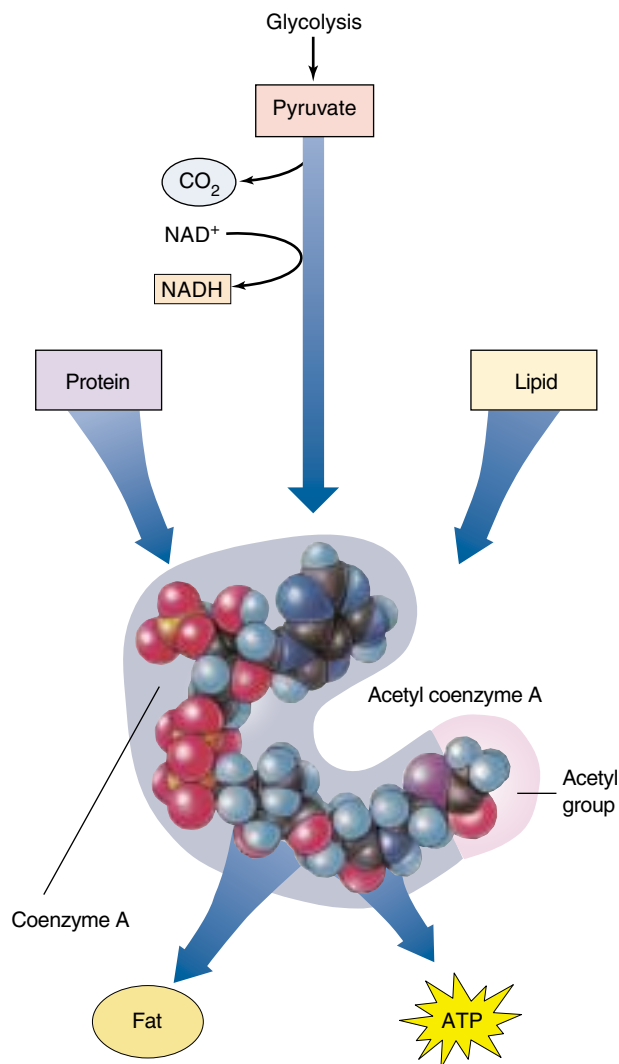


FIGURE 9.11

The oxidation of pyruvate. This complex reaction involves the reduction of NAD^+ to NADH and is thus a significant source of metabolic energy. Its product, acetyl-CoA, is the starting material for the Krebs cycle. Almost all molecules that are catabolized for energy are converted into acetyl-CoA, which is then channeled into fat synthesis or into ATP production.

tive pathway is inhibited, and acetyl-CoA is channeled into fatty acid synthesis. This explains why many animals (humans included) develop fat reserves when they consume more food than their bodies require. Alternatively, when ATP levels are low, the oxidative pathway is stimulated, and acetyl-CoA flows into energy-producing oxidative metabolism.

In the second energy-harvesting stage of glucose catabolism, pyruvate is decarboxylated, yielding acetyl-CoA, NADH, and CO_2 . This process occurs within the mitochondrion.

Stage Three: The Krebs Cycle

After glycolysis catabolizes glucose to produce pyruvate, and pyruvate is oxidized to form acetyl-CoA, the third stage of extracting energy from glucose begins. In this third stage, acetyl-CoA is oxidized in a series of nine reactions called the Krebs cycle. These reactions occur in the matrix of mitochondria. In this cycle, the two-carbon acetyl group of acetyl-CoA combines with a four-carbon molecule called oxaloacetate (figure 9.12). The resulting six-carbon molecule then goes through a sequence of electron-yielding oxidation reactions, during which two CO_2 molecules split off, restoring oxaloacetate. The oxaloacetate is then recycled to bind to another acetyl group. In each turn of the cycle, a new acetyl group replaces the two CO_2 molecules lost, and

more electrons are extracted to drive proton pumps that generate ATP.

Overview of the Krebs Cycle

The nine reactions of the Krebs cycle occur in two steps:

Step A: Priming. Three reactions prepare the six-carbon molecule for energy extraction. First, acetyl-CoA joins the cycle, and then chemical groups are rearranged.

Step B: Energy extraction. Four of the six reactions in this step are oxidations in which electrons are removed, and one generates an ATP equivalent directly by substrate-level phosphorylation.

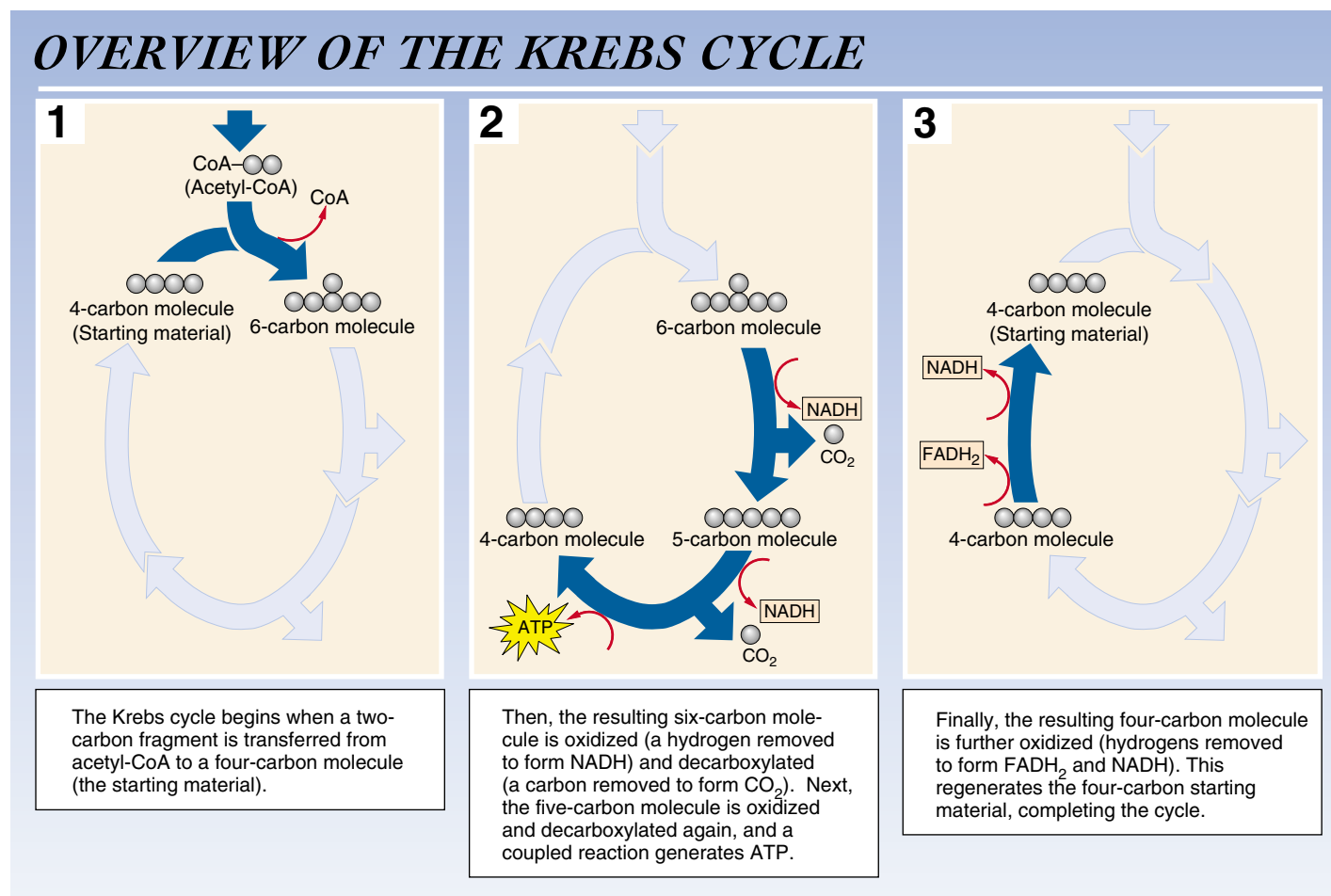


FIGURE 9.12
How the Krebs cycle works.

The Reactions of the Krebs Cycle

The **Krebs cycle** consists of nine sequential reactions that cells use to extract energetic electrons and drive the synthesis of ATP (figure 9.13). A two-carbon group from acetyl-CoA enters the cycle at the beginning, and two CO₂ molecules and several electrons are given off during the cycle.

Reaction 1: Condensation. The two-carbon group from acetyl-CoA joins with a four-carbon molecule, oxaloacetate, to form a six-carbon molecule, citrate. This condensation reaction is irreversible, committing the two-carbon acetyl group to the Krebs cycle. The reaction is inhibited when the cell's ATP concentration is high and stimulated when it is low. Hence, when the cell possesses ample amounts of ATP, the Krebs cycle shuts down and acetyl-CoA is channeled into fat synthesis.

Reactions 2 and 3: Isomerization. Before the oxidation reactions can begin, the hydroxyl (—OH) group of citrate must be repositioned. This is done in two steps: first, a water molecule is removed from one carbon; then, water is added to a different carbon. As a result, an —H group and an —OH group change positions. The product is an isomer of citrate called isocitrate.

Reaction 4: The First Oxidation. In the first energy-yielding step of the cycle, isocitrate undergoes an oxidative decarboxylation reaction. First, isocitrate is oxidized, yielding a pair of electrons that reduce a molecule of NAD⁺ to NADH. Then the oxidized intermediate is decarboxylated; the central carbon atom splits off to form CO₂, yielding a five-carbon molecule called α-ketoglutarate.

Reaction 5: The Second Oxidation. Next, α-ketoglutarate is decarboxylated by a multienzyme complex similar to pyruvate dehydrogenase. The succinyl group left after the removal of CO₂ joins to coenzyme A, forming succinyl-CoA. In the process, two electrons are extracted, and they reduce another molecule of NAD⁺ to NADH.

Reaction 6: Substrate-Level Phosphorylation. The linkage between the four-carbon succinyl group and CoA is a high-energy bond. In a coupled reaction similar to those that take place in glycolysis, this bond is cleaved, and the

energy released drives the phosphorylation of guanosine diphosphate (GDP), forming guanosine triphosphate (GTP). GTP is readily converted into ATP, and the four-carbon fragment that remains is called succinate.

Reaction 7: The Third Oxidation. Next, succinate is oxidized to fumarate. The free energy change in this reaction is not large enough to reduce NAD⁺. Instead, flavin adenine dinucleotide (FAD) is the electron acceptor. Unlike NAD⁺, FAD is not free to diffuse within the mitochondrion; it is an integral part of the inner mitochondrial membrane. Its reduced form, FADH₂, contributes electrons to the electron transport chain in the membrane.

Reactions 8 and 9: Regeneration of Oxaloacetate. In the final two reactions of the cycle, a water molecule is added to fumarate, forming malate. Malate is then oxidized, yielding a four-carbon molecule of oxaloacetate and two electrons that reduce a molecule of NAD⁺ to NADH. Oxaloacetate, the molecule that began the cycle, is now free to combine with another two-carbon acetyl group from acetyl-CoA and reinitiate the cycle.

The Products of the Krebs Cycle

In the process of aerobic respiration, glucose is entirely consumed. The six-carbon glucose molecule is first cleaved into a pair of three-carbon pyruvate molecules during glycolysis. One of the carbons of each pyruvate is then lost as CO₂ in the conversion of pyruvate to acetyl-CoA; two other carbons are lost as CO₂ during the oxidations of the Krebs cycle. All that is left to mark the passing of the glucose molecule into six CO₂ molecules is its energy, some of which is preserved in four ATP molecules and in the reduced state of 12 electron carriers. Ten of these carriers are NADH molecules; the other two are FADH₂.

The Krebs cycle generates two ATP molecules per molecule of glucose, the same number generated by glycolysis. More importantly, the Krebs cycle and the oxidation of pyruvate harvest many energized electrons, which can be directed to the electron transport chain to drive the synthesis of much more ATP.

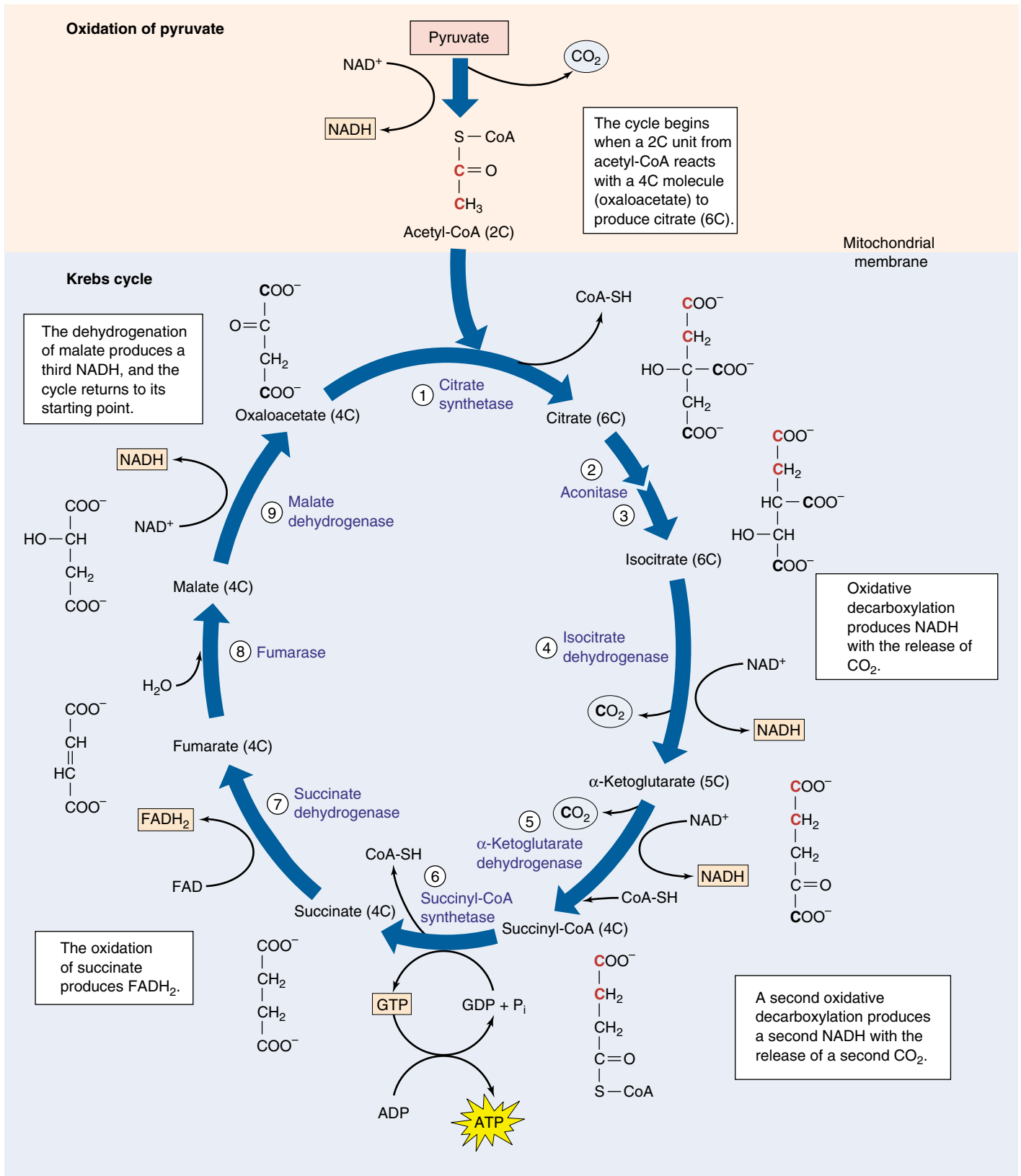


FIGURE 9.13

The Krebs cycle. This series of reactions takes place within the matrix of the mitochondrion. For the complete breakdown of a molecule of glucose, the two molecules of acetyl-CoA produced by glycolysis and pyruvate oxidation will each have to make a trip around the Krebs cycle. Follow the different carbons through the cycle, and notice the changes that occur in the carbon skeletons of the molecules as they proceed through the cycle.

Harvesting Energy by Extracting Electrons

To understand how cells direct some of the energy released during glucose catabolism into ATP production, we need to take a closer look at the electrons in the C—H bonds of the glucose molecule. We stated in chapter 8 that when an electron is removed from one atom and donated to another, the electron's potential energy of position is also transferred. In this process, the atom that receives the electron is reduced. We spoke of reduction in an all-or-none fashion, as if it involved the complete transfer of an electron from one atom to another. Often this is just what happens. However, sometimes a reduction simply changes the *degree of sharing* within a covalent bond. Let us now revisit that discussion and consider what happens when the transfer of electrons is incomplete.

A Closer Look at Oxidation-Reduction

The catabolism of glucose is an oxidation-reduction reaction. The covalent electrons in the C—H bonds of glucose are shared approximately equally between the C and H atoms because carbon and hydrogen nuclei have about the same affinity for valence electrons (that is, they exhibit similar *electronegativity*). However, when the carbon atoms of glucose react with oxygen to form carbon dioxide, the electrons in the new covalent bonds take a different position. Instead of being shared equally, the electrons that were associated with the carbon atoms in glucose shift far toward the oxygen atom in CO_2 because oxygen is very electronegative. Since these electrons are pulled farther from the carbon atoms, the carbon atoms of glucose have been oxidized (loss of electrons) and the oxygen atoms reduced (gain of electrons). Similarly, when the hydrogen atoms of glucose combine with oxygen atoms to form water, the oxygen atoms draw the shared electrons strongly toward them; again, oxygen is reduced and glucose is oxidized. In this reaction, oxygen is an oxidizing (electron-attracting) agent because it oxidizes the atoms of glucose.

Releasing Energy

The key to understanding the oxidation of glucose is to focus on the energy of the shared electrons. In a covalent bond, energy must be added to remove an electron from an atom, just as energy must be used to roll a boulder up a hill. The more electronegative the atom, the steeper the energy hill that must be climbed to pull an electron away from it. However, energy is released when an electron is shifted away from a less electronegative atom and *closer* to a more electronegative atom, just as energy is released when a boulder is allowed to roll down a hill. In the catabolism of glucose, energy is released when glucose is oxidized, as electrons relocate closer to oxygen (figure 9.14).

Glucose is an energy-rich food because it has an abundance of C—H bonds. Viewed in terms of oxidation-reduction, glucose possesses a wealth of electrons held far from their atoms, all with the potential to move closer toward oxygen. In oxidative respiration, energy is released not simply because the hydrogen atoms of the C—H bonds are transferred from glucose to oxygen, but because the positions of the valence electrons shift. This shift releases energy that can be used to make ATP.

Harvesting the Energy in Stages

It is generally true that the larger the release of energy in any single step, the more of that energy is released as heat (random molecular motion) and the less there is available to be channeled into more useful paths. In the combustion of gasoline, the same amount of energy is released whether all of the gasoline in a car's gas tank explodes at once, or whether the gasoline burns in a series of very small explosions inside the cylinders. By releasing the energy in gasoline a little at a time, the harvesting efficiency is greater and

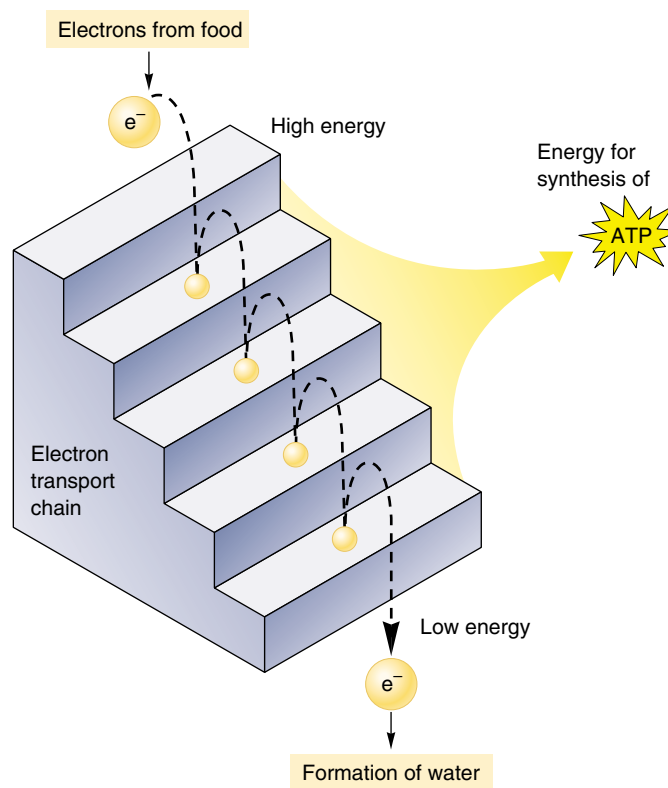


FIGURE 9.14
How electron transport works. This diagram shows how ATP is generated when electrons transfer from one energy level to another. Rather than releasing a single explosive burst of energy, electrons “fall” to lower and lower energy levels in steps, releasing stored energy with each fall as they tumble to the lowest (most electronegative) electron acceptor.

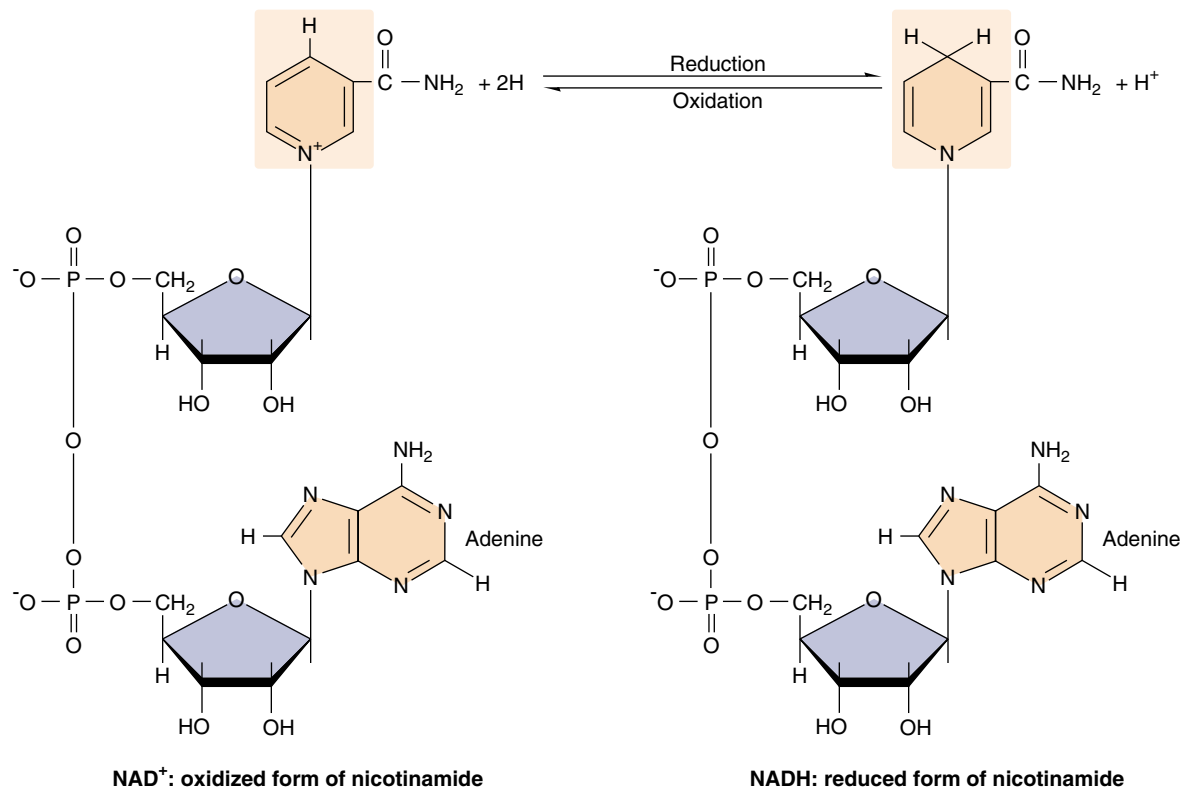


FIGURE 9.15
NAD⁺ and NADH.
 This dinucleotide serves as an “electron shuttle” during cellular respiration. NAD⁺ accepts electrons from catabolized macromolecules and is reduced to NADH.

more of the energy can be used to push the pistons and move the car.

The same principle applies to the oxidation of glucose inside a cell. If all of the hydrogens were transferred to oxygen in one explosive step, releasing all of the free energy at once, the cell would recover very little of that energy in a useful form. Instead, cells burn their fuel much as a car does, a little at a time. The six hydrogens in the C—H bonds of glucose are stripped off in stages in the series of enzyme-catalyzed reactions collectively referred to as glycolysis and the Krebs cycle. We have had a great deal to say about these reactions already in this chapter. Recall that the hydrogens are removed by transferring them to a coenzyme carrier, NAD⁺ (figure 9.15). Discussed in chapter 8, NAD⁺ is a very versatile electron acceptor, shuttling energy-bearing electrons throughout the cell. In harvesting the energy of glucose, NAD⁺ acts as the primary electron acceptor.

Following the Electrons

As you examine these reactions, try not to become confused by the changes in electrical charge. Always *follow the electrons*. Enzymes extract two hydrogens—that is, two electrons and two protons—from glucose and transfer both electrons and one of the protons to NAD⁺. The other proton is released as a hydrogen ion, H⁺, into the surrounding solution. This transfer converts NAD⁺ into NADH; that is,

two negative electrons and one positive proton are added to one positively charged NAD⁺ to form NADH, which is electrically neutral.

Energy captured by NADH is *not* harvested all at once. Instead of being transferred directly to oxygen, the two electrons carried by NADH are passed along the **electron transport chain** if oxygen is present. This chain consists of a series of molecules, mostly proteins, embedded within the inner membranes of mitochondria. NADH delivers electrons to the top of the electron transport chain and oxygen captures them at the bottom. The oxygen then joins with hydrogen ions to form water. At each step in the chain, the electrons move to a slightly more electronegative carrier, and their positions shift slightly. Thus, the electrons move *down* an energy gradient. The entire process releases a total of 53 kcal/mole (222 kJ/mole) under standard conditions. The transfer of electrons along this chain allows the energy to be extracted gradually. In the next section, we will discuss how this energy is put to work to drive the production of ATP.

The catabolism of glucose involves a series of oxidation-reduction reactions that release energy by repositioning electrons closer to oxygen atoms. Energy is thus harvested from glucose molecules in gradual steps, using NAD⁺ as an electron carrier.

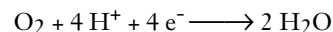
Stage Four: The Electron Transport Chain

The NADH and FADH₂ molecules formed during the first three stages of aerobic respiration each contain a pair of electrons that were gained when NAD⁺ and FAD were reduced. The NADH molecules carry their electrons to the inner mitochondrial membrane, where they transfer the electrons to a series of membrane-associated proteins collectively called the electron transport chain.

Moving Electrons through the Electron Transport Chain

The first of the proteins to receive the electrons is a complex, membrane-embedded enzyme called **NADH dehydrogenase**. A carrier called ubiquinone then passes the electrons to a protein-cytochrome complex called the *bc*₁ complex. This complex, along with others in the chain, operates as a proton pump, driving a proton out across the membrane. Cytochromes are respiratory proteins that contain heme groups, complex carbon rings with many alternating single and double bonds and an iron atom in the center.

The electron is then carried by another carrier, *cytochrome c*, to the cytochrome oxidase complex. This complex uses four such electrons to reduce a molecule of oxygen, each oxygen then combines with two hydrogen ions to form water:



This series of membrane-associated electron carriers is collectively called the electron transport chain (figure 9.16).

NADH contributes its electrons to the first protein of the electron transport chain, NADH dehydrogenase. FADH₂, which is always attached to the inner mitochondrial membrane, feeds its electrons into the electron transport chain later, to ubiquinone.

It is the availability of a plentiful electron acceptor (often oxygen) that makes oxidative respiration possible. As we'll see in chapter 10, the electron transport chain used in aerobic respiration is similar to, and may well have evolved from, the chain employed in aerobic photosynthesis.

The electron transport chain is a series of five membrane-associated proteins. Electrons delivered by NADH and FADH₂ are passed from protein to protein along the chain, like a baton in a relay race.

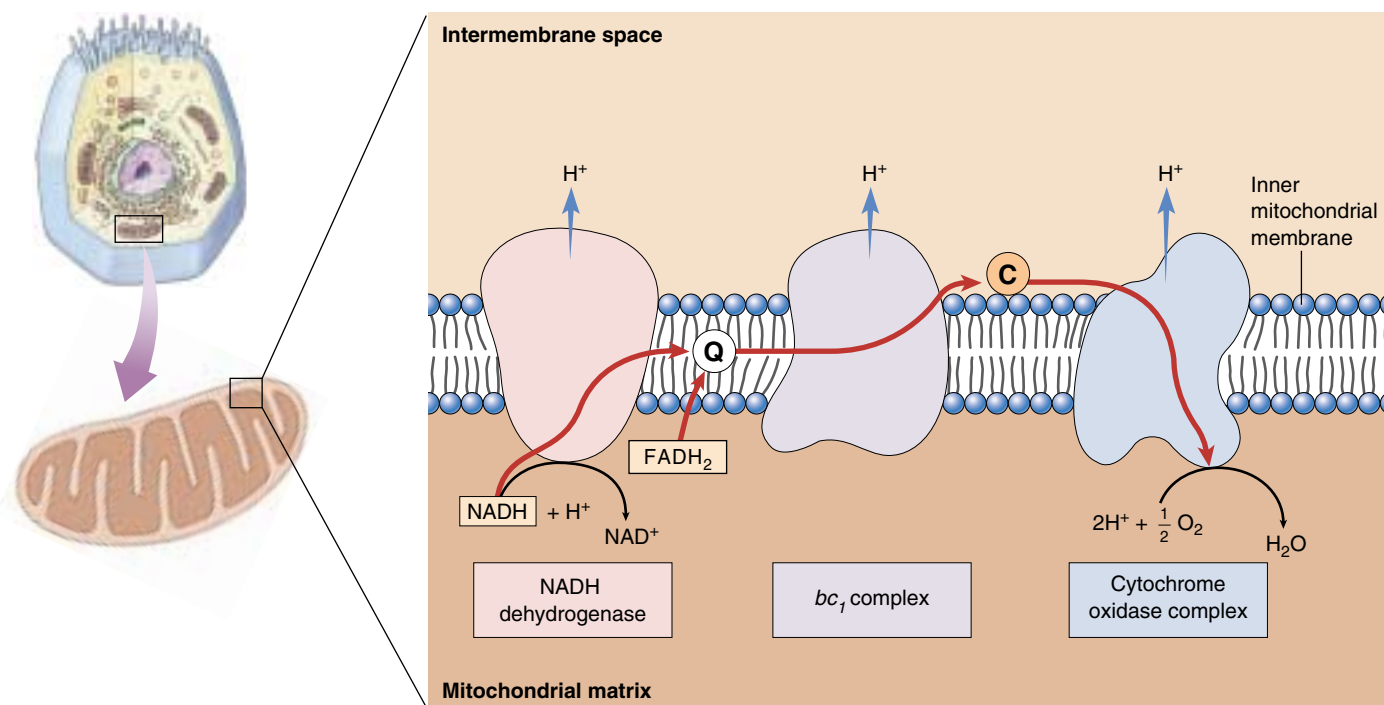


FIGURE 9.16

The electron transport chain. High-energy electrons harvested from catabolized molecules are transported (red arrows) by mobile electron carriers (ubiquinone, marked Q, and cytochrome c, marked C) along a chain of membrane proteins. Three proteins use portions of the electrons' energy to pump protons (blue arrows) out of the matrix and into the intermembrane space. The electrons are finally donated to oxygen to form water.

Building an Electrochemical Gradient

In eukaryotes, aerobic metabolism takes place within the mitochondria present in virtually all cells. The internal compartment, or matrix, of a mitochondrion contains the enzymes that carry out the reactions of the Krebs cycle. As the electrons harvested by oxidative respiration are passed along the electron transport chain, the energy they release transports protons out of the matrix and into the outer compartment, sometimes called the intermembrane space. Three transmembrane proteins in the inner mitochondrial membrane (see figure 9.16) actually accomplish the transport. The flow of excited electrons induces a change in the shape of these pump proteins, which causes them to transport protons across the membrane. The electrons contributed by NADH activate all three of these proton pumps, while those contributed by FADH_2 activate only two.

Producing ATP: Chemiosmosis

As the proton concentration in the intermembrane space rises above that in the matrix, the matrix becomes slightly negative in charge. This internal negativity attracts the positively charged protons and induces them to reenter the matrix. The higher outer concentration tends to drive protons back in by diffusion; since membranes are relatively impermeable to ions, most of the protons that reenter the matrix pass through special proton channels in the inner mitochondrial membrane. When the protons pass through, these channels synthesize ATP from $\text{ADP} + \text{P}_i$ within the matrix. The ATP is then transported by facilitated diffusion out of the mitochondrion and into the cell's cytoplasm. Because the chemical formation of ATP is driven by a diffusion force similar to osmosis, this process is referred to as **chemiosmosis** (figure 9.17).

Thus, the electron transport chain uses electrons harvested in aerobic respiration to pump a large number of protons across the inner mitochondrial membrane. Their subsequent reentry into the mitochondrial matrix drives the synthesis of ATP by chemiosmosis. Figure 9.18 summarizes the overall process.

The electrons harvested from glucose are pumped out of the mitochondrial matrix by the electron transport chain. The return of the protons into the matrix generates ATP.

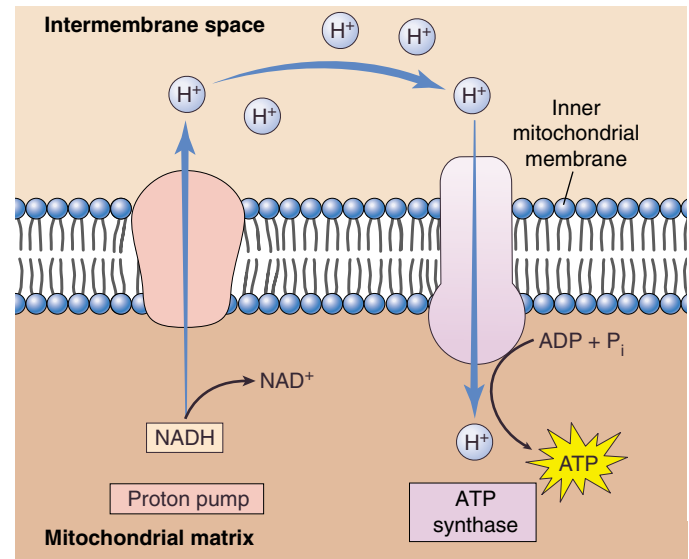


FIGURE 9.17

Chemiosmosis. NADH transports high-energy electrons harvested from the catabolism of macromolecules to “proton pumps” that use the energy to pump protons out of the mitochondrial matrix. As a result, the concentration of protons in the intermembrane space rises, inducing protons to diffuse back into the matrix. Many of the protons pass through special channels that couple the reentry of protons to the production of ATP.

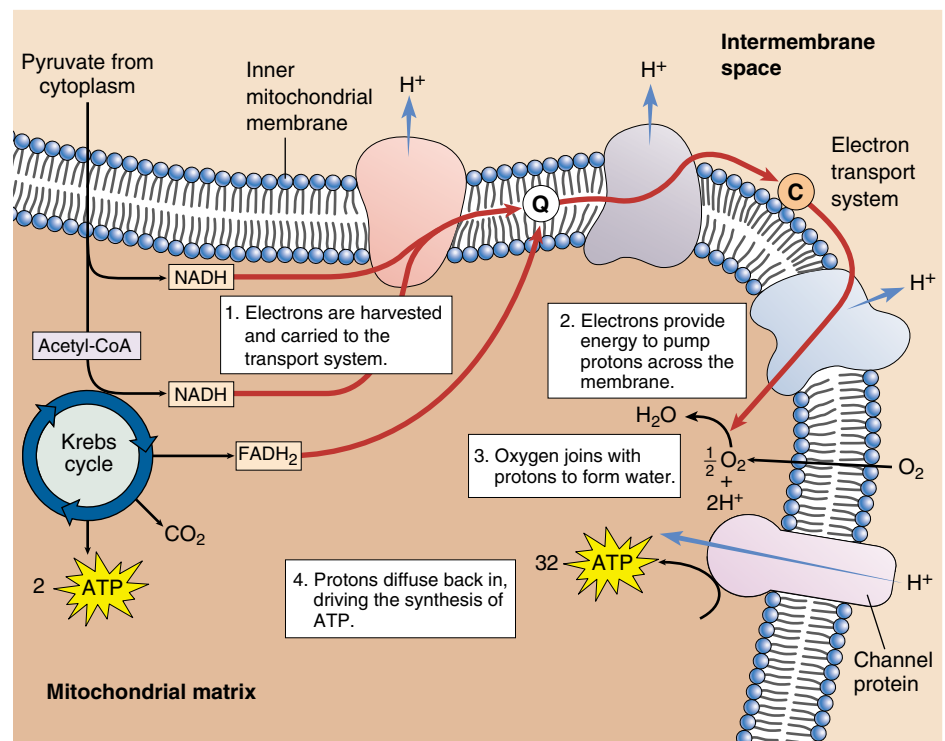


FIGURE 9.18

ATP generation during the Krebs cycle and electron transport chain. This process begins with pyruvate, the product of glycolysis, and ends with the synthesis of ATP.

Summarizing Aerobic Respiration

How much metabolic energy does a cell actually gain from the electrons harvested from a molecule of glucose, using the electron transport chain to produce ATP by chemiosmosis?

Theoretical Yield

The chemiosmotic model suggests that one ATP molecule is generated for each proton pump activated by the electron transport chain. Since the electrons from NADH activate three pumps and those from FADH₂ activate two, we would expect each molecule of NADH and FADH₂ to generate three and two ATP molecules, respectively. However, because eukaryotic cells carry out glycolysis in their cytoplasm and the Krebs cycle within their mitochondria, they must transport the two molecules of NADH produced during glycolysis across the mitochondrial membranes, which requires one ATP per molecule of NADH. Thus, the net ATP production is decreased by two. Therefore, the overall ATP production resulting from aerobic respiration *theoretically* should be 4 (from substrate-level phosphorylation during glycolysis) + 30 (3 from each of 10 molecules of NADH) + 4 (2 from each of 2 molecules of FADH₂) – 2 (for transport of glycolytic NADH) = 36 molecules of ATP (figure 9.19).

Actual Yield

The amount of ATP *actually* produced in a eukaryotic cell during aerobic respiration is somewhat lower than 36, for two reasons. First, the inner mitochondrial membrane is somewhat “leaky” to protons, allowing some of them to reenter the matrix without passing through ATP-generating channels. Second, mitochondria often use the proton gradient generated by chemiosmosis for purposes other than ATP synthesis (such as transporting pyruvate into the matrix). Consequently, the actual measured values of ATP generated by NADH and FADH₂ are closer to 2.5 for each NADH and 1.5 for each FADH₂. With these corrections, the overall harvest of ATP from a molecule of glucose in a eukaryotic cell is closer to 4 (from substrate-level phosphorylation) + 25 (2.5 from each of 10 molecules of NADH) + 3 (1.5 from each of 2 molecules of FADH₂) – 2 (transport of glycolytic NADH) = 30 molecules of ATP.

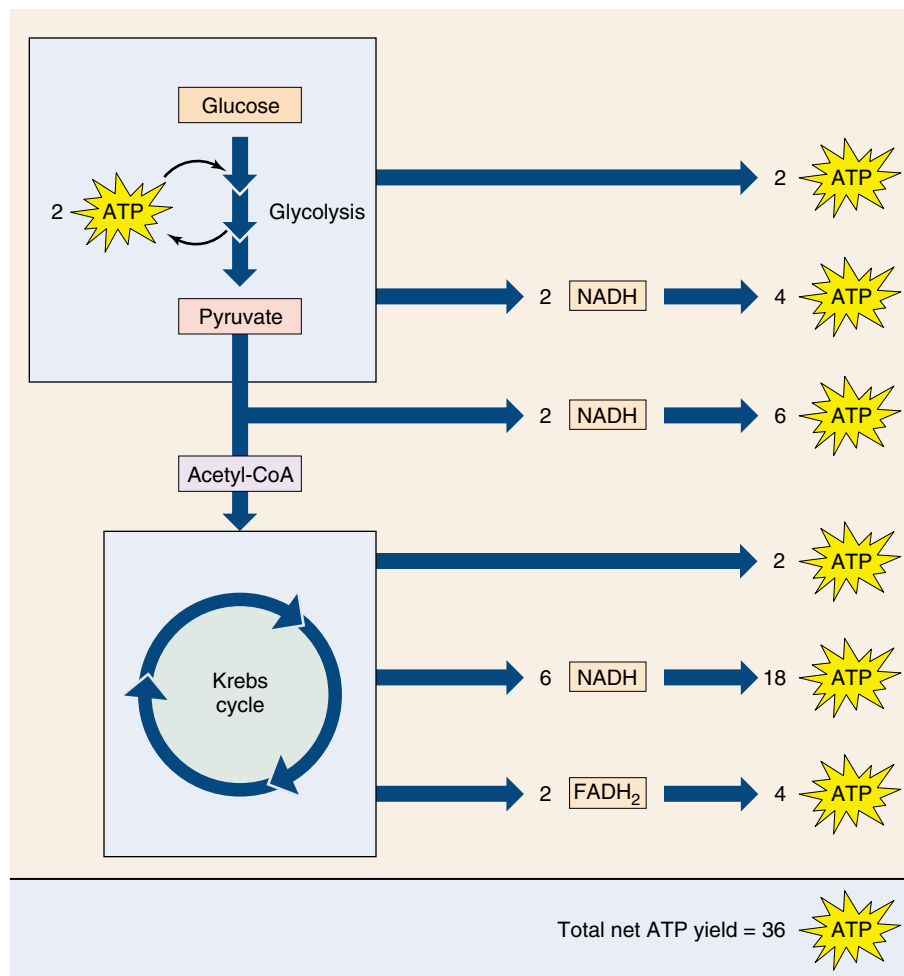


FIGURE 9.19
Theoretical ATP yield. The theoretical yield of ATP harvested from glucose by aerobic respiration totals 36 molecules.

The catabolism of glucose by aerobic respiration, in contrast to glycolysis, is quite efficient. Aerobic respiration in a eukaryotic cell harvests about $7.3 \times 30 \div 686 = 32\%$ of the energy available in glucose. (By comparison, a typical car converts only about 25% of the energy in gasoline into useful energy.) The efficiency of oxidative respiration at harvesting energy establishes a natural limit on the maximum length of food chains.

The high efficiency of aerobic respiration was one of the key factors that fostered the evolution of heterotrophs. With this mechanism for producing ATP, it became feasible for nonphotosynthetic organisms to derive metabolic energy exclusively from the oxidative breakdown of other organisms. As long as some organisms captured energy by photosynthesis, others could exist solely by feeding on them.

Oxidative respiration produces approximately 30 molecules of ATP from each molecule of glucose in eukaryotic cells. This represents more than half of the energy in the chemical bonds of glucose.

Regulating Aerobic Respiration

When cells possess plentiful amounts of ATP, the key reactions of glycolysis, the Krebs cycle, and fatty acid breakdown are inhibited, slowing ATP production. The regulation of these biochemical pathways by the level of ATP is an example of feedback inhibition. Conversely, when ATP levels in the cell are low, ADP levels are high; and ADP activates enzymes in the pathways of carbohydrate catabolism to stimulate the production of more ATP.

Control of glucose catabolism occurs at two key points of the catabolic pathway (figure 9.20). The control point in glycolysis is the enzyme phosphofructokinase, which catalyzes reaction 3, the conversion of fructose phosphate to fructose biphosphate. This is the first reaction of glycolysis that is not readily reversible, committing the substrate to the glycolytic sequence. High levels of ADP relative to ATP (implying a need to convert more ADP to ATP) stimulate phosphofructokinase, committing more sugar to the catabolic pathway; so do low levels of citrate (implying the Krebs cycle is not running at full tilt and needs more input). The main control point in the oxidation of pyruvate occurs at the committing step in the Krebs cycle with the enzyme pyruvate decarboxylase. It is inhibited by high levels of NADH (implying no more is needed).

Another control point in the Krebs cycle is the enzyme citrate synthetase, which catalyzes the first reaction, the conversion of oxaloacetate and acetyl-CoA into citrate. High levels of ATP inhibit citrate synthetase (as well as pyruvate decarboxylase and two other Krebs cycle enzymes), shutting down the catabolic pathway.

Relative levels of ADP and ATP regulate the catabolism of glucose at key committing reactions.

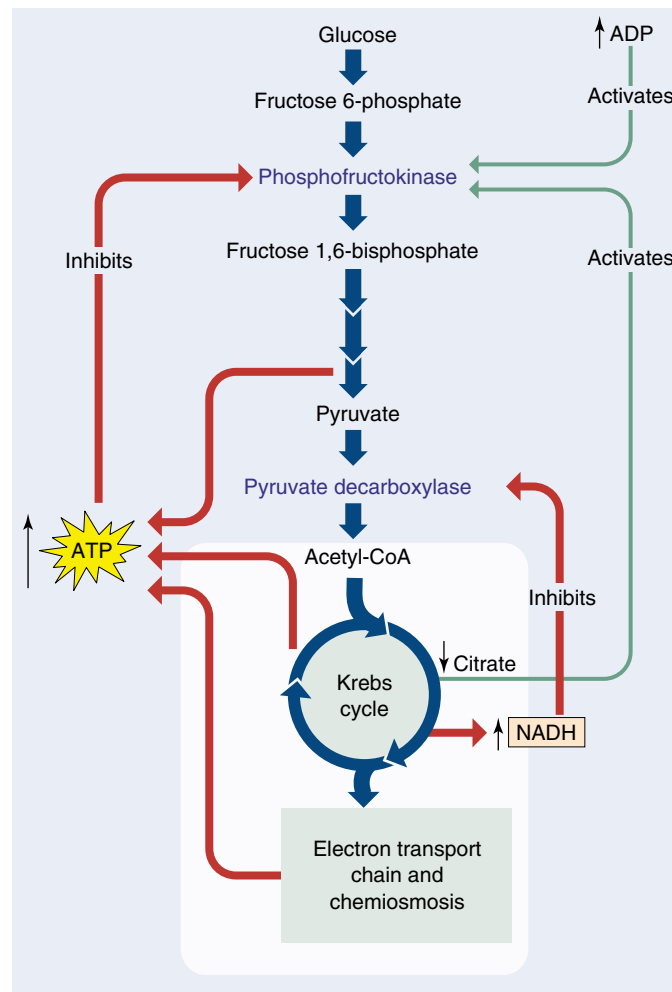


FIGURE 9.20
Control of glucose catabolism. The relative levels of ADP and ATP control the catabolic pathway at two key points: the committing reactions of glycolysis and the Krebs cycle.

A Vocabulary of ATP Generation

aerobic respiration The portion of cellular respiration that requires oxygen as an electron acceptor; it includes pyruvate oxidation, the Krebs cycle, and the electron transport chain.

anaerobic respiration Cellular respiration in which inorganic electron acceptors other than oxygen are used; it includes glycolysis.

cellular respiration The oxidation of organic molecules to produce ATP in

which the final electron acceptor is organic; it includes aerobic and anaerobic respiration.

chemiosmosis The passage of high-energy electrons along the electron transport chain, which is coupled to the pumping of protons across a membrane and the return of protons to the original side of the membrane through ATP-generating channels.

fermentation Alternative ATP-producing pathway performed by some cells in the absence of oxygen, in which the final electron acceptor is an organic molecule.

maximum efficiency The maximum number of ATP molecules generated by

oxidizing a substance, relative to the free energy of that substance; in organisms, the actual efficiency is usually less than the maximum.

oxidation The loss of an electron. In cellular respiration, high-energy electrons are stripped from food molecules, oxidizing them.

photosynthesis The chemiosmotic generation of ATP and complex organic molecules powered by the energy derived from light.

substrate-level phosphorylation The generation of ATP by the direct transfer of a phosphate group to ADP from another phosphorylated molecule.

9.3 Catabolism of proteins and fats can yield considerable energy.

Glucose Is Not the Only Source of Energy

Thus far we have discussed oxidative respiration of glucose, which organisms obtain from the digestion of carbohydrates or from photosynthesis. Other organic molecules than glucose, particularly proteins and fats, are also important sources of energy (figure 9.21).

Cellular Respiration of Protein

Proteins are first broken down into their individual amino acids. The nitrogen-containing side group (the amino group) is then removed from each amino acid in a process called **deamination**. A series of reactions convert the carbon chain that remains into a molecule that takes part in glycolysis or the Krebs cycle. For example, alanine is converted into pyruvate, glutamate into α -ketoglutarate (figure 9.22), and aspartate into oxaloacetate. The reactions of glycolysis and the Krebs cycle then extract the high-energy electrons from these molecules and put them to work making ATP.

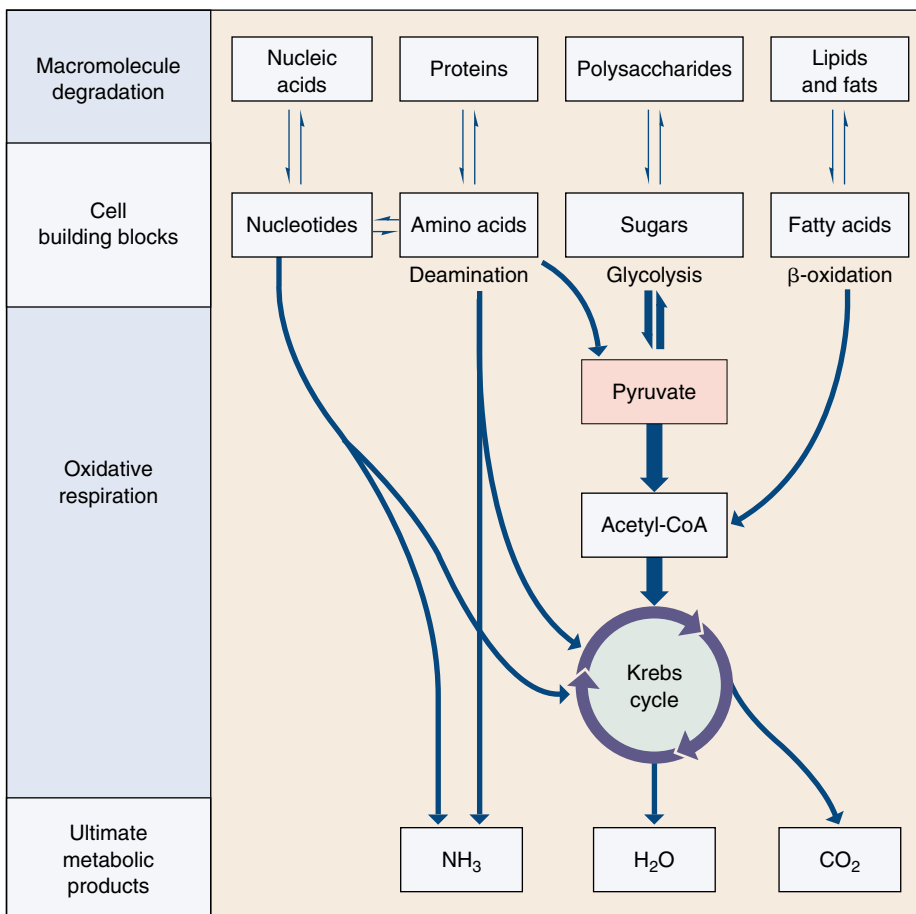
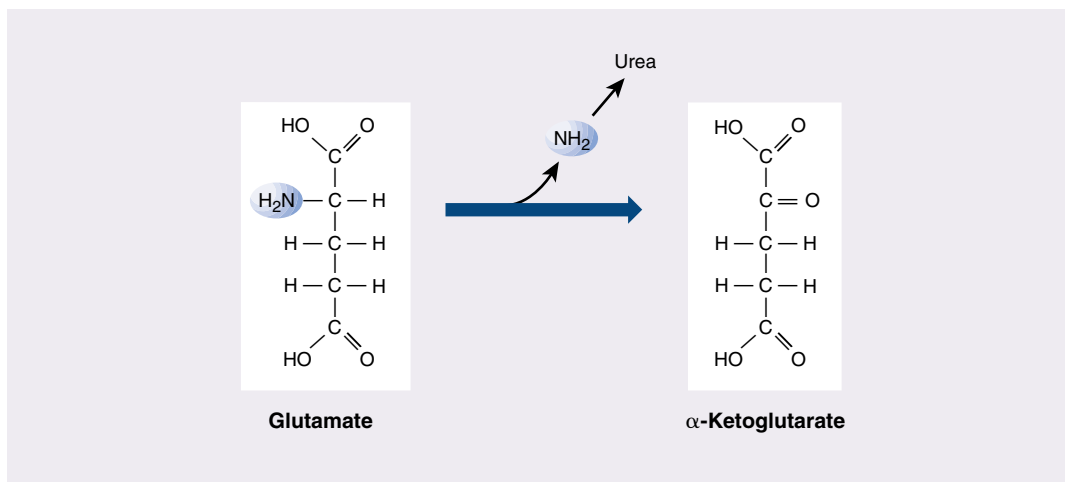


FIGURE 9.21
How cells extract chemical energy. All eukaryotes and many prokaryotes extract energy from organic molecules by oxidizing them. The first stage of this process, breaking down macromolecules into their constituent parts, yields little energy. The second stage, oxidative or aerobic respiration, extracts energy, primarily in the form of high-energy electrons, and produces water and carbon dioxide.

FIGURE 9.22

Deamination. After proteins are broken down into their amino acid constituents, the amino groups are removed from the amino acids to form molecules that participate in glycolysis and the Krebs cycle. For example, the amino acid glutamate becomes α -ketoglutarate, a Krebs cycle molecule, when it loses its amino group.



Cellular Respiration of Fat

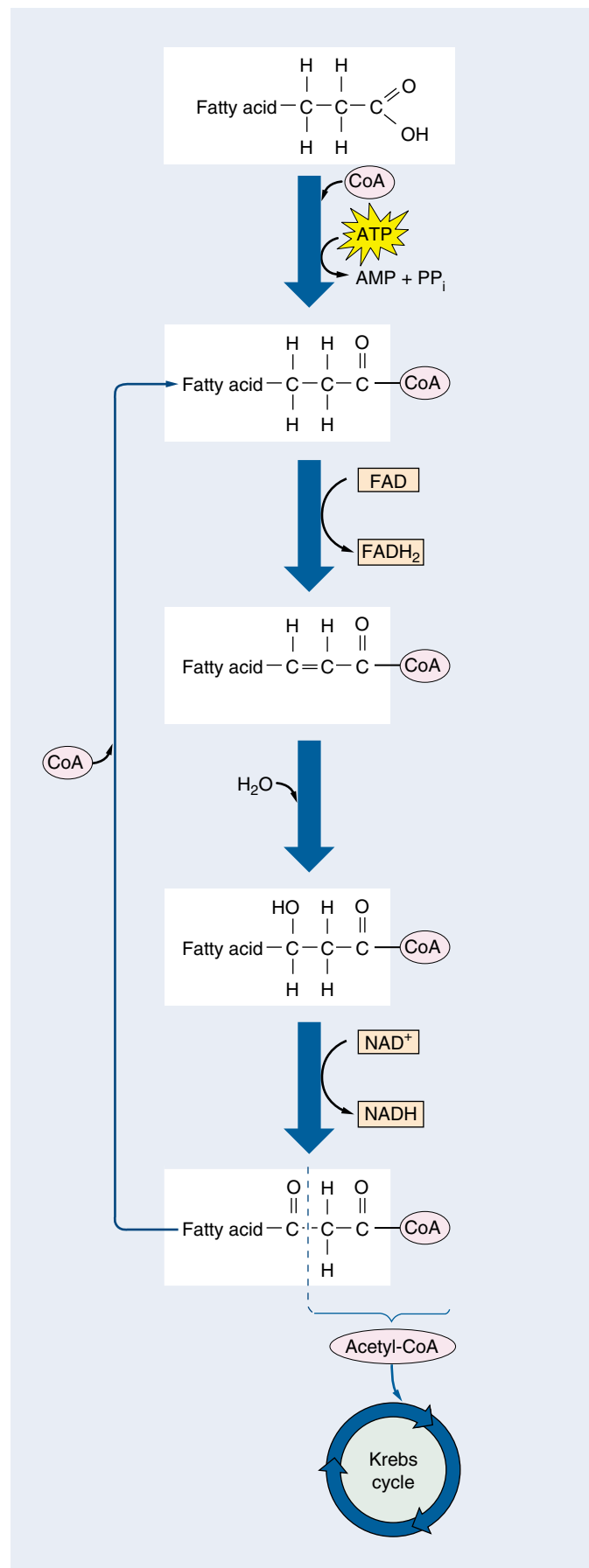
Fats are broken down into fatty acids plus glycerol. The tails of fatty acids typically have 16 or more —CH_2 links, and the many hydrogen atoms in these long tails provide a rich harvest of energy. Fatty acids are oxidized in the matrix of the mitochondrion. Enzymes there remove the two-carbon acetyl groups from the end of each fatty acid tail until the entire fatty acid is converted into acetyl groups (figure 9.23). Each acetyl group then combines with coenzyme A to form acetyl-CoA. This process is known as β -oxidation.

How much ATP does the catabolism of fatty acids produce? Let's compare a hypothetical six-carbon fatty acid with the six-carbon glucose molecule, which we've said yields about 30 molecules of ATP in a eukaryotic cell. Two rounds of β -oxidation would convert the fatty acid into three molecules of acetyl-CoA. Each round requires one molecule of ATP to prime the process, but it also produces one molecule of NADH and one of FADH_2 . These molecules together yield four molecules of ATP (assuming 2.5 ATPs per NADH and 1.5 ATPs per FADH_2). The oxidation of each acetyl-CoA in the Krebs cycle ultimately produces an additional 10 molecules of ATP. Overall, then, the ATP yield of a six-carbon fatty acid would be approximately 8 (from two rounds of β -oxidation) $- 2$ (for priming those two rounds) $+ 30$ (from oxidizing the three acetyl-CoAs) $= 36$ molecules of ATP. Therefore, the respiration of a six-carbon fatty acid yields 20% more ATP than the respiration of glucose. Moreover, a fatty acid of that size would weigh less than two-thirds as much as glucose, so a gram of fatty acid contains more than twice as many kilocalories as a gram of glucose. That is why fat is a storage molecule for excess energy in many types of animals. If excess energy were stored instead as carbohydrate, as it is in plants, animal bodies would be much bulkier.

Proteins, fats, and other organic molecules are also metabolized for energy. The amino acids of proteins are first deaminated, while fats undergo a process called β -oxidation.

FIGURE 9.23

β -oxidation. Through a series of reactions known as β -oxidation, the last two carbons in a fatty acid tail combine with coenzyme A to form acetyl-CoA, which enters the Krebs cycle. The fatty acid, now two carbons shorter, enters the pathway again and keeps reentering until all its carbons have been used to form acetyl-CoA molecules. Each round of β -oxidation uses one molecule of ATP and generates one molecule each of FADH_2 and NADH, not including the molecules generated from the Krebs cycle.



Metabolic Efficiency and the Length of Food Chains

It has been estimated that a heterotroph limited to glycolysis captures only 3.5% of the energy in the food it consumes. Hence, if such a heterotroph preserves 3.5% of the energy in the autotrophs it consumes, then any other heterotrophs that consume the first heterotroph will capture through glycolysis 3.5% of the energy in it, or 0.12% of the energy available in the original autotrophs. A very large base of autotrophs would thus be needed to support a small number of heterotrophs.

When organisms became able to extract energy from organic molecules by oxidative metabolism, this constraint became far less severe, because the efficiency of oxidative respiration is estimated to be about 52 to 63%. This increased efficiency results in the transmission of much more energy from one trophic level to another than does glycolysis. (A trophic level is a step in the movement of energy through an ecosystem.) The efficiency of oxidative metabolism has made possible the evolution of food chains, in which autotrophs are consumed by heterotrophs, which are consumed by other heterotrophs, and so on. You will read more about food chains in chapter 28.

Even with oxidative metabolism, approximately two-thirds of the available energy is lost at each trophic level, and that puts a limit on how long a food chain can be. Most food chains, like the one illustrated in figure 9.A, involve only three or rarely four trophic levels. Too much energy is lost at each transfer to allow chains to be much longer than that. For example, it would be impossible for a large human population to subsist by eating lions captured from the Serengeti Plain of Africa; the amount of grass available there would not support enough zebras and other herbivores to maintain the number of lions needed to feed the human population. Thus, the ecological complexity of our world is fixed in a fundamental way by the chemistry of oxidative respiration.



Stage 1: Photosynthesizers



Stage 2: Herbivores



Stage 3: Carnivore



Stage 4: Scavengers



Stage 5: Refuse utilizers

FIGURE 9.A

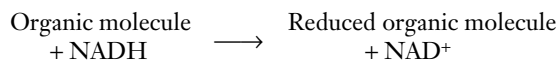
A food chain in the savannas, or open grasslands, of East Africa. Stage 1: *Photosynthesizer*. The grass under these palm trees grows actively during the hot, rainy season, capturing the energy of the sun and storing it in molecules of glucose, which are then converted into starch and stored in the grass. Stage 2: *Herbivore*. These large antelopes, known as wildebeests, consume the grass and transfer some of its stored energy into their own bodies. Stage 3: *Carnivore*. The lion feeds on wildebeests and other animals, capturing part of their stored energy and storing it in its own body. Stage 4: *Scavenger*. This hyena and the vultures occupy the same stage in the food chain as the lion. They are also consuming the body of the dead wildebeest, which has been abandoned by the lion. Stage 5: *Refuse utilizer*. These butterflies, mostly *Precis octavia*, are feeding on the material left in the hyena's dung after the food the hyena consumed had passed through its digestive tract. At each of these four levels, only about a third or less of the energy present is used by the recipient.

9.4 Cells can metabolize food without oxygen.

Fermentation

In the absence of oxygen, aerobic metabolism cannot occur, and cells must rely exclusively on glycolysis to produce ATP. Under these conditions, the hydrogen atoms generated by glycolysis are donated to organic molecules in a process called **fermentation**.

Bacteria carry out more than a dozen kinds of fermentations, all using some form of organic molecule to accept the hydrogen atom from NADH and thus recycle NAD⁺:



Often the reduced organic compound is an organic acid—such as acetic acid, butyric acid, propionic acid, or lactic acid—or an alcohol.

Ethanol Fermentation

Eukaryotic cells are capable of only a few types of fermentation. In one type, which occurs in single-celled fungi called yeast, the molecule that accepts hydrogen from NADH is pyruvate, the end product of glycolysis itself. Yeast enzymes remove a terminal CO₂ group from pyruvate through decarboxylation, producing a two-carbon molecule called acetaldehyde. The CO₂ released causes bread made with yeast to rise, while bread made without yeast (unleavened bread) does not. The acetaldehyde accepts a hydrogen atom from NADH, producing NAD⁺ and **ethanol** (ethyl alcohol). This particular type of fermentation is of great interest to humans, since it is the source of the ethanol in wine and beer (figure 9.24). Ethanol is a by-product of fermentation that is actually toxic to yeast; as it approaches a concentration of about 12%, it begins to kill the yeast. That explains why naturally fermented wine contains only about 12% ethanol.

Lactic Acid Fermentation

Most animal cells regenerate NAD⁺ without decarboxylation. Muscle cells, for example, use an enzyme called lactate dehydrogenase to transfer a hydrogen atom from NADH back to the pyruvate that is produced by glycolysis. This reaction converts pyruvate into lactic acid and regenerates NAD⁺ from NADH. It therefore closes the metabolic circle, allowing glycolysis to continue as long as glucose is available. Circulating blood removes excess lactate (the ionized form of lactic acid) from muscles, but when removal cannot keep pace with production, the accumulating lactic acid interferes with muscle function and contributes to muscle fatigue.

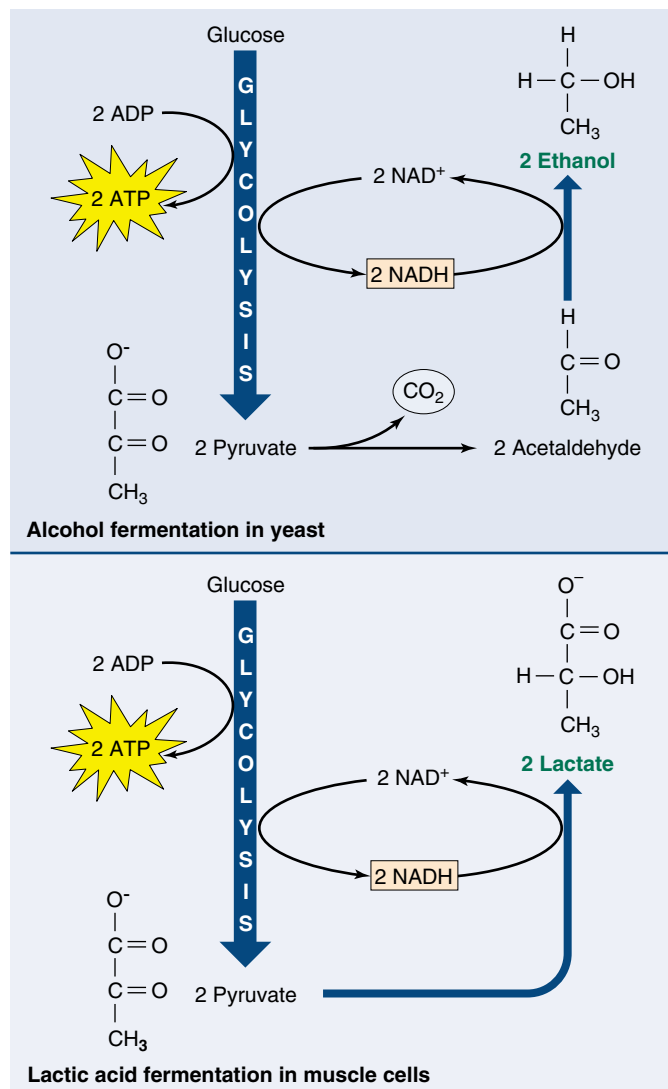


FIGURE 9.24

How wine is made. Yeasts carry out the conversion of pyruvate to ethanol. This takes place naturally in grapes left to ferment on vines, as well as in fermentation vats containing crushed grapes. When the ethanol concentration reaches about 12%, its toxic effects kill the yeast; what remains is wine. Muscle cells convert pyruvate into lactate, which is less toxic than ethanol. However, lactate is still toxic enough to produce a painful sensation in muscles during heavy exercise, when oxygen in the muscles is depleted.

In fermentation, which occurs in the absence of oxygen, the electrons that result from the glycolytic breakdown of glucose are donated to an organic molecule, regenerating NAD⁺ from NADH.

**Summary****Questions****Media Resources****9.1 Cells harvest the energy in chemical bonds.**

- The reactions of cellular respiration are oxidation-reduction (redox) reactions. Those that require a net input of free energy are coupled to the cleavage of ATP, which releases free energy.
- The mechanics of cellular respiration are often dictated by electron behavior, which is in turn influenced by the presence of electron acceptors. Some atoms, such as oxygen, are very electronegative and thus behave as good oxidizing agents.

1. What is the difference between an autotroph and a heterotroph? How does each obtain energy?
2. What is the difference between digestion and catabolism? Which provides more energy?

9.2 Cellular respiration oxidizes food molecules.

- In eukaryotic cells, the oxidative respiration of pyruvate takes place within the matrix of mitochondria.
- The electrons generated in the process are passed along the electron transport chain, a sequence of electron carriers in the inner mitochondrial membrane.
- Some of the energy released by passage of electrons along the electron transport chain is used to pump protons out of the mitochondrial matrix. The reentry of protons into the matrix is coupled to the production of ATP. This process is called chemiosmosis. The ATP then leaves the mitochondrion by facilitated diffusion.

3. Where in a eukaryotic cell does glycolysis occur? What is the net production of ATP during glycolysis, and why is it different from the number of ATP molecules synthesized during glycolysis?
4. By what two mechanisms can the NADH that results from glycolysis be converted back into NAD⁺?
5. What is the theoretical maximum number of ATP molecules produced during the oxidation of a glucose molecule by these processes? Why is the actual number of ATP molecules produced usually lower than the theoretical maximum?



- Exploration: Oxidative Respiration
- Art Activity: Aerobic Cellular Respiration
- Art Activity: Organization of Cristae



- Electron Transport and ATP



- Glycolysis
- Krebs Cycle
- Electron Transport

9.3 Catabolism of proteins and fats can yield considerable energy.

- The catabolism of fatty acids begins with β -oxidation and provides more energy than the catabolism of carbohydrates.

6. How is acetyl-CoA produced during the aerobic oxidation of carbohydrates, and what happens to it? How is it produced during the aerobic oxidation of fatty acids, and what happens to it then?

9.4 Cells can metabolize food without oxygen.

- Fermentation is an anaerobic process that uses an organic molecule instead of oxygen as a final electron acceptor.
- It occurs in bacteria as well as eukaryotic cells, including yeast and the muscle cells of animals.

7. How do the amounts of ATP produced by the aerobic oxidation of glucose and fatty acids compare? Which type of substance contains more energy on a per-weight basis?



- Fermentation