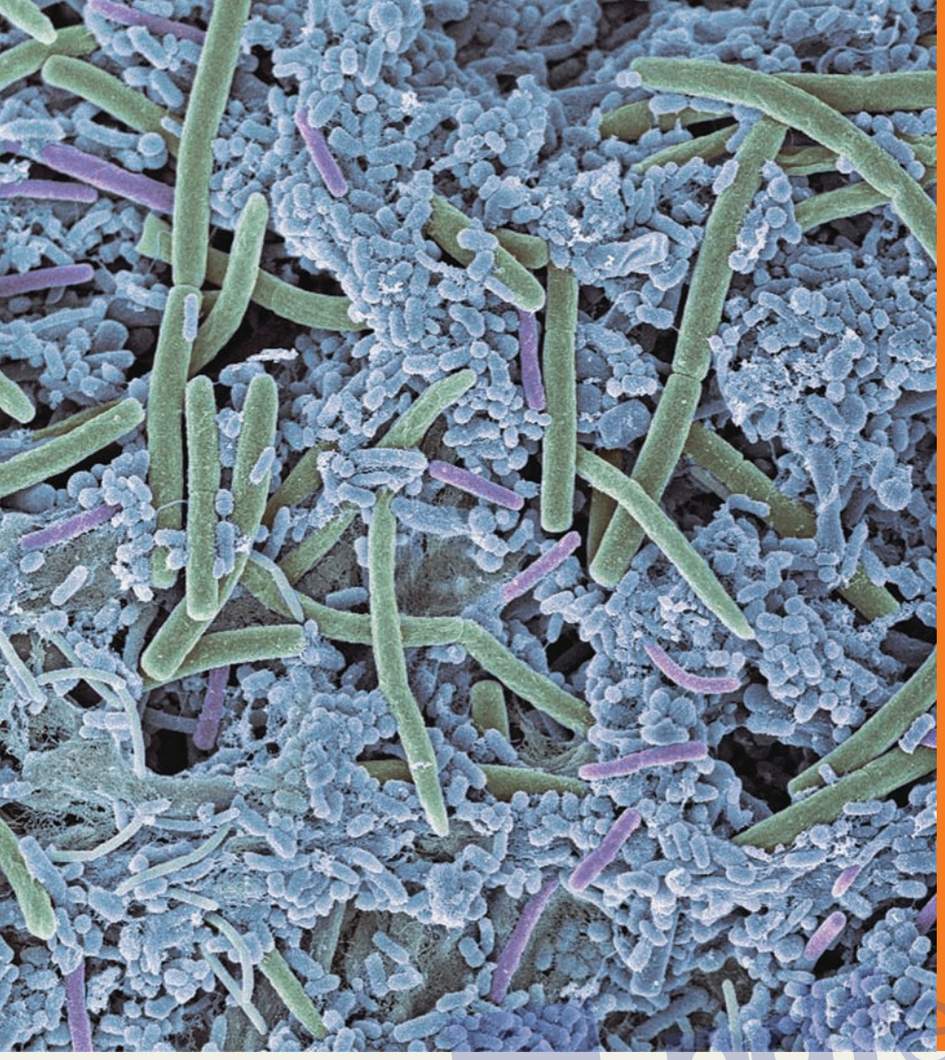

5

Microbial Metabolism

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Now that you are familiar with the structure of prokaryotic cells, we can discuss the activities that enable these microbes to thrive. The life-support processes of even the most structurally simple organism involve a large number of complex biochemical reactions. Most, although not all, of the biochemical processes of bacteria also occur in eukaryotic microbes and in the cells of multicellular organisms, including humans. However, the reactions that are unique to bacteria are fascinating because they allow microorganisms to do things we cannot do. For example, some bacteria can live on cellulose, whereas others can live on petroleum. Through their metabolism, bacteria recycle elements after other organisms have used them. Still other bacteria can live on diets of such inorganic substances as carbon dioxide, iron, sulfur, hydrogen gas, and ammonia. Microbial metabolism allows some microorganisms to grow in or on the human body as shown in dental plaque in the photograph. An example of the bacterial metabolism that contributes to dental caries is discussed in the Clinical Case.

This chapter examines some representative chemical reactions that either produce energy (the catabolic reactions) or use energy (the anabolic reactions) in microorganisms. We will also look at how these various reactions are integrated within the cell.

Catabolic and Anabolic Reactions

LEARNING OBJECTIVES

5-1 Define *metabolism*, and describe the fundamental differences between anabolism and catabolism.

5-2 Identify the role of ATP as an intermediate between catabolism and anabolism.

We use the term **metabolism** to refer to the sum of all chemical reactions within a living organism. Because chemical reactions either release or require energy, metabolism can be viewed as an energy-balancing act. Accordingly, metabolism can be divided into two classes of chemical reactions: those that release energy and those that require energy.

In living cells, the enzyme-regulated chemical reactions that release energy are generally the ones involved in **catabolism,** the breakdown of complex organic compounds into simpler ones. These reactions are called *catabolic,* or *degradative,* reactions. Catabolic reactions are generally *hydrolytic reactions* (reactions which use water and in which chemical bonds are broken), and they are *exergonic* (produce more energy than they consume). An example of catabolism occurs when cells break down sugars into carbon dioxide and water.

The enzyme-regulated energy-requiring reactions are mostly involved in **anabolism,** the building of complex organic molecules from simpler ones. These reactions are called *anabolic,* or *biosynthetic,* reactions. Anabolic processes often involve *dehydration synthesis* reactions (reactions that release water), and they are *endergonic* (consume more energy than they produce). Examples of anabolic processes are the formation of proteins from amino acids, nucleic acids from nucleotides, and polysaccharides from simple sugars. These biosynthetic reactions generate the materials for cell growth.

Clinical Case: More Than a Sweet Tooth

Dr. Antonia Rivera is a pediatric dentist in St. Louis, Missouri. Her latest patient, 7-year-old Micah Thompson, has just left the office with strict instructions about brushing and flossing regularly. What most worries Dr. Rivera, however, is that Micah is her seventh patient this week to present with multiple dental caries, or cavities. Dr. Rivera is used to seeing some increase in tooth decay after Halloween and Easter, but why are all these children getting cavities in the middle of the summer? When possible, she has been speaking to each of the patient's parents or grandparents, but no one has noticed anything out of the ordinary in the children's diets.

Why do so many of Dr. Rivera's patients have multiple dental caries? Read on to find out.

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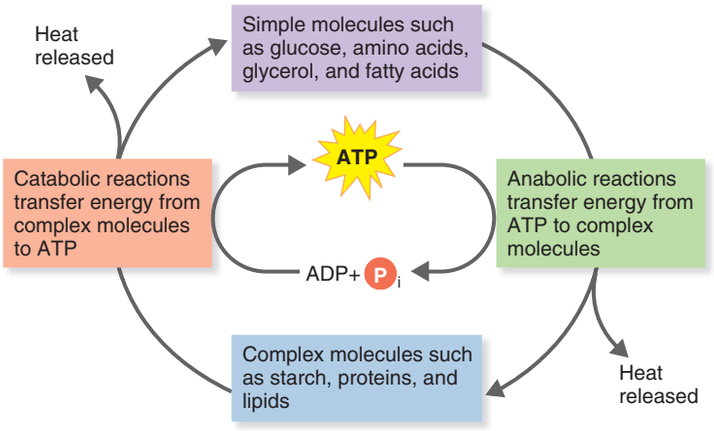


Figure 5.1 The role of ATP in coupling anabolic and catabolic reactions. When complex molecules are split apart (catabolism), some of the energy is transferred to and trapped in ATP, and the rest is given off as heat. When simple molecules are combined to form complex molecules (anabolism), ATP provides the energy for synthesis, and again some energy is given off as heat.

Q How does ATP provide the energy for synthesis?

Catabolic reactions provide building blocks for anabolic reactions and furnish the energy needed to drive anabolic reactions. This coupling of energy-requiring and energy-releasing reactions is made possible through the molecule adenosine triphosphate (ATP). (You can review its structure in Figure 2.18, page 48.) ATP stores energy derived from catabolic reactions and releases it later to drive anabolic reactions and perform other cellular work. Recall from Chapter 2 that a molecule of ATP consists of an adenine, a ribose, and three phosphate groups. When the terminal phosphate group is split from ATP, adenosine diphosphate (ADP) is formed, and energy is released to drive anabolic reactions. Using P to represent a phosphate group (P_i represents inorganic phosphate, which is not bound to any other molecule), we write this reaction as follows:

$$ATP \rightarrow ADP + P_i + \text{energy}$$

Then, the energy from catabolic reactions is used to combine ADP and a P_i to resynthesize ATP:

$$ADP + P_i + \text{energy} \rightarrow ATP$$

Thus, anabolic reactions are coupled to ATP breakdown, and catabolic reactions are coupled to ATP synthesis. This concept of coupled reactions is very important; you will see why by the end of this chapter. For now, you should know that the chemical composition of a living cell is constantly changing: some molecules are broken down while others are being synthesized. This balanced flow of chemicals and energy maintains the life of a cell.

The role of ATP in coupling anabolic and catabolic reactions is shown in Figure 5.1. Only part of the energy released in catabolism is actually available for cellular functions because part

of the energy is lost to the environment as heat. Because the cell must use energy to maintain life, it has a continuous need for new external sources of energy.

Before we discuss how cells produce energy, let's first consider the principal properties of a group of proteins involved in almost all biologically important chemical reactions: enzymes. A cell's **metabolic pathways** (sequences of chemical reactions) are determined by its enzymes, which are in turn determined by the cell's genetic makeup. **Animation** Metabolism: Overview

CHECK YOUR UNDERSTANDING

- Distinguish catabolism from anabolism. **5-1**
- How is ATP an intermediate between catabolism and anabolism? **5-2**

Enzymes

LEARNING OBJECTIVES

5-3 Identify the components of an enzyme.

5-4 Describe the mechanism of enzymatic action.

5-5 List the factors that influence enzymatic activity.

5-6 Distinguish competitive and noncompetitive inhibition.

5-7 Define *ribozyme*.

Collision Theory

We indicated in Chapter 2 that chemical reactions occur when chemical bonds are formed or broken. For reactions to take place, atoms, ions, or molecules must collide. The **collision theory** explains how chemical reactions occur and how certain factors affect the rates of those reactions. The basis of the collision theory is that all atoms, ions, and molecules are continuously moving and are thus continuously colliding with one another. The energy transferred by the particles in the collision can disrupt their electron structures enough to break chemical bonds or form new bonds.

Several factors determine whether a collision will cause a chemical reaction: the velocities of the colliding particles, their energy, and their specific chemical configurations. Up to a point, the higher the particles' velocities, the more probable that their collision will cause a reaction. Also, each chemical reaction requires a specific level of energy. But even if colliding particles possess the minimum energy needed for reaction, no reaction will take place unless the particles are properly oriented toward each other.

Let's assume that molecules of substance AB (the reactant) are to be converted to molecules of substances A and B (the products). In a given population of molecules of substance AB, at a specific temperature, some molecules possess relatively little energy; the majority of the population possesses an average amount of energy; and a small portion of the population has high energy. If only the high-energy AB molecules are able to react and be converted to A and B molecules, then only relatively few molecules at any one time possess enough energy to react in a collision. The collision energy required for a chemical reaction is its **activation energy,** which is the amount of energy needed to disrupt the stable electronic configuration of any specific molecule so that the electrons can be rearranged.

The **reaction rate**—the frequency of collisions containing sufficient energy to bring about a reaction—depends on the number of reactant molecules at or above the activation energy level. One way to increase the reaction rate of a substance is to raise its temperature. By causing the molecules to move faster, heat increases both the frequency of collisions and the number of molecules that attain activation energy. The number of collisions also increases when pressure is increased or when the reactants are more concentrated (because the distance between molecules is thereby decreased). In living systems, enzymes increase the reaction rate without raising the temperature.

Enzymes and Chemical Reactions

Substances that can speed up a chemical reaction without being permanently altered themselves are called **catalysts.** In living cells, **enzymes** serve as biological catalysts. As catalysts, enzymes are specific. Each acts on a specific substance, called the enzyme's **substrate** (or substrates, when there are two or more reactants), and each catalyzes only one reaction. For example, sucrose (table sugar) is the substrate of the enzyme sucrase, which catalyzes the hydrolysis of sucrose to glucose and fructose.

As catalysts, enzymes typically accelerate chemical reactions. The three-dimensional enzyme molecule has an *active site,* a region that interacts with a specific chemical substance (see Figure 5.4).

The enzyme orients the substrate into a position that increases the probability of a reaction. The **enzyme–substrate complex** formed by the temporary binding of enzyme and reactants enables the collisions to be more effective and lowers the activation energy of the reaction (**Figure 5.2**). The enzyme therefore speeds up the reaction by increasing the number of AB molecules that attain sufficient activation energy to react.

An enzyme's ability to accelerate a reaction without the need for an increase in temperature is crucial to living systems because a significant temperature increase would destroy cellular proteins. The crucial function of enzymes, therefore, is to speed up biochemical reactions at a temperature that is compatible with the normal functioning of the cell.

Enzyme Specificity and Efficiency

The specificity of enzymes is made possible by their structures. Enzymes are generally large globular proteins that range in molecular weight from about 10,000 to several million. Each of the thousands of known enzymes has a characteristic three-dimensional shape with a specific surface configuration as a result of its primary, secondary, and tertiary structures (see Figure 2.15, page 45). The unique configuration of each enzyme enables it to "find" the correct substrate from among the large number of diverse molecules in the cell.

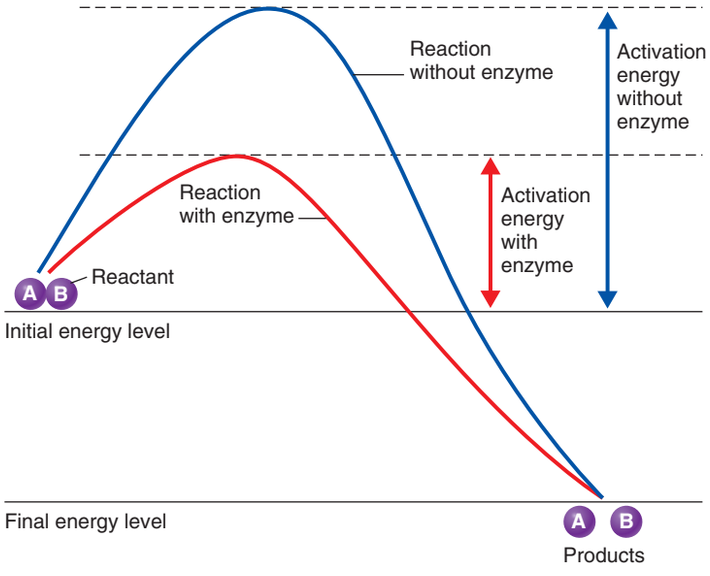


Figure 5.2 Energy requirements of a chemical reaction. This graph shows the progress of the reaction AB → A + B both without (blue line) and with (red line) an enzyme. The presence of an enzyme lowers the activation energy of the reaction (see arrows). Thus, more molecules of reactant AB are converted to products A and B because more molecules of reactant AB possess the activation energy needed for the reaction.

Q Why does a chemical reaction require increased activation energy without an enzyme as a biological catalyst?

Enzymes are extremely efficient. Under optimum conditions, they can catalyze reactions at rates 10^8 to 10^{10} times (up to 10 billion times) higher than those of comparable reactions without enzymes. The **turnover number** (maximum number of substrate molecules an enzyme molecule converts to product each second) is generally between 1 and 10,000 and can be as high as 500,000. For example, the enzyme DNA polymerase I, which participates in the synthesis of DNA, has a turnover number of 15, whereas the enzyme lactate dehydrogenase, which removes hydrogen atoms from lactic acid, has a turnover number of 1000.

Many enzymes exist in the cell in both active and inactive forms. The rate at which enzymes switch between these two forms is determined by the cellular environment.

Naming Enzymes

The names of enzymes usually end in *-ase.* All enzymes can be grouped into six classes, according to the type of chemical reaction they catalyze (**Table 5.1**). Enzymes within each of the major classes are named according to the more specific types of reactions they assist. For example, the class called *oxidoreductases* is involved with oxidation-reduction reactions (described shortly). Enzymes in the oxidoreductase class that remove hydrogen from a substrate are called *dehydrogenases;* those that add molecular oxygen (O_2) are called *oxidases.* As you will see later, dehydrogenase and oxidase enzymes have even more specific names, such as lactate dehydrogenase and cytochrome oxidase, depending on the specific substrates on which they act.

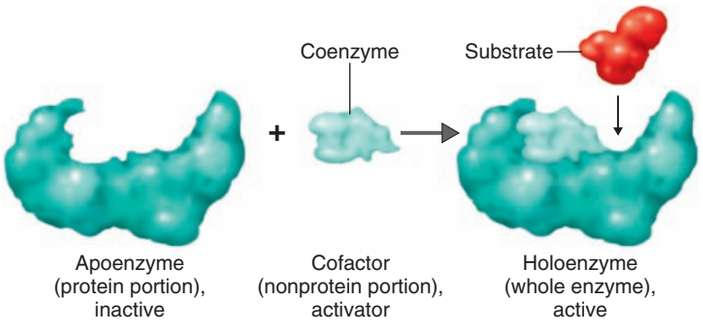


Figure 5.3 Components of a holoenzyme. Many enzymes require both an apoenzyme (protein portion) and a cofactor (nonprotein portion) to become active. The cofactor can be a metal ion, or if it is an organic molecule, it is called a coenzyme (as shown here). The apoenzyme and cofactor together make up the holoenzyme, or whole enzyme. The substrate is the reactant acted upon by the enzyme.

Q How does the enzyme–substrate complex lower the activation energy of the reaction?

Enzyme Components

Although some enzymes consist entirely of proteins, most consist of both a protein portion, called an **apoenzyme,** and a nonprotein component, called a **cofactor.** Ions of iron, zinc, magnesium, or calcium are examples of cofactors. If the cofactor is an organic molecule, it is called a **coenzyme.** Apoenzymes are inactive by themselves; they must be activated by cofactors. Together, the apoenzyme and cofactor form a **holoenzyme,** or whole, active enzyme (**Figure 5.3**). If the cofactor is removed, the apoenzyme will not function.

Coenzymes may assist the enzyme by accepting atoms removed from the substrate or by donating atoms required by the substrate. Some coenzymes act as electron carriers, removing electrons from the substrate and donating them to other molecules in subsequent reactions. Many coenzymes are derived from vitamins (**Table 5.2**). Two of the most important coenzymes in cellular metabolism are **nicotinamide adenine dinucleotide (NAD^+)** and **nicotinamide adenine dinucleotide phosphate ($NADP^+$).** Both compounds contain derivatives of the B vitamin niacin (nicotinic acid), and both function as electron carriers. Whereas NAD^+ is primarily involved in catabolic (energy-yielding) reactions, $NADP^+$ is primarily involved in anabolic (energy-requiring) reactions. The flavin coenzymes, such as **flavin mononucleotide (FMN)** and **flavin adenine dinucleotide (FAD),** contain derivatives of the B vitamin riboflavin and are also electron carriers. Another important coenzyme, **coenzyme A (CoA),** contains a derivative of pantothenic acid, another B vitamin. This coenzyme plays an important role in the synthesis and breakdown of fats and in a series of oxidizing reactions called the Krebs cycle.

TABLE 5.1 Enzyme Classification Based on Type of Chemical Reaction Catalyzed

Class	Type of Chemical Reaction Catalyzed	Examples
Oxidoreductase	Oxidation-reduction, in which oxygen and hydrogen are gained or lost	Cytochrome oxidase, lactate dehydrogenase
Transferase	Transfer of functional groups, such as an amino group, acetyl group, or phosphate group	Acetate kinase, alanine deaminase
Hydrolase	Hydrolysis (addition of water)	Lipase, sucrase
Lyase	Removal of groups of atoms without hydrolysis	Oxalate decarboxylase, isocitrate lyase
Isomerase	Rearrangement of atoms within a molecule	Glucose-phosphate isomerase, alanine racemase
Ligase	Joining of two molecules (using energy usually derived from the breakdown of ATP)	Acetyl-CoA synthetase, DNA ligase

We will come across all of these coenzymes in our discussion of metabolism later in the chapter.

As noted earlier, some cofactors are metal ions, including iron, copper, magnesium, manganese, zinc, calcium, and cobalt. Such cofactors may help catalyze a reaction by forming a bridge between the enzyme and a substrate. For example, magnesium (Mg^{2+}) is required by many phosphorylating enzymes (enzymes that transfer a phosphate group from ATP to another substrate). The Mg^{2+} can form a link between the enzyme and the ATP molecule. Most trace elements required by living cells are probably used in some such way to activate cellular enzymes.

The Mechanism of Enzymatic Action

Enzymes lower the activation energy of chemical reactions. The general sequence of events in enzyme action is as follows (Figure 5.4a):

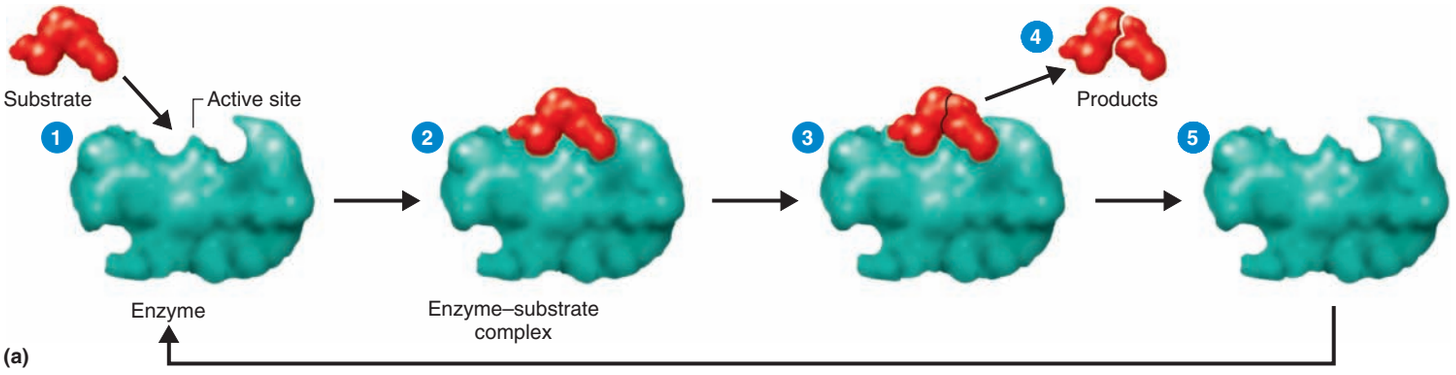
1. The surface of the substrate contacts a specific region of the surface of the enzyme molecule, called the **active site.**
2. A temporary intermediate compound forms, called an **enzyme–substrate complex.**
3. The substrate molecule is transformed by the rearrangement of existing atoms, the breakdown of the substrate molecule, or in combination with another substrate molecule.
4. The transformed substrate molecules—the products of the reaction—are released from the enzyme molecule because they no longer fit in the active site of the enzyme.
5. The unchanged enzyme is now free to react with other substrate molecules.

As a result of these events, an enzyme speeds up a chemical reaction.

TABLE 5.2 Selected Vitamins and Their Coenzymatic Functions

Vitamin	Function
Vitamin B_1 (Thiamine)	Part of coenzyme cocarboxylase; has many functions, including the metabolism of pyruvic acid
Vitamin B_2 (Riboflavin)	Coenzyme in flavoproteins; active in electron transfers
Niacin (Nicotinic Acid)	Part of NAD molecule*; active in electron transfers
Vitamin B_6 (Pyridoxine)	Coenzyme in amino acid metabolism
Vitamin B_{12} (Cyanocobalamin)	Coenzyme (methyl cyanocobalamide) involved in the transfer of methyl groups; active in amino acid metabolism
Pantothenic Acid	Part of coenzyme A molecule; involved in the metabolism of pyruvic acid and lipids
Biotin	Involved in carbon dioxide fixation reactions and fatty acid synthesis
Folic Acid	Coenzyme used in the synthesis of purines and pyrimidines
Vitamin E	Needed for cellular and macromolecular syntheses
Vitamin K	Coenzyme used in electron transport (naphthoquinones and quinones)

*NAD = nicotinamide adenine dinucleotide



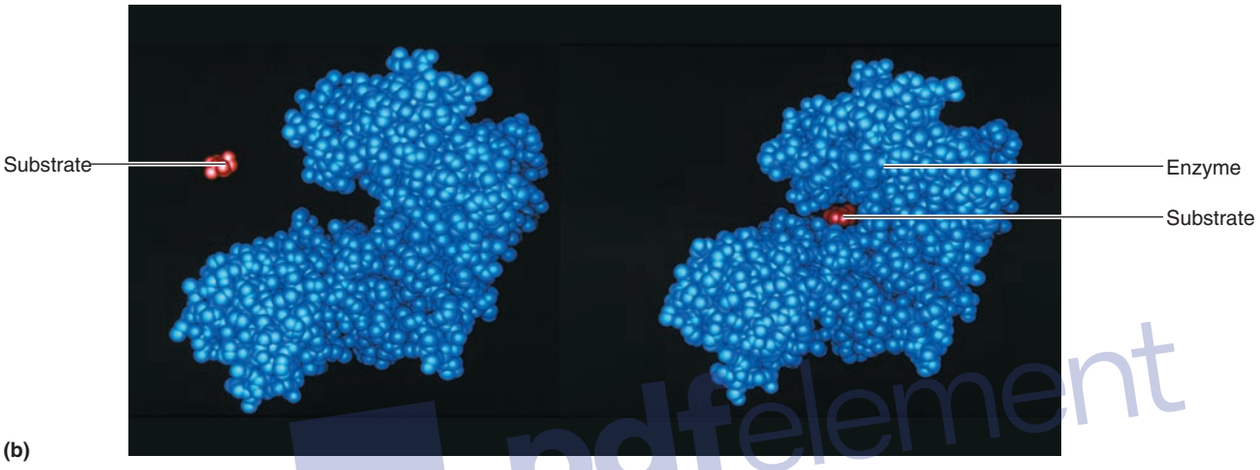


Figure 5.4 The mechanism of enzymatic action. (**a**) 1 The substrate contacts the active site on the enzyme to form 2 an enzyme–substrate complex. 3 The substrate is then transformed into products, 4 the products are released, and 5 the enzyme is recovered unchanged. In the example shown, the transformation into products involves a breakdown of the substrate into two products. Other transformations, however, may occur. (**b**) Left: A molecular model of the enzyme in step 1 of part (a). The active site of the enzyme can be seen here as a groove on the surface of the protein. Right: As the enzyme and substrate meet in step 2 of part (a), the enzyme changes shape slightly to fit together more tightly with the substrate.

Give an example of enzymatic specifity.

As mentioned earlier, enzymes have *specificity* for particular substrates. For example, a specific enzyme may be able to hydrolyze a peptide bond only between two specific amino acids. Other enzymes can hydrolyze starch but not cellulose; even though both starch and cellulose are polysaccharides composed of glucose subunits, the orientations of the subunits in the two polysaccharides differ. Enzymes have this specificity because the three-dimensional shape of the active site fits the substrate somewhat as a lock fits with its key (Figure 5.4b). However, the active site and substrate are flexible, and they change shape somewhat as they meet to fit together more tightly. The substrate is usually much smaller than the enzyme, and relatively few of the enzyme's amino acids make up the active site.

A certain compound can be a substrate for several different enzymes that catalyze different reactions, so the fate of a compound depends on the enzyme that acts on it. At least four different enzymes can act on glucose 6-phosphate, a molecule important in cell metabolism, and each reaction will yield a different product. MM **Animations** Enzymes: Overview, Steps in a Reaction

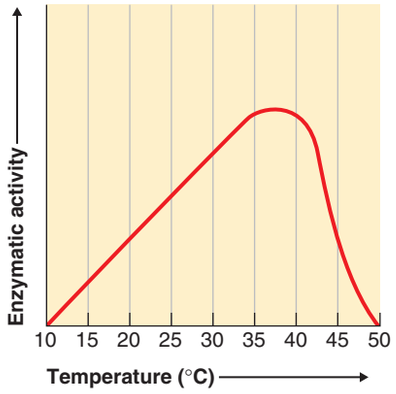
Factors Influencing Enzymatic Activity

Enzymes are subject to various cellular controls. Two primary types are the control of enzyme *synthesis* (see Chapter 8) and the control of enzyme *activity* (how much enzyme is present versus how active it is).

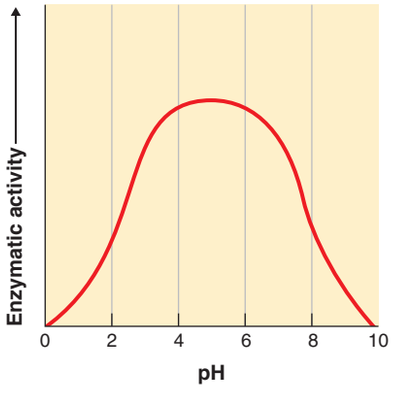
Several factors influence the activity of an enzyme. Among the more important are temperature, pH, substrate concentration, and the presence or absence of inhibitors.

Temperature

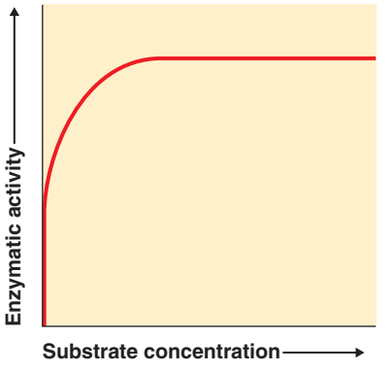
The rate of most chemical reactions increases as the temperature increases. Molecules move more slowly at lower temperatures than at higher temperatures and so may not have enough energy



(a) Temperature. The enzymatic activity (rate of reaction catalyzed by the enzyme) increases with increasing temperature until the enzyme, a protein, is denatured by heat and inactivated. At this point, the reaction rate falls steeply.



(b) pH. The enzyme illustrated is most active at about pH 5.0.



(c) Substrate concentration. With increasing concentration of substrate molecules, the rate of reaction increases until the active sites on all the enzyme molecules are filled, at which point the maximum rate of reaction is reached.

Figure 5.5 **Factors that influence enzymatic activity, plotted for a hypothetical enzyme.**

Q How will this enzyme act at 25°C? At 45°C? At pH 7?

to cause a chemical reaction. For enzymatic reactions, however, elevation beyond a certain temperature (the optimal temperature) drastically reduces the rate of reaction (Figure 5.5a). The optimal temperature for most disease-producing bacteria in the human body is between 35°C and 40°C. The rate of reaction declines beyond the optimal temperature because of the enzyme's **denaturation,** the loss of its characteristic three-dimensional structure (tertiary configuration) (Figure 5.6). Denaturation of a protein involves the breakage of hydrogen bonds and other noncovalent bonds; a common example is the transformation of uncooked egg white (a protein called albumin) to a hardened state by heat.

Denaturation of an enzyme changes the arrangement of the amino acids in the active site, altering its shape and causing the enzyme to lose its catalytic ability. In some cases, denaturation is partially or fully reversible. However, if denaturation continues until the enzyme has lost its solubility and coagulates, the enzyme cannot regain its original properties. Enzymes can also be denatured by concentrated acids, bases, heavy-metal ions (such as lead, arsenic, or mercury), alcohol, and ultraviolet radiation.

pH

Most enzymes have an optimum pH at which their activity is characteristically maximal. Above or below this pH value, enzyme activity, and therefore the reaction rate, decline (Figure 5.5b). When the H^+ concentration (pH) in the medium is changed drastically, the protein's three-dimensional structure is altered. Extreme changes in pH can cause denaturation. Acids (and bases) alter a protein's three-dimensional structure because the H^+ (and OH^-) compete with hydrogen and ionic bonds in an enzyme, resulting in the enzyme's denaturation.

Substrate Concentration

There is a maximum rate at which a certain amount of enzyme can catalyze a specific reaction. Only when the concentration of substrate(s) is extremely high can this maximum rate be attained. Under conditions of high substrate concentration, the enzyme is said to be in **saturation;** that is, its active site is always occupied by substrate or product molecules. In this condition, a further increase in substrate concentration will not affect the reaction rate because all active sites are already in use (Figure 5.5c). Under normal cellular conditions, enzymes are not saturated with substrate(s). At any given time, many of the enzyme molecules are inactive for lack of substrate; thus, the substrate concentration is likely to influence the rate of reaction.

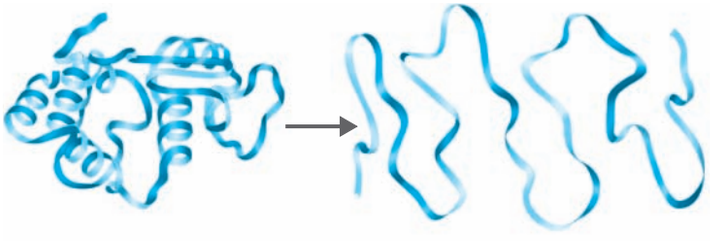


Figure 5.6 **Denaturation of a protein.** Breakage of the noncovalent bonds (such as hydrogen bonds) that hold the active protein in its three-dimensional shape renders the denatured protein nonfunctional.

Q When is denaturation irreversible?

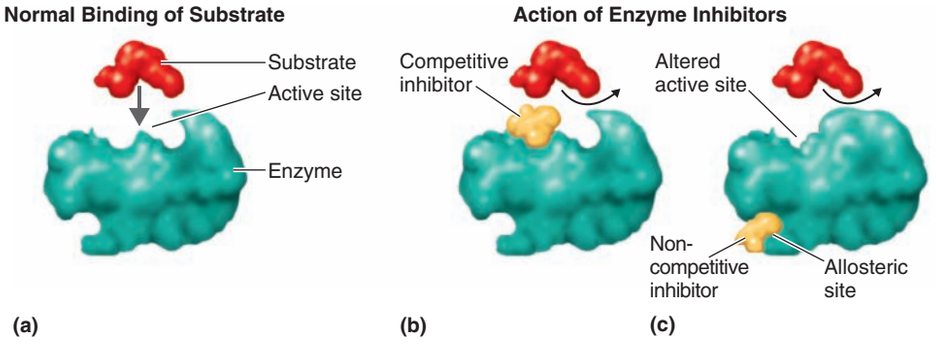


Figure 5.7 Enzyme inhibitors. (**a**) An uninhibited enzyme and its normal substrate. (**b**) A competitive inhibitor. (**c**) One type of noncompetitive inhibitor, causing allosteric inhibition.

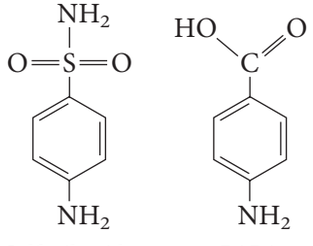
How do competitive inhibitors operate in comparison to noncompetitive inhibitors?

Inhibitors

An effective way to control the growth of bacteria is to control their enzymes. Certain poisons, such as cyanide, arsenic, and mercury, combine with enzymes and prevent them from functioning. As a result, the cells stop functioning and die.

Enzyme inhibitors are classified as either competitive or noncompetitive inhibitors (Figure 5.7). **Competitive inhibitors** fill the active site of an enzyme and compete with the normal substrate for the active site. A competitive inhibitor can do this because its shape and chemical structure are similar to those of the normal substrate (Figure 5.7b). However, unlike the substrate, it does not undergo any reaction to form products. Some competitive inhibitors bind irreversibly to amino acids in the active site, preventing any further interactions with the substrate. Others bind reversibly, alternately occupying and leaving the active site; these slow the enzyme's interaction with the substrate. Increasing the substrate concentration can overcome reversible competitive inhibition. As active sites become available, more substrate molecules than competitive inhibitor molecules are available to attach to the active sites of enzymes.

One good example of a competitive inhibitor is sulfanilamide (a sulfa drug), which inhibits the enzyme whose normal substrate is *para*-aminobenzoic acid (PABA):



Sulfanilamide PABA

PABA is an essential nutrient used by many bacteria in the synthesis of folic acid, a vitamin that functions as a coenzyme. When sulfanilamide is administered to bacteria, the enzyme that normally converts PABA to folic acid combines instead with the sulfanilamide. Folic acid is not synthesized, and the bacteria cannot grow. Because human cells do not use PABA to make their folic acid, sulfanilamide can kill bacteria but does not harm human cells.

Noncompetitive inhibitors do not compete with the substrate for the enzyme's active site; instead, they interact with another part of the enzyme (Figure 5.7c). In this process, called **allosteric** ("other space") **inhibition,** the inhibitor binds to a site on the enzyme other than the substrate's binding site, called the **allosteric site.** This binding causes the active site to change its shape, making it nonfunctional. As a result, the enzyme's activity is reduced. This effect can be either reversible or irreversible, depending on whether the active site can return to its original shape. In some cases, allosteric interactions can activate an enzyme rather than inhibit it. Another type of noncompetitive inhibition can operate on enzymes that require metal ions for their activity. Certain chemicals can bind or tie up the metal ion activators and thus prevent an enzymatic reaction. Cyanide can bind the iron in iron-containing enzymes, and fluoride can bind calcium or magnesium. Substances such as cyanide and fluoride are sometimes called *enzyme poisons* because they permanently inactivate enzymes. **Animations** Enzymes: Competitive Inhibition, Noncompetitive Inhibition

Feedback Inhibition

Allosteric inhibitors play a role in a kind of biochemical control called **feedback inhibition,** or **end-product inhibition.** This control mechanism stops the cell from making more of a substance than it needs and thereby wasting chemical resources. In some metabolic reactions, several steps are required for the synthesis of a particular chemical compound, called the *end-product.* The process is similar to an assembly line, with each step catalyzed by a separate enzyme (Figure 5.8). In many anabolic pathways, the final product can allosterically inhibit the activity of one of the enzymes earlier in the pathway. This phenomenon is feedback inhibition.

Feedback inhibition generally acts on the first enzyme in a metabolic pathway (similar to shutting down an assembly line by stopping the first worker). Because the enzyme is inhibited, the product of the first enzymatic reaction in the pathway is not synthesized. Because that unsynthesized product would

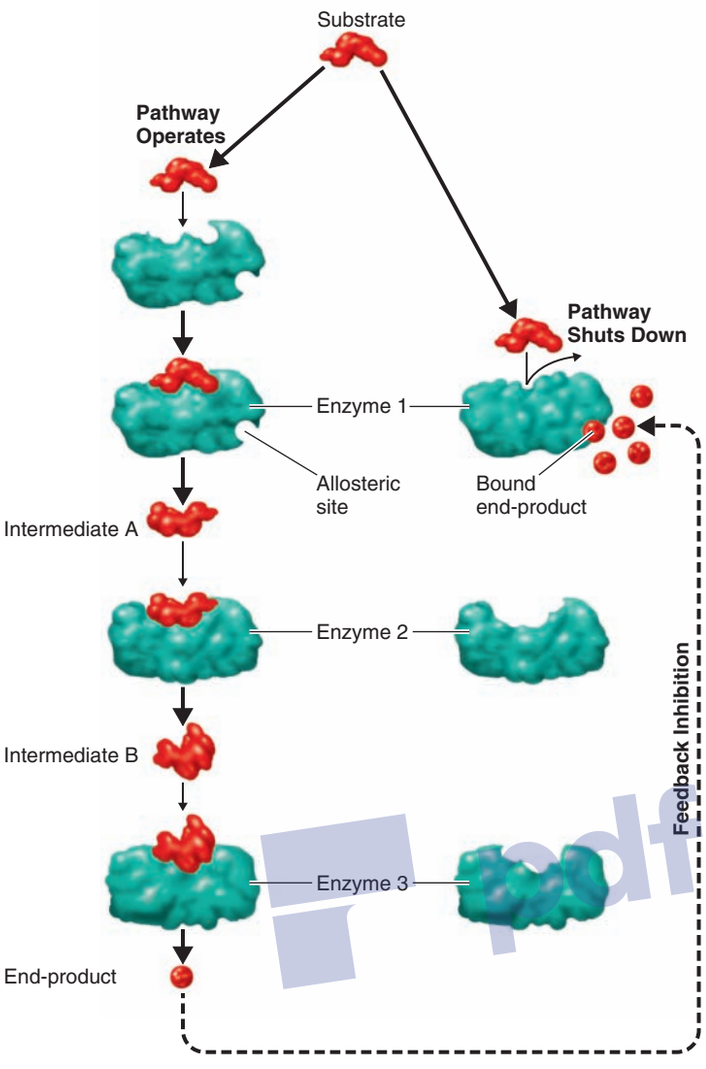


Figure 5.8 **Feedback inhibition.**

Q Explain the differences between competitive inhibition and feedback inhibition.

normally be the substrate for the second enzyme in the pathway, the second reaction stops immediately as well. Thus, even though only the first enzyme in the pathway is inhibited, the entire pathway shuts down, and no new end-product is formed. By inhibiting the first enzyme in the pathway, the cell also keeps metabolic intermediates from accumulating. As the cell uses up the existing end-product, the first enzyme's allosteric site more often remains unbound, and the pathway resumes activity.

The bacterium *E. coli* can be used to demonstrate feedback inhibition in the synthesis of the amino acid isoleucine, which is required for the cell's growth. In this metabolic pathway, the amino acid threonine is enzymatically converted to isoleucine in five steps. If isoleucine is added to the growth medium for *E. coli,* it inhibits the first enzyme in the pathway, and the bacteria stop synthesizing isoleucine. This condition is maintained until the supply of isoleucine is depleted. This type of feedback inhibition is also involved in regulating the cells' production of other amino acids, as well as vitamins, purines, and pyrimidines.

Ribozymes

Prior to 1982, it was believed that only protein molecules had enzymatic activity. Researchers working on microbes discovered a unique type of RNA called a **ribozyme.** Like protein enzymes, ribozymes function as catalysts, have active sites that bind to substrates, and are not used up in a chemical reaction. Ribozymes specifically act on strands of RNA by removing sections and splicing together the remaining pieces. In this respect, ribozymes are more restricted than protein enzymes in terms of the diversity of substrates with which they interact.

CHECK YOUR UNDERSTANDING

- What is a coenzyme? **5-3**
- Why is enzyme specificity important? **5-4**
- What happens to an enzyme below its optimal temperature? Above its optimal temperature? **5-5**
- Why is feedback inhibition noncompetitive inhibition? **5-6**
- What is a ribozyme? **5-7**

Energy Production

LEARNING OBJECTIVES

5-8 Explain the term *oxidation-reduction.*

5-9 List and provide examples of three types of phosphorylation reactions that generate ATP.

5-10 Explain the overall function of metabolic pathways.

Nutrient molecules, like all molecules, have energy associated with the electrons that form bonds between their atoms. When it is spread throughout the molecule, this energy is difficult for the cell to use. Various reactions in catabolic pathways, however, concentrate the energy into the bonds of ATP, which serves as a convenient energy carrier. ATP is generally referred to as having "high-energy" bonds. Actually, a better term is probably *unstable bonds.* Although the amount of energy in these bonds is not exceptionally large, it can be released quickly and easily. In a sense, ATP is similar to a highly flammable liquid such as kerosene. Although a large log might eventually burn to produce more heat than a cup of kerosene, the kerosene is easier to ignite and provides heat more quickly and conveniently. In a similar way, the "high-energy" unstable bonds of ATP provide the cell with readily available energy for anabolic reactions.

Before discussing the catabolic pathways, we will consider two general aspects of energy production: the concept of oxidation-reduction and the mechanisms of ATP generation.

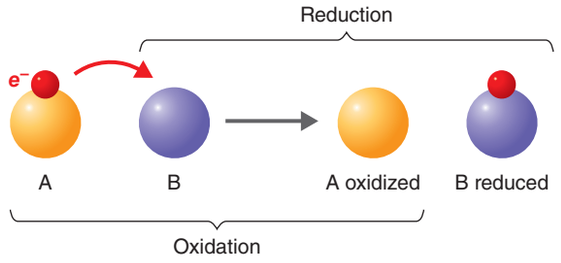


Figure 5.9 Oxidation-reduction. An electron is transferred from molecule A to molecule B. In the process, molecule A is oxidized, and molecule B is reduced.

Q How do oxidation and reduction differ?

Oxidation-Reduction Reactions

Oxidation is the removal of electrons (e^-) from an atom or molecule, a reaction that often produces energy. Figure 5.9 shows an example of an oxidation in which molecule A loses an electron to molecule B. Molecule A has undergone oxidation (meaning that it has lost one or more electrons), whereas molecule B has undergone **reduction** (meaning that it has gained one or more electrons).* Oxidation and reduction reactions are always coupled; in other words, each time one substance is oxidized, another is simultaneously reduced. The pairing of these reactions is called **oxidation-reduction** or a **redox reaction.**

In many cellular oxidations, electrons and protons (hydrogen ions, H^+) are removed at the same time; this is equivalent to the removal of hydrogen atoms, because a hydrogen atom is made up of one proton and one electron (see Table 2.2, page 28). Because most biological oxidations involve the loss of hydrogen atoms, they are also called **dehydrogenation** reactions. Figure 5.10 shows an example of a biological oxidation. An organic molecule is oxidized by the loss of two hydrogen atoms, and a molecule of NAD^+ is reduced. Recall from our earlier discussion of coenzymes that NAD^+ assists enzymes by accepting hydrogen atoms removed from the substrate, in this case the organic molecule. As shown in Figure 5.10, NAD^+ accepts two electrons and one proton. One proton (H^+) is left over and is released into the surrounding medium. The reduced coenzyme, NADH, contains more energy than NAD^+. This energy can be used to generate ATP in later reactions.

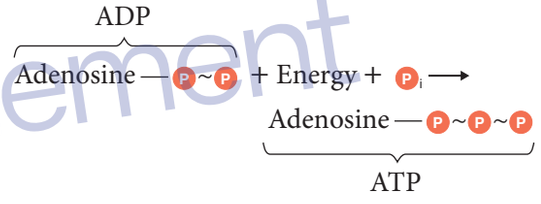
An important point to remember about biological oxidation-reduction reactions is that cells use them in catabolism to extract energy from nutrient molecules. Cells take nutrients, some of which serve as energy sources, and degrade them from highly reduced compounds (with many hydrogen atoms) to highly oxidized compounds. For example, when a cell oxidizes a molecule of glucose ($C_6H_{12}O_6$) to CO_2 and H_2O, the energy in the glucose molecule is removed in a stepwise manner and ultimately is trapped by ATP, which can then serve as an energy source for energy-requiring reactions. Compounds such as glucose that have many hydrogen atoms are highly reduced compounds, containing a large amount of potential energy. Thus, glucose is a valuable nutrient for organisms. MM **Animation** Oxidation-Reduction Reactions

CHECK YOUR UNDERSTANDING

Why is glucose such an important molecule for organisms? 5-8

The Generation of ATP

Much of the energy released during oxidation-reduction reactions is trapped within the cell by the formation of ATP. Specifically, an inorganic phosphate group, P_i, is added to ADP with the input of energy to form ATP:



The symbol ~ designates a "high-energy" bond—that is, one that can readily be broken to release usable energy. The high-energy bond that attaches the third P in a sense contains the energy stored in this reaction. When this P is removed, usable energy is released. The addition of P to a chemical compound is called **phosphorylation.** Organisms use three mechanisms of phosphorylation to generate ATP from ADP.

Substrate-Level Phosphorylation

In **substrate-level phosphorylation,** ATP is usually generated when a high-energy P is directly transferred from a phosphorylated compound (a substrate) to ADP. Generally, the P has acquired its energy during an earlier reaction in which the substrate itself was oxidized. The following example shows only the carbon skeleton and the P of a typical substrate:

$$C—C—C \sim P + ADP \rightarrow C—C—C + ATP$$

Oxidative Phosphorylation

In **oxidative phosphorylation,** electrons are transferred from organic compounds to one group of electron carriers (usually to NAD^+ and FAD). Then, the electrons are passed through a

*The terms do not seem logical until one considers the history of the discovery of these reactions. When mercury is heated, it gains weight as mercuric oxide is formed; this was called *oxidation*. Later it was determined that the mercury actually *lost* electrons, and the observed *gain* in oxygen was a direct result of this. Oxidation, therefore, is a *loss* of electrons, and reduction is a *gain* of electrons, but the gain and loss of electrons is not usually apparent as chemical-reaction equations are usually written. For example, in the equations for aerobic respiration on page 130, notice that each carbon in glucose had only one oxygen originally, and later, as carbon dioxide, each carbon now has two oxygens. However, the gain or loss of electrons actually responsible for this is not apparent.

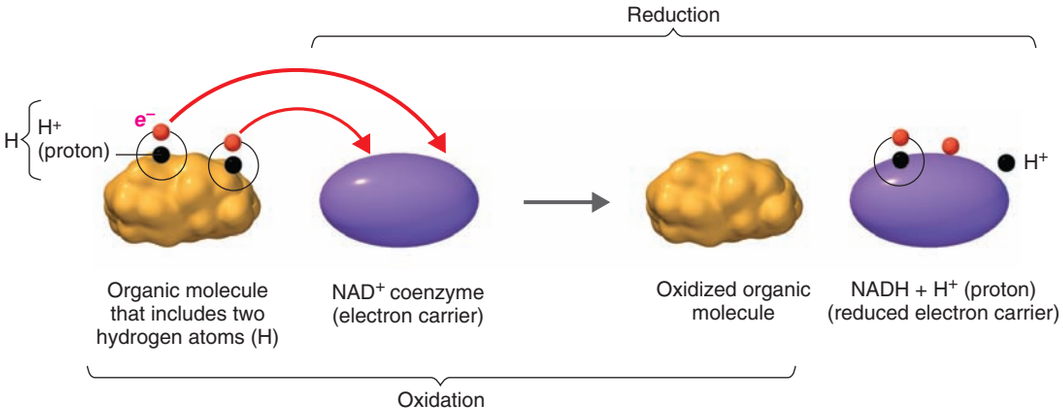


Figure 5.10 Representative biological oxidation. Two electrons and two protons (altogether equivalent to two hydrogen atoms) are transferred from an organic substrate molecule to a coenzyme, NAD^+. NAD^+ actually receives one hydrogen atom and one electron, and one proton is released into the medium. NAD^+ is reduced to NADH, which is a more energy-rich molecule.

Q How do organisms use oxidation-reduction reactions?

series of different electron carriers to molecules of oxygen (O_2) or other oxidized inorganic and organic molecules. This process occurs in the plasma membrane of prokaryotes and in the inner mitochondrial membrane of eukaryotes. The sequence of electron carriers used in oxidative phosphorylation is called an **electron transport chain (system)** (see Figure 5.14). The transfer of electrons from one electron carrier to the next releases energy, some of which is used to generate ATP from ADP through a process called *chemiosmosis,* to be described on page 128.

Photophosphorylation

The third mechanism of phosphorylation, **photophosphorylation,** occurs only in photosynthetic cells, which contain light-trapping pigments such as chlorophylls. In photosynthesis, organic molecules, especially sugars, are synthesized with the energy of light from the energy-poor building blocks carbon dioxide and water. Photophosphorylation starts this process by converting light energy to the chemical energy of ATP and NADPH, which, in turn, are used to synthesize organic molecules. As in oxidative phosphorylation, an electron transport chain is involved.

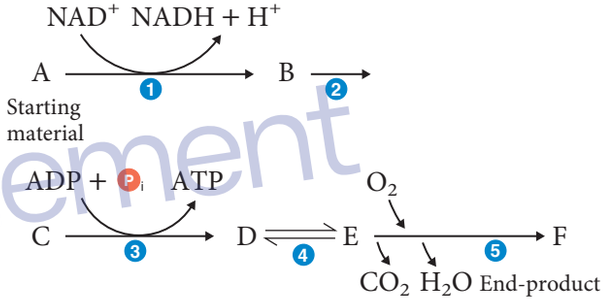
CHECK YOUR UNDERSTANDING

Outline the three ways that ATP is generated. **5-9**

Metabolic Pathways of Energy Production

Organisms release and store energy from organic molecules by a series of controlled reactions rather than in a single burst. If the energy were released all at once, as a large amount of heat, it could not be readily used to drive chemical reactions and would, in fact, damage the cell. To extract energy from organic compounds and store it in chemical form, organisms pass electrons from one compound to another through a series of oxidation-reduction reactions.

As noted earlier, a sequence of enzymatically catalyzed chemical reactions occurring in a cell is called a metabolic pathway. Below is a hypothetical metabolic pathway that converts starting material A to end-product F in a series of five steps:



The first step is the conversion of molecule A to molecule B. The curved arrow indicates that the reduction of coenzyme NAD^+ to NADH is coupled to that reaction; the electrons and protons come from molecule A. Similarly, the two arrows in 3 show a coupling of two reactions. As C is converted to D, ADP is converted to ATP; the energy needed comes from C as it transforms into D. The reaction converting D to E is readily reversible, as indicated by the double arrow. In the fifth step, the curved arrow leading from O_2 indicates that O_2 is a reactant. The curved arrows leading to CO_2 and H_2O indicate that these substances are secondary products produced in the reaction, in addition to F, the end-product that (presumably) interests us the most. Secondary products such as CO_2 and H_2O shown here are sometimes called *by-products* or *waste products.* Keep in mind that almost every reaction in a metabolic pathway is catalyzed by a specific enzyme; sometimes the name of the enzyme is printed near the arrow.

CHECK YOUR UNDERSTANDING

What is the purpose of metabolic pathways? **5-10**

Carbohydrate Catabolism

LEARNING OBJECTIVES

5-11 Describe the chemical reactions of glycolysis.

5-12 Identify the functions of the pentose phosphate and Entner-Doudoroff pathways.

5-13 Explain the products of the Krebs cycle.

5-14 Describe the chemiosmotic model for ATP generation.

5-15 Compare and contrast aerobic and anaerobic respiration.

5-16 Describe the chemical reactions of, and list some products of, fermentation.

Most microorganisms oxidize carbohydrates as their primary source of cellular energy. **Carbohydrate catabolism,** the breakdown of carbohydrate molecules to produce energy, is therefore of great importance in cell metabolism. Glucose is the most common carbohydrate energy source used by cells. Microorganisms can also catabolize various lipids and proteins for energy production (page 133).

To produce energy from glucose, microorganisms use two general processes: *cellular respiration* and *fermentation*. (In discussing cellular respiration, we frequently refer to the process simply as respiration, but it should not be confused with breathing.) Both cellular respiration and fermentation usually start with the same first step, glycolysis, but follow different subsequent pathways (Figure 5.11). Before examining the details of glycolysis, respiration, and fermentation, we will first look at a general overview of the processes.

As shown in Figure 5.11, the respiration of glucose typically occurs in three principal stages: glycolysis, the Krebs cycle, and the electron transport chain (system).

1. Glycolysis is the oxidation of glucose to pyruvic acid with the production of some ATP and energy-containing NADH.
2. The Krebs cycle is the oxidation of acetyl CoA (a derivative of pyruvic acid) to carbon dioxide, with the production of some ATP, energy-containing NADH, and another reduced electron carrier, $FADH_2$ (the reduced form of flavin adenine dinucleotide).
3. In the electron transport chain (system), NADH and $FADH_2$ are oxidized, contributing the electrons they have carried from the substrates to a "cascade" of oxidation-reduction reactions involving a series of additional electron carriers. Energy from these reactions is used to generate a considerable amount of ATP. In respiration, most of the ATP is generated in the third step.

Because respiration involves a long series of oxidation-reduction reactions, the entire process can be thought of as involving a flow of electrons from the energy-rich glucose molecule to the relatively energy-poor CO_2 and H_2O molecules. The coupling of ATP production to this flow is somewhat analogous to producing electrical power by using energy from a flowing stream. Carrying the analogy further, you could imagine a stream flowing down a gentle slope during glycolysis and the Krebs cycle, supplying energy to turn two old-fashioned waterwheels. Then the stream rushes down a steep slope in the electron transport chain, supplying energy for a large modern power plant. In a similar way, glycolysis and the Krebs cycle generate a small amount of ATP and also supply the electrons that generate a great deal of ATP at the electron transport chain stage.

Typically, the initial stage of fermentation is also glycolysis (Figure 5.11). However, once glycolysis has taken place, the pyruvic acid is converted into one or more different products, depending on the type of cell. These products might include alcohol (ethanol) and lactic acid. Unlike respiration, there is no Krebs cycle or electron transport chain in fermentation. Accordingly, the ATP yield, which comes only from glycolysis, is much lower.

Glycolysis

Glycolysis, the oxidation of glucose to pyruvic acid, is usually the first stage in carbohydrate catabolism. Most microorganisms use this pathway; in fact, it occurs in most living cells.

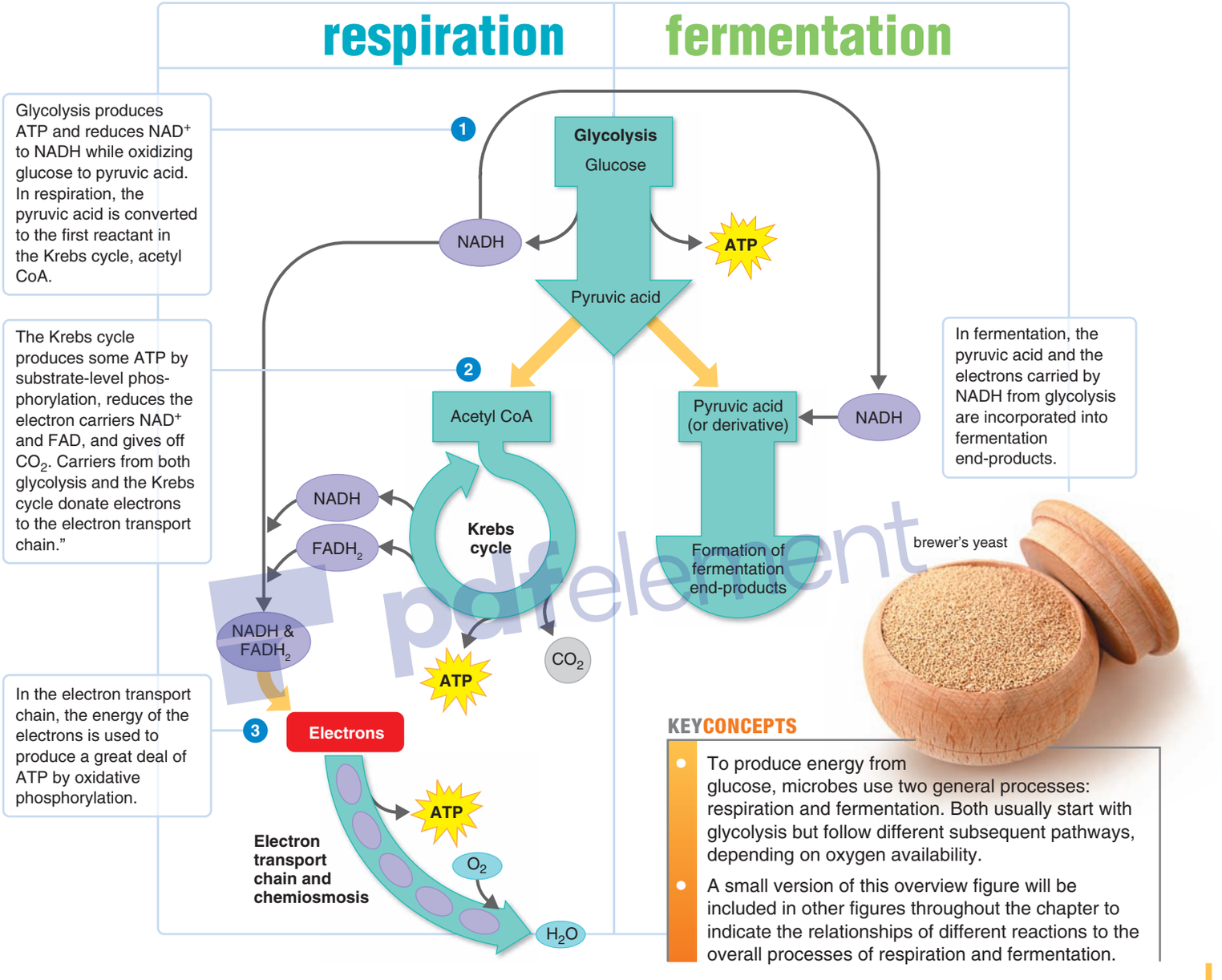
Glycolysis is also called the *Embden-Meyerhof pathway*. The word *glycolysis* means splitting of sugar, and this is exactly what happens. The enzymes of glycolysis catalyze the splitting of glucose, a six-carbon sugar, into two three-carbon sugars. These sugars are then oxidized, releasing energy, and their atoms are rearranged to form two molecules of pyruvic acid. During glycolysis NAD^+ is reduced to NADH, and there is a net production of two ATP molecules by substrate-level phosphorylation. Glycolysis does not require oxygen; it can occur whether oxygen is present or not. This pathway is a series of ten chemical reactions, each catalyzed by a different enzyme. The steps are outlined in Figure 5.12; see also Figure A.2 in Appendix A for a more detailed representation of glycolysis.

To summarize the process, glycolysis consists of two basic stages, a preparatory stage and an energy-conserving stage:

1. First, in the preparatory stage (steps 1–4 in Figure 5.12), two molecules of ATP are used as a six-carbon glucose molecule is phosphorylated, restructured, and split into two three-carbon compounds: glyceraldehyde 3-phosphate (GP) and dihydroxyacetone phosphate (DHAP). 5 DHAP is readily converted to GP. (The reverse reaction may also occur.) The conversion of DHAP into GP means that from this point on in glycolysis, two molecules of GP are fed into the remaining chemical reactions.
2. In the energy-conserving stage (steps 6–10), the two three-carbon molecules are oxidized in several steps to two molecules of pyruvic acid. In these reactions, two molecules of NAD^+ are reduced to NADH, and four molecules of ATP are formed by substrate-level phosphorylation.

FOUNDATION FIGURE **5.11**

An Overview of Respiration and Fermentation



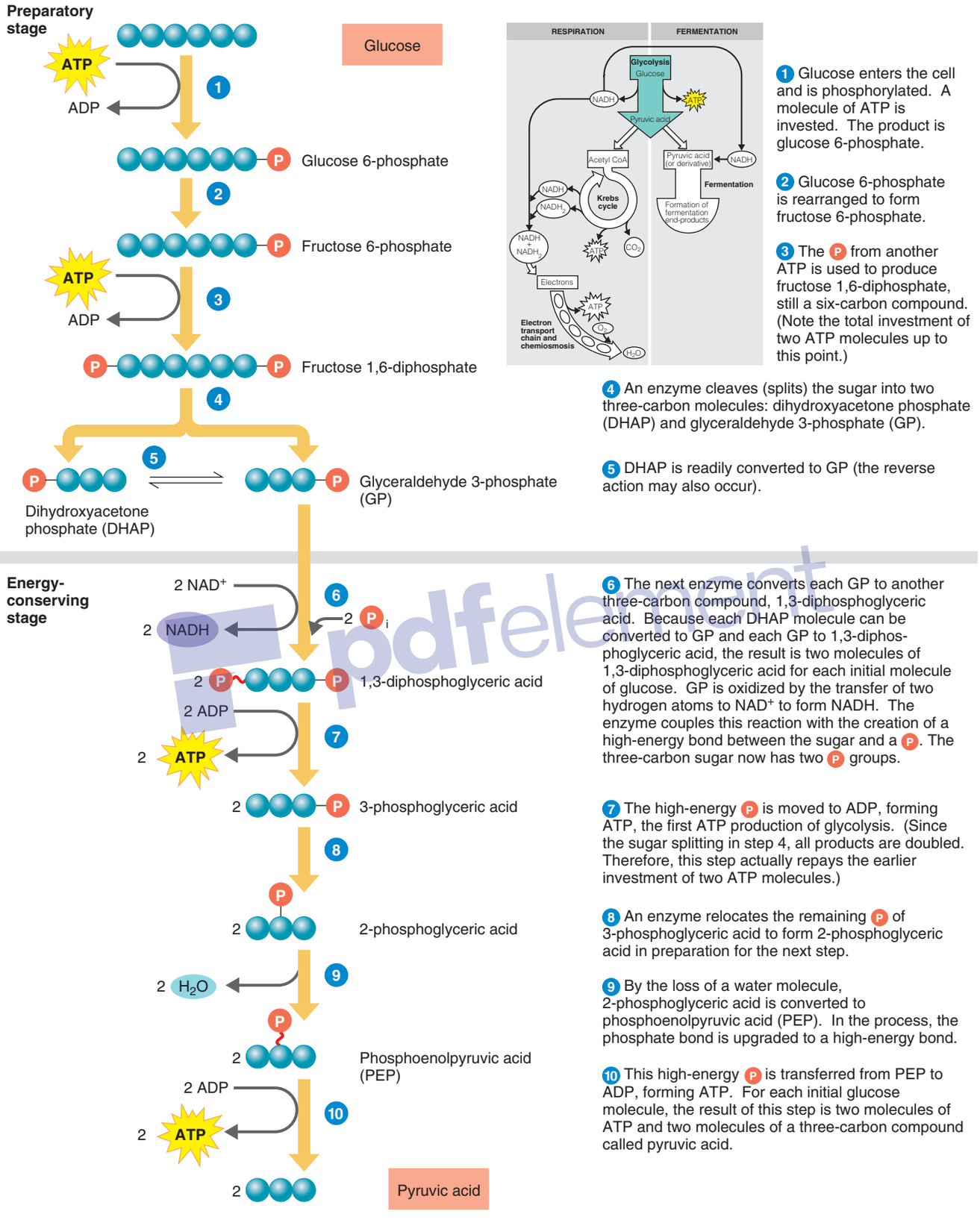
Because two molecules of ATP were needed to get glycolysis started and four molecules of ATP are generated by the process, *there is a net gain of two molecules of ATP for each molecule of glucose that is oxidized.* **Animations** Glycolysis: Overview, Steps

Alternatives to Glycolysis

Many bacteria have another pathway in addition to glycolysis for the oxidation of glucose. The most common alternative is the pentose phosphate pathway; another alternative is the Entner-Doudoroff pathway.

The Pentose Phosphate Pathway

The **pentose phosphate pathway** (or *hexose monophosphate shunt*) operates simultaneously with glycolysis and provides a means for the breakdown of five-carbon sugars (pentoses) as well as glucose (see Figure A.3 in Appendix A for a more



1 Glucose enters the cell and is phosphorylated. A molecule of ATP is invested. The product is glucose 6-phosphate.

2 Glucose 6-phosphate is rearranged to form fructose 6-phosphate.

3 The P from another ATP is used to produce fructose 1,6-diphosphate, still a six-carbon compound. (Note the total investment of two ATP molecules up to this point.)

4 An enzyme cleaves (splits) the sugar into two three-carbon molecules: dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GP).

5 DHAP is readily converted to GP (the reverse action may also occur).

6 The next enzyme converts each GP to another three-carbon compound, 1,3-diphosphoglyceric acid. Because each DHAP molecule can be converted to GP and each GP to 1,3-diphosphoglyceric acid, the result is two molecules of 1,3-diphosphoglyceric acid for each initial molecule of glucose. GP is oxidized by the transfer of two hydrogen atoms to NAD^+ to form NADH. The enzyme couples this reaction with the creation of a high-energy bond between the sugar and a P. The three-carbon sugar now has two P groups.

7 The high-energy P is moved to ADP, forming ATP, the first ATP production of glycolysis. (Since the sugar splitting in step 4, all products are doubled. Therefore, this step actually repays the earlier investment of two ATP molecules.)

8 An enzyme relocates the remaining P of 3-phosphoglyceric acid to form 2-phosphoglyceric acid in preparation for the next step.

9 By the loss of a water molecule, 2-phosphoglyceric acid is converted to phosphoenolpyruvic acid (PEP). In the process, the phosphate bond is upgraded to a high-energy bond.

10 This high-energy P is transferred from PEP to ADP, forming ATP. For each initial glucose molecule, the result of this step is two molecules of ATP and two molecules of a three-carbon compound called pyruvic acid.

Figure 5.12 An outline of the reactions of glycolysis (Embden-Meyerhof pathway). The inset indicates the relationship of glycolysis to the overall processes of respiration and fermentation. A more detailed version of glycolysis is presented in Figure A.2 in Appendix A.

Q What is glycolysis?

detailed representation of the pentose phosphate pathway). A key feature of this pathway is that it produces important intermediate pentoses used in the synthesis of (1) nucleic acids, (2) glucose from carbon dioxide in photosynthesis, and (3) certain amino acids. The pathway is an important producer of the reduced coenzyme NADPH from $NADP^+$. The pentose phosphate pathway yields a net gain of only one molecule of ATP for each molecule of glucose oxidized. Bacteria that use the pentose phosphate pathway include *Bacillus subtilis* (sub′ til-us), *E. coli, Leuconostoc mesenteroides* (lü-kō-nos′ tok mes-en-ter-oi′ dēz), and *Enterococcus faecalis* (fē-kāl′ is).

The Entner-Doudoroff Pathway

From each molecule of glucose, the **Entner-Doudoroff pathway** produces two molecules of NADPH and one molecule of ATP for use in cellular biosynthetic reactions (see Figure A.4 in Appendix A for a more detailed representation). Bacteria that have the enzymes for the Entner-Doudoroff pathway can metabolize glucose without either glycolysis or the pentose phosphate pathway. The Entner-Doudoroff pathway is found in some gram-negative bacteria, including *Rhizobium, Pseudomonas* (sū-dō-mō′ nas), and *Agrobacterium* (ag-rō-bak-ti′ rē-um); it is generally not found among gram-positive bacteria. Tests for the ability to oxidize glucose by this pathway are sometimes used to identify *Pseudomonas* in the clinical laboratory.

CHECK YOUR UNDERSTANDING

- What happens during the preparatory and energy-conserving stages of glycolysis? **5-11**
- What is the value of the pentose phosphate and Entner-Doudoroff pathways if they produce only one ATP molecule? **5-12**

Cellular Respiration

After glucose has been broken down to pyruvic acid, the pyruvic acid can be channeled into the next step of either fermentation (page 130) or cellular respiration (see Figure 5.11). **Cellular respiration,** or simply **respiration,** is defined as an ATP-generating process in which molecules are oxidized and the final electron acceptor is (almost always) an inorganic molecule. An essential feature of respiration is the operation of an electron transport chain.

There are two types of respiration, depending on whether an organism is an **aerobe,** which uses oxygen, or an **anaerobe,** which does not use oxygen and may even be killed by it. In **aerobic respiration,** the final electron acceptor is O_2; in **anaerobic respiration,** the final electron acceptor is an inorganic molecule other than O_2 or, rarely, an organic molecule. First we will describe respiration as it typically occurs in an aerobic cell.

Aerobic Respiration

The Krebs Cycle The **Krebs cycle,** also called the *tricarboxylic acid (TCA) cycle* or *citric acid cycle,* is a series of biochemical reactions in which the large amount of potential chemical energy stored in acetyl CoA is released step by step (see Figure 5.11). In this cycle, a series of oxidations and reductions transfer that potential energy, in the form of electrons, to electron carrier coenzymes, chiefly NAD^+. The pyruvic acid derivatives are oxidized; the coenzymes are reduced.

Pyruvic acid, the product of glycolysis, cannot enter the Krebs cycle directly. In a preparatory step, it must lose one molecule of CO_2 and become a two-carbon compound (**Figure 5.13**, at top). This process is called **decarboxylation.** The two-carbon compound, called an *acetyl group,* attaches to coenzyme A through a high-energy bond; the resulting complex is known as *acetyl coenzyme A (acetyl CoA).* During this reaction, pyruvic acid is also oxidized, and NAD^+ is reduced to NADH.

Remember that the oxidation of one glucose molecule produces two molecules of pyruvic acid, so for each molecule of glucose, two molecules of CO_2 are released in this preparatory step, two molecules of NADH are produced, and two molecules of acetyl CoA are formed. Once the pyruvic acid has undergone decarboxylation and its derivative (the acetyl group) has attached to CoA, the resulting acetyl CoA is ready to enter the Krebs cycle.

As acetyl CoA enters the Krebs cycle, CoA detaches from the acetyl group. The two-carbon acetyl group combines with a four-carbon compound called oxaloacetic acid to form the six-carbon citric acid. This synthesis reaction requires energy, which is provided by the cleavage of the high-energy bond between the acetyl group and CoA. The formation of citric acid is thus the first step in the Krebs cycle. The major chemical reactions of this cycle are outlined in Figure 5.13; a more detailed representation of the Krebs cycle is provided in Figure A.5 in Appendix A. Keep in mind that each reaction is catalyzed by a specific enzyme.

The chemical reactions of the Krebs cycle fall into several general categories; one of these is decarboxylation. For example, in step 3 isocitric acid, a six-carbon compound, is decarboxylated to the five-carbon compound called α-ketoglutaric acid. Another decarboxylation takes place in step 4. Because one decarboxylation has taken place in the preparatory step and two in the Krebs cycle, all three carbon atoms in pyruvic acid are eventually released as CO_2 by the Krebs cycle. This represents the conversion to CO_2 of all six carbon atoms contained in the original glucose molecule.

Another general category of Krebs cycle chemical reactions is oxidation-reduction. For example, in step 3, two hydrogen atoms are lost during the conversion of the six-carbon isocitric acid to a five-carbon compound. In other words, the six-carbon compound is oxidized. Hydrogen atoms are also released in the Krebs cycle in steps 4, 6, and 8 and are picked up by the coenzymes NAD^+ and FAD. Because NAD^+ picks up two electrons but only one additional proton, its reduced form is represented as NADH; however, FAD picks up two complete hydrogen atoms and is reduced to $FADH_2$.

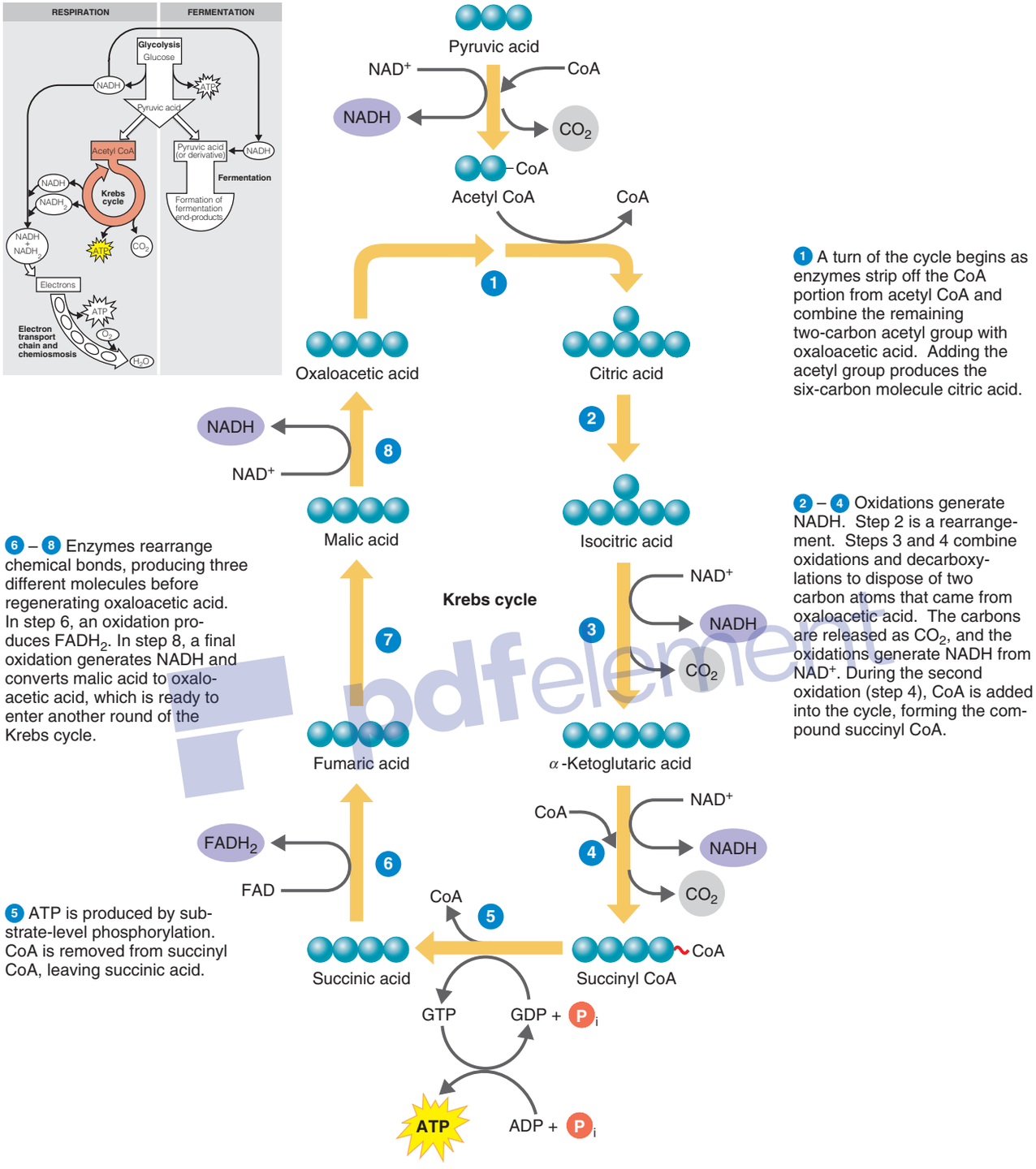


Figure 5.13 The Krebs cycle. The inset indicates the relationship of the Krebs cycle to the overall process of respiration. A more detailed version of the Krebs cycle is presented in Figure A.5 in Appendix A.

Q What are the products of the Krebs cycle?

If we look at the Krebs cycle as a whole, we see that for every two molecules of acetyl CoA that enter the cycle, four molecules of CO_2 are liberated by decarboxylation, six molecules of NADH and two molecules of $FADH_2$ are produced by oxidation-reduction reactions, and two molecules of ATP are generated by substrate-level phosphorylation. A molecule of guanosine triphosphate (GTP), formed from guanosine diphosphate (GDP + P_i), is similar to ATP and serves as an intermediary at this point in the cycle. Many of the intermediates in the Krebs cycle also play a role in other pathways, especially in amino acid biosynthesis (page 144).

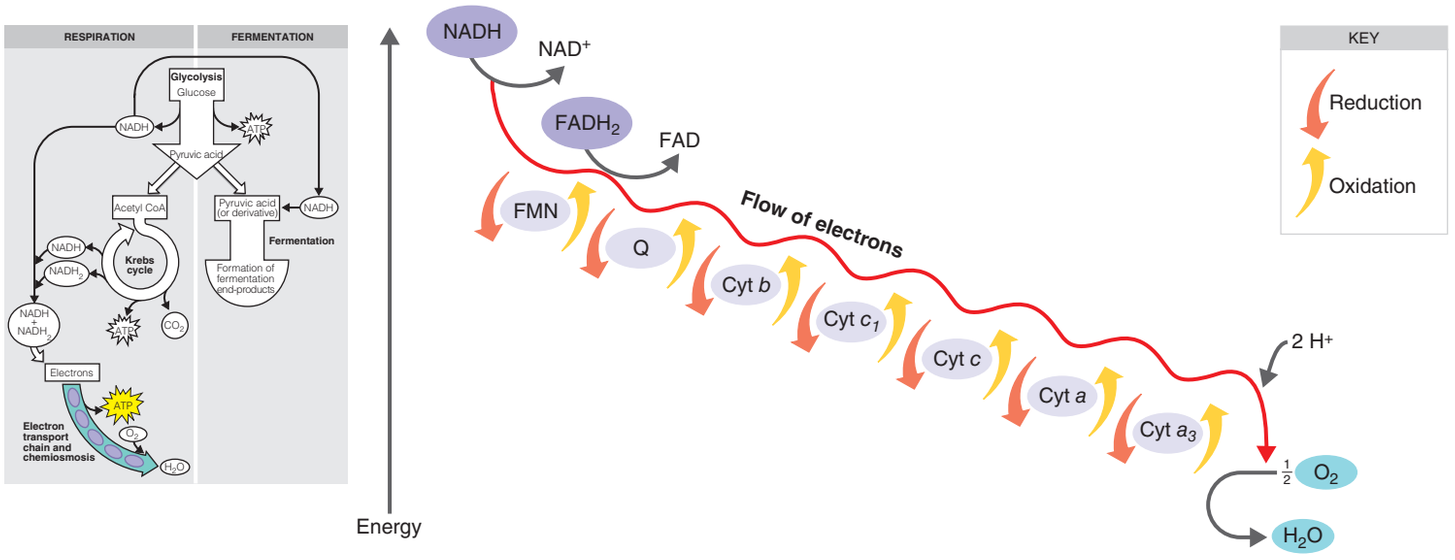


Figure 5.14 **An electron transport chain (system).** The inset indicates the relationship of the electron transport chain to the overall process of respiration. In the mitochondrial electron transport chain shown, the electrons pass along the chain in a gradual and stepwise fashion, so energy is released in manageable quantities. To learn where ATP is formed, see Figure 5.16.

Q What are the functions of the electron transport chain?

The CO_2 produced in the Krebs cycle is ultimately liberated into the atmosphere as a gaseous by-product of aerobic respiration. (Humans produce CO_2 from the Krebs cycle in most cells of the body and discharge it through the lungs during exhalation.) The reduced coenzymes NADH and $FADH_2$ are the most important products of the Krebs cycle because they contain most of the energy originally stored in glucose. During the next phase of respiration, a series of reductions indirectly transfers the energy stored in those coenzymes to ATP. These reactions are collectively called the electron transport chain. MM **Animations** Krebs Cycle: Overview, Steps

The Electron Transport Chain (System) An **electron transport chain (system)** consists of a sequence of carrier molecules that are capable of oxidation and reduction. As electrons are passed through the chain, there occurs a stepwise release of energy, which is used to drive the chemiosmotic generation of ATP, to be described shortly. The final oxidation is irreversible. In eukaryotic cells, the electron transport chain is contained in the inner membrane of mitochondria; in prokaryotic cells, it is found in the plasma membrane.

There are three classes of carrier molecules in electron transport chains. The first are **flavoproteins.** These proteins contain flavin, a coenzyme derived from riboflavin (vitamin B_2), and are capable of performing alternating oxidations and reductions. One important flavin coenzyme is flavin mononucleotide (FMN). The second class of carrier molecules are **cytochromes,** proteins with an iron-containing group (heme) capable of existing alternately as a reduced form (Fe^{2+}) and an oxidized form (Fe^{3+}). The cytochromes involved in electron transport chains include cytochrome *b* (cyt *b*), cytochrome c_1 (cyt c_1), cytochrome *c* (cyt *c*), cytochrome *a* (cyt *a*), and cytochrome a_3 (cyt a_3). The third class is known as **ubiquinones,** or **coenzyme Q,** symbolized Q; these are small nonprotein carriers.

The electron transport chains of bacteria are somewhat diverse, in that the particular carriers used by a bacterium and the order in which they function may differ from those of other bacteria and from those of eukaryotic mitochondrial systems. Even a single bacterium may have several types of electron transport chains. However, keep in mind that all electron transport chains achieve the same basic goal: to release energy as electrons are transferred from higher-energy compounds to lower-energy compounds. Much is known about the electron transport chain in the mitochondria of eukaryotic cells, so this is the chain we will describe.

The first step in the mitochondrial electron transport chain involves the transfer of high-energy electrons from NADH to FMN, the first carrier in the chain (**Figure 5.14**). This transfer actually involves the passage of a hydrogen atom with two electrons to FMN, which then picks up an additional H^+ from the surrounding aqueous medium. As a result of the first transfer, NADH is oxidized to NAD^+, and FMN is reduced to $FMNH_2$. In the second step in the electron transport chain, $FMNH_2$ passes $2H^+$ to the other side of the mitochondrial membrane (see Figure 5.16) and passes two electrons to Q. As a result, $FMNH_2$ is oxidized to FMN. Q also picks up an additional $2H^+$ from the surrounding aqueous medium and releases it on the other side of the membrane.

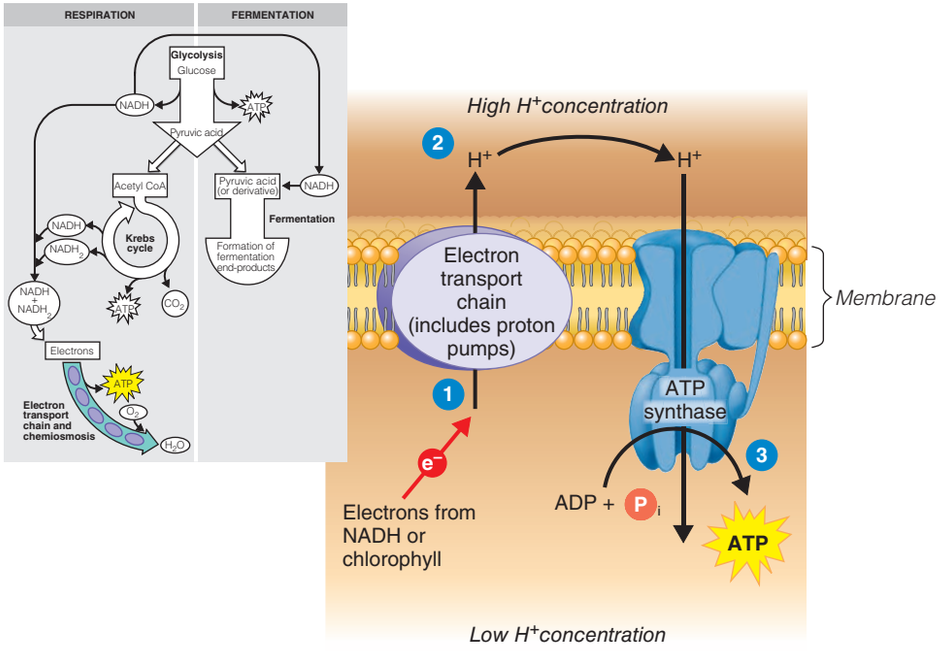


Figure 5.15 Chemiosmosis. An overview of the mechanism of chemiosmosis. The membrane shown could be a prokaryotic plasma membrane, a eukaryotic mitochondrial membrane, or a photosynthetic thylakoid. The numbered steps are described in the text.

Q What is the proton motive force?

The next part of the electron transport chain involves the cytochromes. Electrons are passed successively from Q to cyt *b*, cyt c_1, cyt *c*, cyt *a*, and cyt a_3. Each cytochrome in the chain is reduced as it picks up electrons and is oxidized as it gives up electrons. The last cytochrome, cyt a_3, passes its electrons to molecular oxygen (O_2), which becomes negatively charged and then picks up protons from the surrounding medium to form H_2O.

Notice that Figure 5.14 shows $FADH_2$, which is derived from the Krebs cycle, as another source of electrons. However, $FADH_2$ adds its electrons to the electron transport chain at a lower level than NADH. Because of this, the electron transport chain produces about one-third less energy for ATP generation when $FADH_2$ donates electrons than when NADH is involved.

An important feature of the electron transport chain is the presence of some carriers, such as FMN and Q, that accept and release protons as well as electrons, and other carriers, such as cytochromes, that transfer electrons only. Electron flow down the chain is accompanied at several points by the active transport (pumping) of protons from the matrix side of the inner mitochondrial membrane to the opposite side of the membrane. The result is a buildup of protons on one side of the membrane. Just as water behind a dam stores energy that can be used to generate electricity, this buildup of protons provides energy for the generation of ATP by the chemiosmotic mechanism.

The Chemiosmotic Mechanism of ATP Generation The mechanism of ATP synthesis using the electron transport chain is called **chemiosmosis.** To understand chemiosmosis, we need to recall several concepts that were introduced in Chapter 4 as part of the section on the movement of materials across membranes (page 91). Recall that substances diffuse passively across membranes from areas of high concentration to areas of low concentration; this diffusion yields energy. Recall also that the movement of substances *against* such a concentration gradient *requires* energy and that, in such an active transport of molecules or ions across biological membranes, the required energy is usually provided by ATP. In chemiosmosis, the energy released when a substance moves along a gradient is used to *synthesize* ATP. The "substance" in this case refers to protons. In respiration, chemiosmosis is responsible for most of the ATP that is generated. The steps of chemiosmosis are as follows (**Figure 5.15** and **Figure 5.16**):

1. As energetic electrons from NADH (or chlorophyll) pass down the electron transport chain, some of the carriers in the chain pump—actively transport—protons across the membrane. Such carrier molecules are called *proton pumps.*
2. The phospholipid membrane is normally impermeable to protons, so this one-directional pumping establishes a proton gradient (a difference in the concentrations of protons on the two sides of the membrane). In addition to a concentration gradient, there is an electrical charge gradient. The excess H^+ on one side of the membrane makes that side positively charged compared with the other side. The resulting electrochemical gradient has potential energy, called the *proton motive force.*
3. The protons on the side of the membrane with the higher proton concentration can diffuse across the membrane only through special protein channels that contain an enzyme called *ATP synthase.* When this flow occurs, energy is released and is used by the enzyme to synthesize ATP from ADP and P_i.

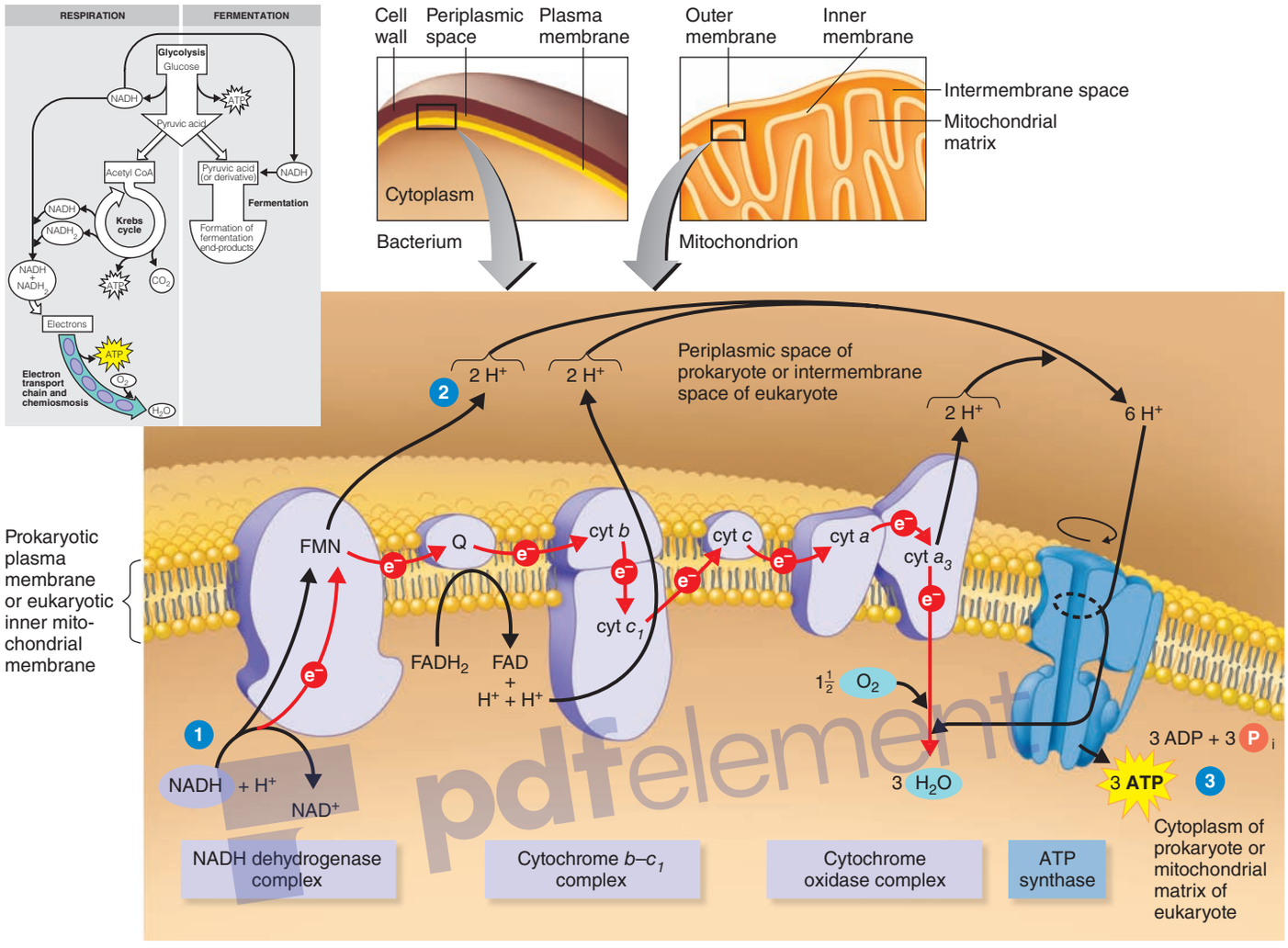


Figure 5.16 Electron transport and the chemiosmotic generation of ATP. Electron carriers are organized into three complexes, and protons (H^+) are pumped across the membrane at three points. In a prokaryotic cell, protons are pumped across the plasma membrane from the cytoplasmic side. In a eukaryotic cell, they are pumped from the matrix side of the mitochondrial membrane to the opposite side. The flow of electrons is indicated with red arrows.

Q Where does chemiosmosis occur in eukaryotes? In prokaryotes?

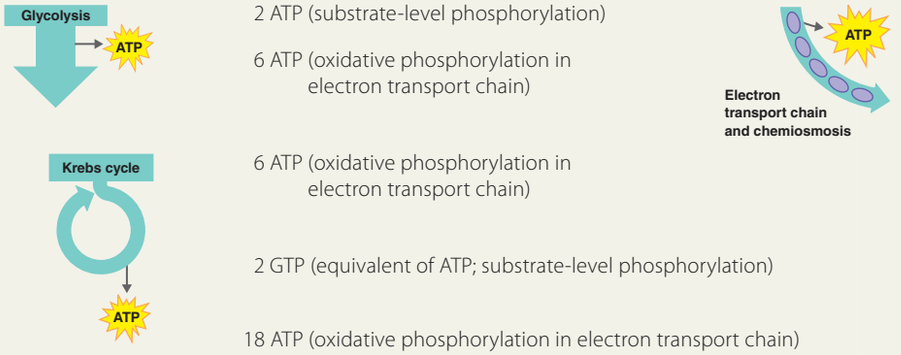
Figure 5.16 shows in detail how the electron transport chain operates in eukaryotes to drive the chemiosmotic mechanism. 1 Energetic electrons from NADH pass down the electron transport chains. Within the inner mitochondrial membrane, the carriers of the electron transport chain are organized into three complexes, with Q transporting electrons between the first and second complexes, and cyt *c* transporting them between the second and third complexes. 2 Three components of the system pump protons: the first and third complexes and Q. At the end of the chain, electrons join with protons and oxygen (O_2) in the matrix fluid to form water (H_2O). Thus, O_2 is the final electron acceptor.

Both prokaryotic and eukaryotic cells use the chemiosmotic mechanism to generate energy for ATP production. However, in eukaryotic cells, 3 the inner mitochondrial membrane contains the electron transport carriers and ATP synthase, whereas in most prokaryotic cells, the plasma membrane does so. An electron transport chain also operates in photophosphorylation and is located in the thylakoid membrane of cyanobacteria and eukaryotic chloroplasts.

A Summary of Aerobic Respiration The electron transport chain regenerates NAD^+ and FAD, which can be used again in glycolysis and the Krebs cycle. The various electron transfers in the electron transport chain generate about 34 molecules of ATP from each molecule of glucose oxidized: approximately three from each of the ten molecules of NADH (a total of 30), and approximately two from each of the two molecules of $FADH_2$ (a total of four). To arrive at the total number of ATP molecules generated for each molecule of glucose, the 34 from chemiosmosis

TABLE 5.3 ATP Yield during Prokaryotic Aerobic Respiration of One Glucose Molecule

Source	ATP Yield (Method)
Glycolysis	
1. Oxidation of glucose to pyruvic acid	2 ATP (substrate-level phosphorylation)
2. Production of 2 NADH	6 ATP (oxidative phosphorylation in electron transport chain)
Preparatory Step	
1. Formation of acetyl CoA produces 2 NADH	6 ATP (oxidative phosphorylation in electron transport chain)
Krebs Cycle	
1. Oxidation of succinyl CoA to succinic acid	2 GTP (equivalent of ATP; substrate-level phosphorylation)
2. Production of 6 NADH	18 ATP (oxidative phosphorylation in electron transport chain)
3. Production of 2 FADH	4 ATP (oxidative phosphorylation in electron transport chain)
	Total: 38 ATP



are added to those generated by oxidation in glycolysis and the Krebs cycle. In aerobic respiration among prokaryotes, a total of 38 molecules of ATP can be generated from one molecule of glucose. Note that four of those ATPs come from substrate-level phosphorylation in glycolysis and the Krebs cycle. **Table 5.3** provides a detailed accounting of the ATP yield during prokaryotic aerobic respiration.

Aerobic respiration among eukaryotes produces a total of only 36 molecules of ATP. There are fewer ATPs than in prokaryotes because some energy is lost when electrons are shuttled across the mitochondrial membranes that separate glycolysis (in the cytoplasm) from the electron transport chain. No such separation exists in prokaryotes. We can now summarize the overall reaction for aerobic respiration in prokaryotes as follows:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Oxygen}}{6\,O_2} + 38\,ADP + 38\,P_i \longrightarrow \underset{\text{Carbon dioxide}}{6\,CO_2} + \underset{\text{Water}}{6\,H_2O} + 38\,ATP$$

A summary of the various stages of aerobic respiration in prokaryotes is presented in **Figure 5.17**.

Anaerobic Respiration

In anaerobic respiration, the final electron acceptor is an inorganic substance other than oxygen (O_2). Some bacteria, such as *Pseudomonas* and *Bacillus,* can use a nitrate ion (NO_3^-) as a final electron acceptor; the nitrate ion is reduced to a nitrite ion (NO_2^-), nitrous oxide (N_2O), or nitrogen gas (N_2). Other bacteria, such as *Desulfovibrio* (dē-sul-fō-vib′ rē-ō), use sulfate (SO_4^{2-}) as the final electron acceptor to form hydrogen sulfide (H_2S). Still other bacteria use carbonate (CO_3^{2-}) to form methane (CH_4). Anaerobic respiration by bacteria using nitrate and sulfate as final acceptors is essential for the nitrogen and sulfur cycles that occur in nature. The amount of ATP generated in anaerobic respiration varies with the organism and the pathway. Because only part of the Krebs cycle operates under anaerobic conditions, and because not all the carriers in the electron transport chain participate in anaerobic respiration, the ATP yield is never as high as in aerobic respiration. Accordingly, anaerobes tend to grow more slowly than aerobes. **Animations** Electron Transport Chain: Overview, The Process, Factors Affecting ATP Yield

CHECK YOUR UNDERSTANDING

- What are the principal products of the Krebs cycle? **5-13**
- How do carrier molecules function in the electron transport chain? **5-14**
- Compare the energy yield (ATP) of aerobic and anaerobic respiration. **5-15**

Fermentation

After glucose has been broken down into pyruvic acid, the pyruvic acid can be completely broken down in respiration, as previously described, or it can be converted to an organic product in fermentation, whereupon NAD^+ and $NADP^+$ are regenerated and can enter another round of glycolysis (see Figure 5.11). **Fermentation** can be defined in several ways (see the box, page 134), but we define it here as a process that

1. releases energy from sugars or other organic molecules, such as amino acids, organic acids, purines, and pyrimidines;
2. does not require oxygen (but sometimes can occur in its presence);
3. does not require the use of the Krebs cycle or an electron transport chain;

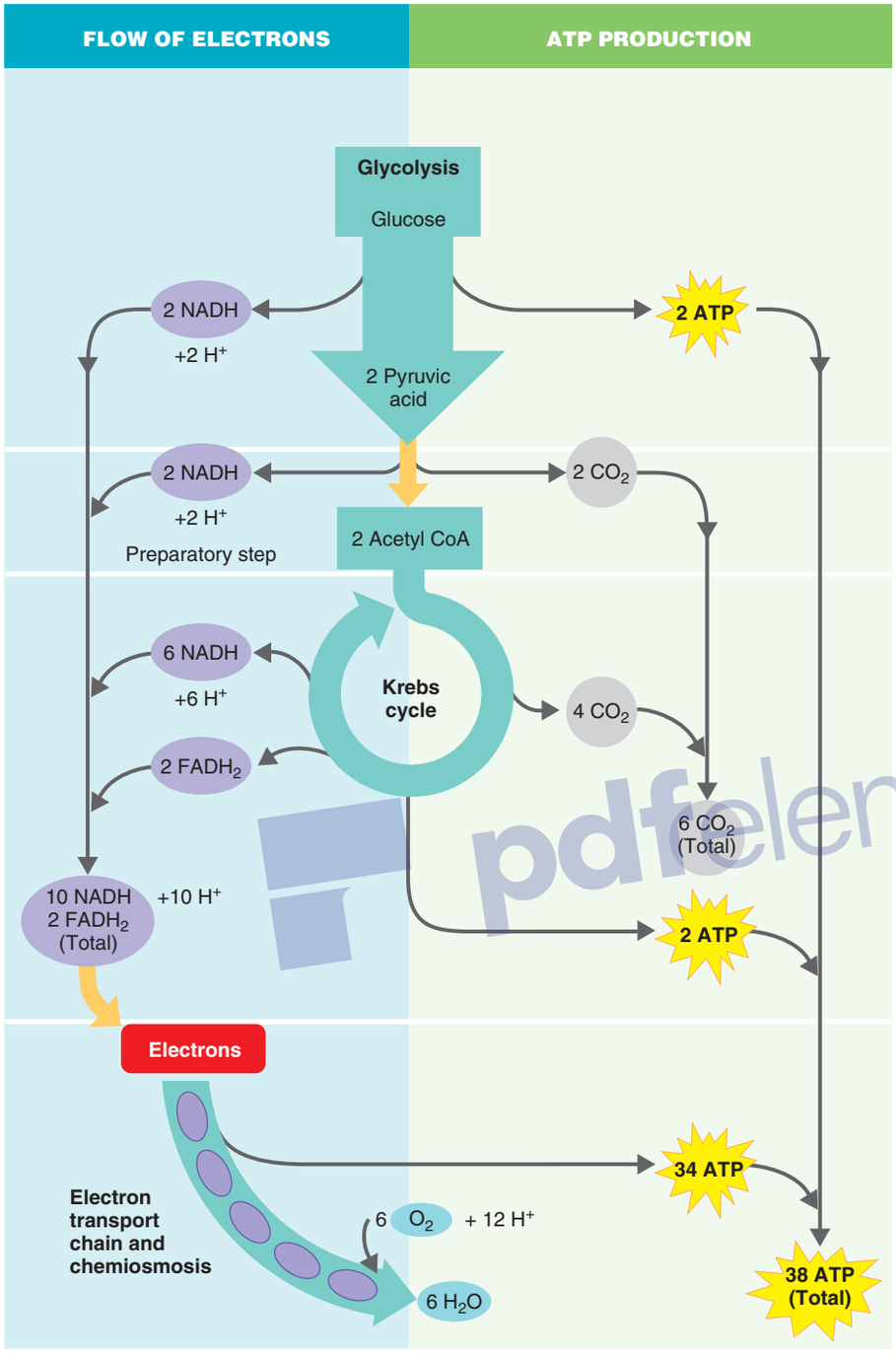


Figure 5.17 A summary of aerobic respiration in prokaryotes. Glucose is broken down completely to carbon dioxide and water, and ATP is generated. This process has three major phases: glycolysis, the Krebs cycle, and the electron transport chain. The preparatory step is between glycolysis and the Krebs cycle. The key event in aerobic respiration is that electrons are picked up from intermediates of glycolysis and the Krebs cycle by NAD^+ or FAD and are carried by NADH or $FADH_2$ to the electron transport chain. NADH is also produced during the conversion of pyruvic acid to acetyl CoA. Most of the ATP generated by aerobic respiration is made by the chemiosmotic mechanism during the electron transport chain phase; this is called oxidative phosphorylation.

Q How do aerobic and anaerobic respiration differ?

4. uses an organic molecule as the final electron acceptor;
5. produces only small amounts of ATP (only one or two ATP molecules for each molecule of starting material) because much of the original energy in glucose remains in the chemical bonds of the organic end-products, such as lactic acid or ethanol.

During fermentation, electrons are transferred (along with protons) from reduced coenzymes (NADH, NADPH) to pyruvic acid or its derivatives (Figure 5.18a). Those final electron acceptors are reduced to the end-products shown in Figure 5.18b. An essential function of the second stage of fermentation is to ensure a steady supply of NAD^+ and $NADP^+$ so that glycolysis can continue. In fermentation, ATP is generated only during glycolysis.

Microorganisms can ferment various substrates; the end-products depend on the particular microorganism, the substrate, and the enzymes that are present and active. Chemical analyses of these end-products are useful in identifying microorganisms.

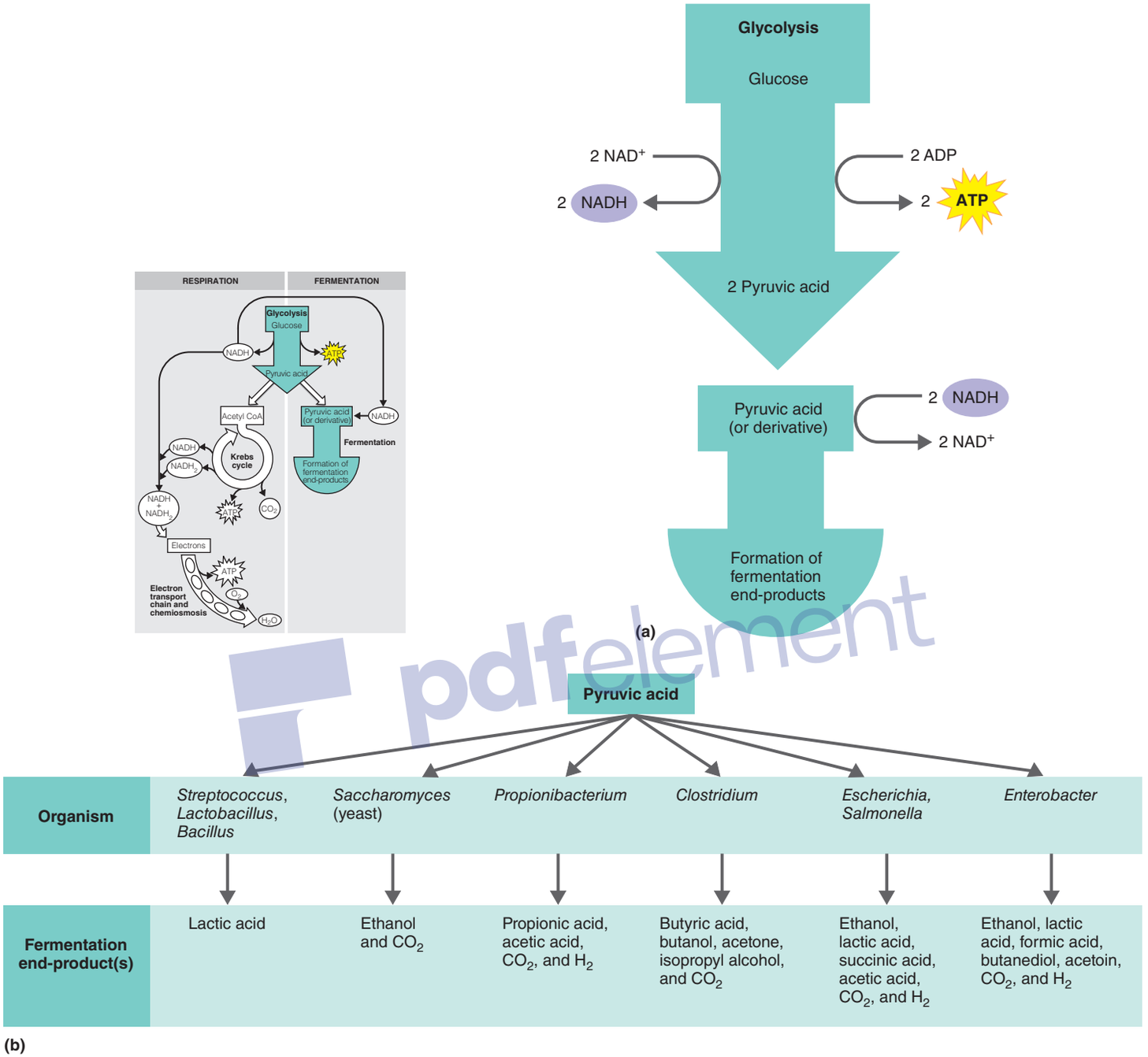


Figure 5.18 Fermentation. The inset indicates the relationship of fermentation to the overall energy-producing processes. (**a**) An overview of fermentation. The first step is glycolysis, the conversion of glucose to pyruvic acid. In the second step, the reduced coenzymes from glycolysis or its alternatives (NADH, NADPH) donate their electrons and hydrogen ions to pyruvic acid or a derivative to form a fermentation end-product. (**b**) End-products of various microbial fermentations.

Q During which phase of fermentation is ATP generated?

We next consider two of the more important processes: lactic acid fermentation and alcohol fermentation.

Lactic Acid Fermentation

During glycolysis, which is the first phase of **lactic acid fermentation,** a molecule of glucose is oxidized to two molecules of pyruvic acid (Figure 5.19; see also Figure 5.10). This oxidation generates the energy that is used to form the two molecules of ATP. In the next step, the two molecules of pyruvic acid are reduced by two molecules of NADH to form two molecules of lactic acid (Figure 5.19a). Because lactic acid is the end-product of the reaction, it undergoes no further oxidation, and most of the

energy produced by the reaction remains stored in the lactic acid. Thus, this fermentation yields only a small amount of energy.

Two important genera of lactic acid bacteria are *Streptococcus* and *Lactobacillus* (lak-tō-bä-sil′lus). Because these microbes produce only lactic acid, they are referred to as **homolactic** (or *homofermentative*). Lactic acid fermentation can result in food spoilage. However, the process can also produce yogurt from milk, sauerkraut from fresh cabbage, and pickles from cucumbers.

Clinical Case

Feeling certain that there must be some connection between the increase in dental caries and the activities of her patients, Dr. Rivera starts to ask more questions about the children's activities. She finds out that they all attend a summer program at the same church in a nearby neighborhood. She also discovers that the culprit isn't candy, but bubblegum. The camp counselors have been giving out bubblegum as an incentive for attendance and good behavior. Although Dr. Rivera is pleased to hear that her patients have all been behaving themselves, she is concerned about the amount of bubblegum they have been chewing on a daily basis. The sucrose in gum causes a decrease in the pH of saliva, and the acid erodes the tooth enamel, thus exposing the tooth to bacterial decay.

If the pH of gum and sucrose is 7, what lowers the salivary pH?

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Alcohol Fermentation

Alcohol fermentation also begins with the glycolysis of a molecule of glucose to yield two molecules of pyruvic acid and two molecules of ATP. In the next reaction, the two molecules of pyruvic acid are converted to two molecules of acetaldehyde and two molecules of CO_2 (**Figure 5.19b**). The two molecules of acetaldehyde are next reduced by two molecules of NADH to form two molecules of ethanol. Again, alcohol fermentation is a low-energy-yield process because most of the energy contained in the initial glucose molecule remains in the ethanol, the end-product.

Alcohol fermentation is carried out by a number of bacteria and yeasts. The ethanol and carbon dioxide produced by the yeast *Saccharomyces* (sak-ä-rō-mī′sēs) are waste products for yeast cells but are useful to humans. Ethanol made by yeasts is the alcohol in alcoholic beverages, and carbon dioxide made by yeasts causes bread dough to rise.

Organisms that produce lactic acid as well as other acids or alcohols are known as **heterolactic** (or *heterofermentative*) and often use the pentose phosphate pathway.

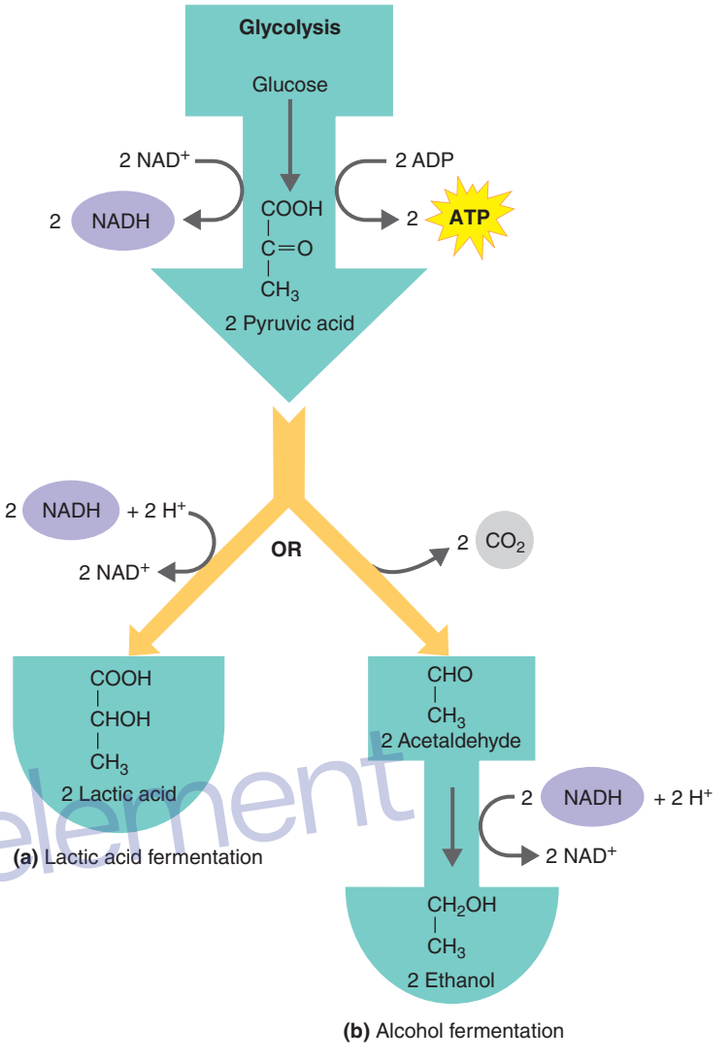

Figure 5.19 **Types of fermentation.**

Q What is the difference between homolactic and heterolactic fermentation?

Table 5.4 lists some of the various microbial fermentations used by industry to convert inexpensive raw materials into useful end-products. **Table 5.5** provides a summary comparison of aerobic respiration, anaerobic respiration, and fermentation.

MM **Animation** Fermentation

CHECK YOUR UNDERSTANDING

List four compounds that can be made from pyruvic acid by an organism that uses fermentation. **5-16**

Lipid and Protein Catabolism

LEARNING OBJECTIVE

5-17 Describe how lipids and proteins undergo catabolism.

Our discussion of energy production has emphasized the oxidation of glucose, the main energy-supplying carbohydrate.

What Is Fermentation?

To many people, *fermentation* simply means the production of alcohol: grains and fruits are fermented to produce beer and wine. If a food soured, you might say it was "off," or fermented. Here are some definitions of *fermentation*. They range from informal, general usage to more scientific definitions.

1. Any spoilage of food by microorganisms (general use)
2. Any process that produces alcoholic beverages or acidic dairy products (general use)
3. Any large-scale microbial process occurring with or without air (common definition used in industry)
4. Any energy-releasing metabolic process that takes place only under anaerobic conditions (becoming more scientific)
5. Any metabolic process that releases energy from a sugar or other organic molecule, does not require oxygen or an electron transport system, and uses an organic molecule as the final electron acceptor (the definition we use in this book).

However, microbes also oxidize lipids and proteins, and the oxidations of all these nutrients are related.

Recall that fats are lipids consisting of fatty acids and glycerol. Microbes produce extracellular enzymes called *lipases* that break fats down into their fatty acid and glycerol components. Each component is then metabolized separately (Figure 5.20). The Krebs cycle functions in the oxidation of glycerol and fatty acids. Many bacteria that hydrolyze fatty acids can use the same enzymes to degrade petroleum products. Although these bacteria are a nuisance when they grow in a fuel storage tank, they are beneficial when they grow in oil spills. Beta-oxidation (the oxidation of fatty acids) of petroleum is illustrated in the box in Chapter 2 (page 32).

Proteins are too large to pass unaided through plasma membranes. Microbes produce extracellular *proteases* and *peptidases*,

TABLE 5.4 Some Industrial Uses for Different Types of Fermentations*

Fermentation End-Product(s)	Industrial or Commercial Use	Starting Material	Microorganism
Ethanol	Beer, wine	Starch, sugar	*Saccharomyces cerevisiae* (yeast, a fungus)
	Fuel	Agricultural wastes	*Saccharomyces cerevisiae* (yeast)
Acetic Acid	Vinegar	Ethanol	*Acetobacter*
Lactic Acid	Cheese, yogurt	Milk	*Lactobacillus, Streptococcus*
	Rye bread	Grain, sugar	*Lactobacillus delbrueckii*
	Sauerkraut	Cabbage	*Lactobacillus plantarum*
	Summer sausage	Meat	*Pediococcus*
Propionic Acid and Carbon Dioxide	Swiss cheese	Lactic acid	*Propionibacterium freudenreichii*
Acetone and Butanol	Pharmaceutical, industrial uses	Molasses	*Clostridium acetobutylicum*
Citric Acid	Flavoring	Molasses	*Aspergillus* (fungus)
Methane	Fuel	Acetic acid	*Methanosarcina*
Sorbose	Vitamin C (ascorbic acid)	Sorbitol	*Gluconobacter*

*Unless otherwise noted, the microorganisms listed are bacteria.

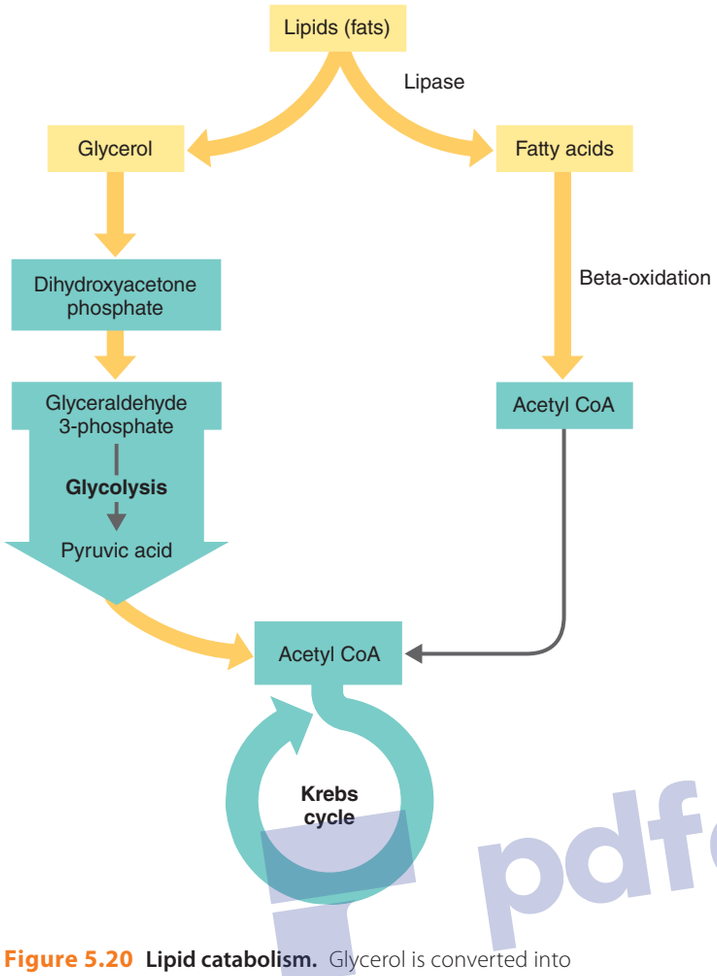

Figure 5.20 Lipid catabolism. Glycerol is converted into dihydroxyacetone phosphate (DHAP) and catabolized via glycolysis and the Krebs cycle. Fatty acids undergo beta-oxidation, in which carbon fragments are split off two at a time to form acetyl CoA, which is catabolized via the Krebs cycle.

Q What is the role of lipases?

enzymes that break down proteins into their component amino acids, which can cross the membranes. However, before amino acids can be catabolized, they must be enzymatically converted to other substances that can enter the Krebs cycle. In one such conversion, called **deamination,** the amino group of an amino acid is removed and converted to an ammonium ion (NH_4^+), which can be excreted from the cell. The remaining organic acid can enter the Krebs cycle. Other conversions involve **decarboxylation** (the removal of —COOH) and **dehydrogenation.**

A summary of the interrelationships of carbohydrate, lipid, and protein catabolism is shown in Figure 5.21.

CHECK YOUR UNDERSTANDING

What are the end-products of lipid and protein catabolism? 5-17

Clinical Case

Dental caries are caused by oral streptococci, including *S. mutans*, *S. salivarius*, and *S. sobrinus*, that attach to tooth surfaces. Oral streptococci ferment sucrose and produce lactic acid, which lowers the salivary pH. Dr. Rivera decides to ask the camp counselors to substitute the bubblegum with a sugarless gum made with xylitol. A study has shown that chewing gum sweetened with xylitol, a naturally occuring sugar alcohol, can significantly lower the number of dental caries in children because it lowers the number of *S. mutans* in the mouth.

Why might xylitol reduce the number of *S. mutans*?

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Biochemical Tests and Bacterial Identification

LEARNING OBJECTIVE

5-18 Provide two examples of the use of biochemical tests to identify bacteria in the laboratory.

Biochemical testing is frequently used to identify bacteria and yeasts because different species produce different enzymes. Such biochemical tests are designed to detect the presence of enzymes.

TABLE 5.5 Aerobic Respiration, Anaerobic Respiration, and Fermentation

Energy-Producing Process	Growth Conditions	Final Hydrogen (Electron) Acceptor	Type of Phosphorylation Used to Generate ATP	ATP Molecules Produced per Glucose Molecule
Aerobic Respiration	Aerobic	Molecular oxygen (O_2)	Substrate-level and oxidative	36 (eukaryotes) 38 (prokaryotes)
Anaerobic Respiration	Anaerobic	Usually an inorganic substance (such as NO_3^-, SO_4^{2-}, or CO_3^{2-}) but not molecular oxygen (O_2)	Substrate-level and oxidative	Variable (fewer than 38 but more than 2)
Fermentation	Aerobic or anaerobic	An organic molecule	Substrate-level	2

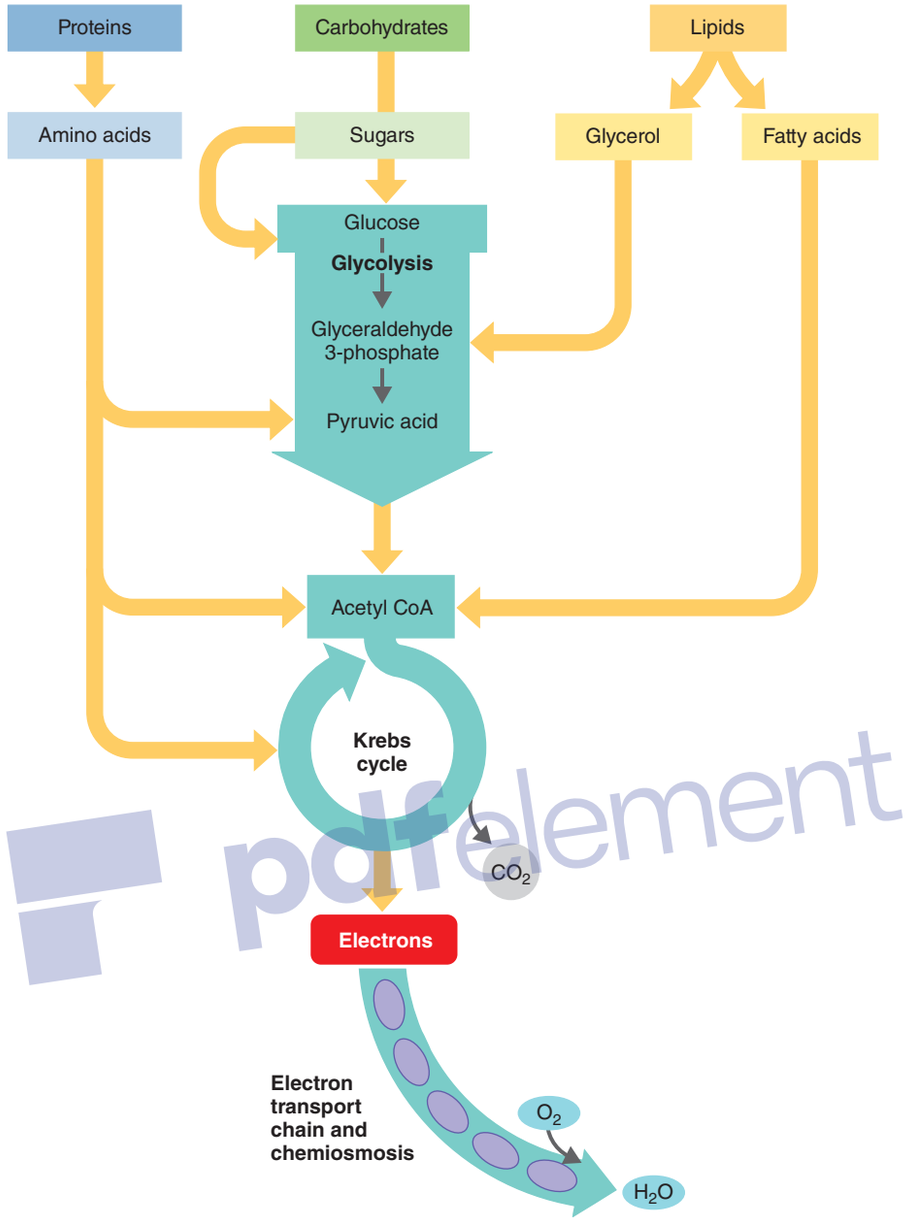

Figure 5.21 Catabolism of various organic food molecules. Proteins, carbohydrates, and lipids can all be sources of electrons and protons for respiration. These food molecules enter glycolysis or the Krebs cycle at various points.

Q What are the catabolic pathways through which high-energy electrons from all kinds of organic molecules flow on their energy-releasing pathways?

One type of biochemical test is the detection of amino acid catabolizing enzymes involved in decarboxylation and dehydrogenation (discussed on page 120; **Figure 5.22**).

Another biochemical test is a **fermentation test.** The test medium contains protein, a single carbohydrate, a pH indicator, and an inverted Durham tube, which is used to capture gas (**Figure 5.23a**). Bacteria inoculated into the tube can use the protein or carbohydrate as a carbon and energy source. If they catabolize the carbohydrate and produce acid, the pH indicator changes color. Some organisms produce gas as well as acid from carbohydrate catabolism. The presence of a bubble in the Durham tube indicates gas formation (**Figure 5.23b–d**).

E. coli ferments the carbohydrate sorbitol. The pathogenic *E. coli* O157 strain, however, does not ferment sorbitol, a characteristic that differentiates it from nonpathogenic, commensal *E. coli*.

Another example of the use of biochemical tests is shown in Figure 10.8 on page 284.

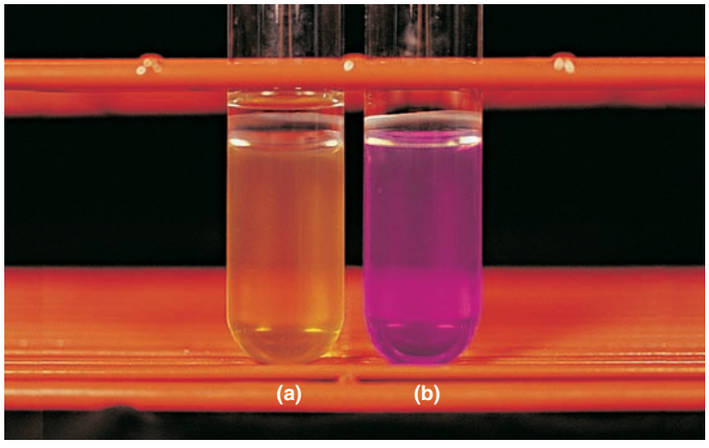

Figure 5.22 Detecting amino acid catabolizing enzymes in the lab. Bacteria are inoculated in tubes containing glucose, a pH indicator, and a specific amino acid. (**a**) The pH indicator turns to yellow when bacteria produce acid from glucose. (**b**) Alkaline products from decarboxylation turn the indicator to purple.

Q What is decarboxylation?

In some instances, the waste products of one microorganism can be used as a carbon and energy source by another species. *Acetobacter* (ä-sē-tō-bak′tėr) bacteria oxidize ethanol made by yeast. *Propionibacterium* (prō-pē-on-ē-bak-ti′re-um) can use lactic acid produced by other bacteria. Propionibacteria convert lactic acid to pyruvic acid in preparation for the Krebs cycle. During the Krebs cycle, propionic acid and CO_2 are made. The holes in Swiss cheese are formed by the accumulation of the CO_2 gas.

Biochemical tests are used to identify bacteria that cause disease. All aerobic bacteria use the electron transport chain (ETC),

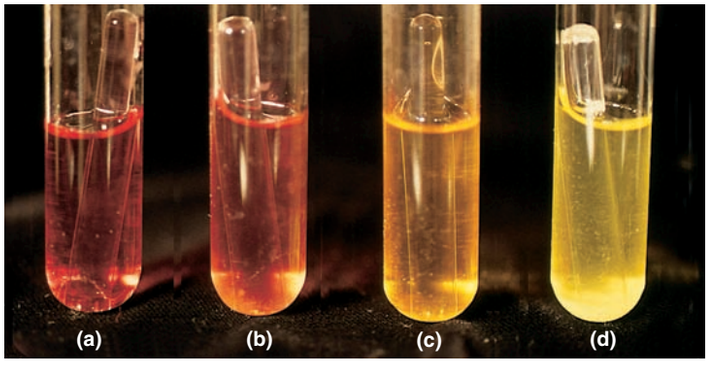

Figure 5.23 A fermentation test. (**a**) An uninoculated fermentation tube containing the carbohydrate mannitol. (**b**) *Staphylococcus epidermidis* grew on the protein but did not use the carbohydrate. This organism is described as mannitol −. (**c**) *Staphylococcus aureus* produced acid but not gas. This species is mannitol +. (**d**) *Escherichia coli* is also mannitol + and produced acid and gas from mannitol. The gas is trapped in the inverted Durham tube.

Q On what is the *S. epidermidis* growing?

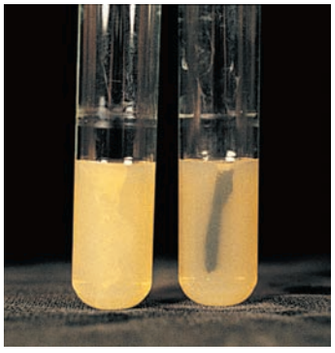

Figure 5.24 Use of peptone iron agar to detect the production of H_2S. H_2S produced in the tube precipitates with iron in the medium as ferrous sulfide.

Q What chemical reaction causes the release of H_2S?

but not all their ETCs are identical. Some bacteria have cytochrome *c*, but others do not. In the former, *cytochrome c oxidase* is the last enzyme, which transfers electrons to oxygen. The oxidase test is routinely used to quickly identify *Neisseria gonorrhoeae*. *Neisseria* is positive for cytochrome oxidase. The oxidase test can also be used to distinguish some gram-negative rods: *Pseudomonas* is oxidase-positive, and *Escherichia* is oxidase-negative.

Shigella causes dysentery. *Shigella* is differentiated from *E. coli* by biochemical tests. Unlike *E. coli*, *Shigella* does not produce gas from lactose and does not produce the enzyme lactate dehydrogenase.

Salmonella bacteria are readily distinguishable from *E. coli* by the production of hydrogen sulfide (H_2S). Hydrogen sulfide is released when the bacteria remove sulfur from amino acids (**Figure 5.24**). The H_2S combines with iron to form a black precipitate in a culture medium.

The box on page 142 describes how biochemical tests were used to determine the cause of disease in a young child in Dallas, Texas.

Clinical Case Resolved

S. mutans cannot ferment xylitol; consequently, it doesn't grow and can't produce acid in the mouth. The camp counselors agree to switch to sugarless gum made with xylitol, and Dr. Rivera is pleased. She understands that there will be other sources of sucrose in the children's diets, but at least her patients are no longer going to be adversely affected by the camp's well-intentioned incentives. Researchers are still investigating ways that antimicrobials and vaccines can be used to reduce bacterial colonization. However, reducing consumption of sucrose-containing gum and candy may be an effective preventive measure.

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CHECK YOUR UNDERSTANDING

- On what biochemical basis are *Pseudomonas* and *Escherichia* differentiated? **5-18**

Photosynthesis

LEARNING OBJECTIVES

5-19 Compare and contrast cyclic and noncyclic photophosphorylation.

5-20 Compare and contrast the light-dependent and light-independent reactions of photosynthesis.

5-21 Compare and contrast oxidative phosphorylation and photophosphorylation.

In all of the metabolic pathways just discussed, organisms obtain energy for cellular work by oxidizing organic compounds. But where do organisms obtain these organic compounds? Some, including animals and many microbes, feed on matter produced by other organisms. For example, bacteria may catabolize compounds from dead plants and animals, or they may obtain nourishment from a living host.

Other organisms synthesize complex organic compounds from simple inorganic substances. The major mechanism for such synthesis is a process called **photosynthesis,** which is carried out by plants and many microbes. Essentially, photosynthesis is the conversion of light energy from the sun into chemical energy. The chemical energy is then used to convert CO_2 from the atmosphere to more reduced carbon compounds, primarily sugars. The word *photosynthesis* summarizes the process: *photo* means light, and *synthesis* refers to the assembly of organic compounds. This synthesis of sugars by using carbon atoms from CO_2 gas is also called **carbon fixation.** Continuation of life as we know it on Earth depends on the recycling of carbon in this way (see Figure 27.3 on page 775). Cyanobacteria, algae, and green plants all contribute to this vital recycling with photosynthesis.

Photosynthesis can be summarized with the following equations:

1. Plants, algae, and cyanobacteria use water as a hydrogen donor, releasing O_2.

$$6\,CO_2 + 12\,H_2O + \text{Light energy} \rightarrow C_6H_{12}O_6 + 6\,H_2O + 6\,O_2$$

2. Purple sulfur and green sulfur bacteria use H_2S as a hydrogen donor, producing sulfur granules.

$$6\,CO_2 + 12\,H_2S + \text{Light energy} \rightarrow C_6H_{12}O_6 + 6\,H_2O + 12\,S$$

In the course of photosynthesis, electrons are taken from hydrogen atoms, an energy-poor molecule, and incorporated into sugar, an energy-rich molecule. The energy boost is supplied by light energy, although indirectly.

Photosynthesis takes place in two stages. In the first stage, called the **light-dependent (light) reactions,** light energy is used to convert ADP and P to ATP. In addition, in the predominant form of the light-dependent reactions, the electron carrier $NADP^+$ is reduced to NADPH. The coenzyme NADPH, like NADH, is an energy-rich carrier of electrons. In the second stage, the **light-independent (dark) reactions,** these electrons are used along with energy from ATP to reduce CO_2 to sugar.
MM **Animation** Photosynthesis: Overview

The Light-Dependent Reactions: Photophosphorylation

Photophosphorylation is one of the three ways ATP is formed, and it occurs only in photosynthetic cells. In this mechanism, light energy is absorbed by chlorophyll molecules in the photosynthetic cell, exciting some of the molecules' electrons. The chlorophyll principally used by green plants, algae, and cyanobacteria is *chlorophyll a*. It is located in the membranous thylakoids of chloroplasts in algae and green plants (see Figure 4.28, page 105) and in the thylakoids found in the photosynthetic structures of cyanobacteria. Other bacteria use *bacteriochlorophylls*.

The excited electrons jump from the chlorophyll to the first of a series of carrier molecules, an electron transport chain similar to that used in respiration. As electrons are passed along the series of carriers, protons are pumped across the membrane, and ADP is converted to ATP by chemiosmosis. Chlorophyll and other pigments are packed into thylakoids of chloroplasts (see Figure 4.28 on page 105) and are called **photosystems.** *Photosystem II* is so numbered because even though it was most likely the first photosystem to evolve, it was the second one discovered. It contains chlorophyll that is sensitive to wavelengths of light of 680 nm. *Photosystem I* contains chlorophyll that is sensitive to wavelengths of light of 700 nm. In **cyclic photophosphorylation,** the electrons released from chlorophyll in photosystem I eventually return to chlorophyll (**Figure 5.25a**). In **noncyclic photophosphorylation,** which is used in oxygenic organisms, the electrons released from the chlorophyll in photosystem II and photosystem I do not return to chlorophyll but become incorporated into NADPH (**Figure 5.25b**). The electrons lost from chlorophyll are replaced by electrons from H_2O. To summarize: the products of noncyclic photophosphorylation are ATP (formed by chemiosmosis using energy released in an electron transport chain), O_2 (from water molecules), and NADPH (in which the hydrogen electrons and protons were derived ultimately from water). MM **Animations** Light Reaction: Cyclic Photophosphorylation; Light Reaction: Noncyclic Photophosphorylation

The Light-Independent Reactions: The Calvin-Benson Cycle

The light-independent (dark) reactions are so named because they require no light directly. They include a complex cyclic pathway called the **Calvin-Benson cycle,** in which CO_2 is "fixed"—that is,

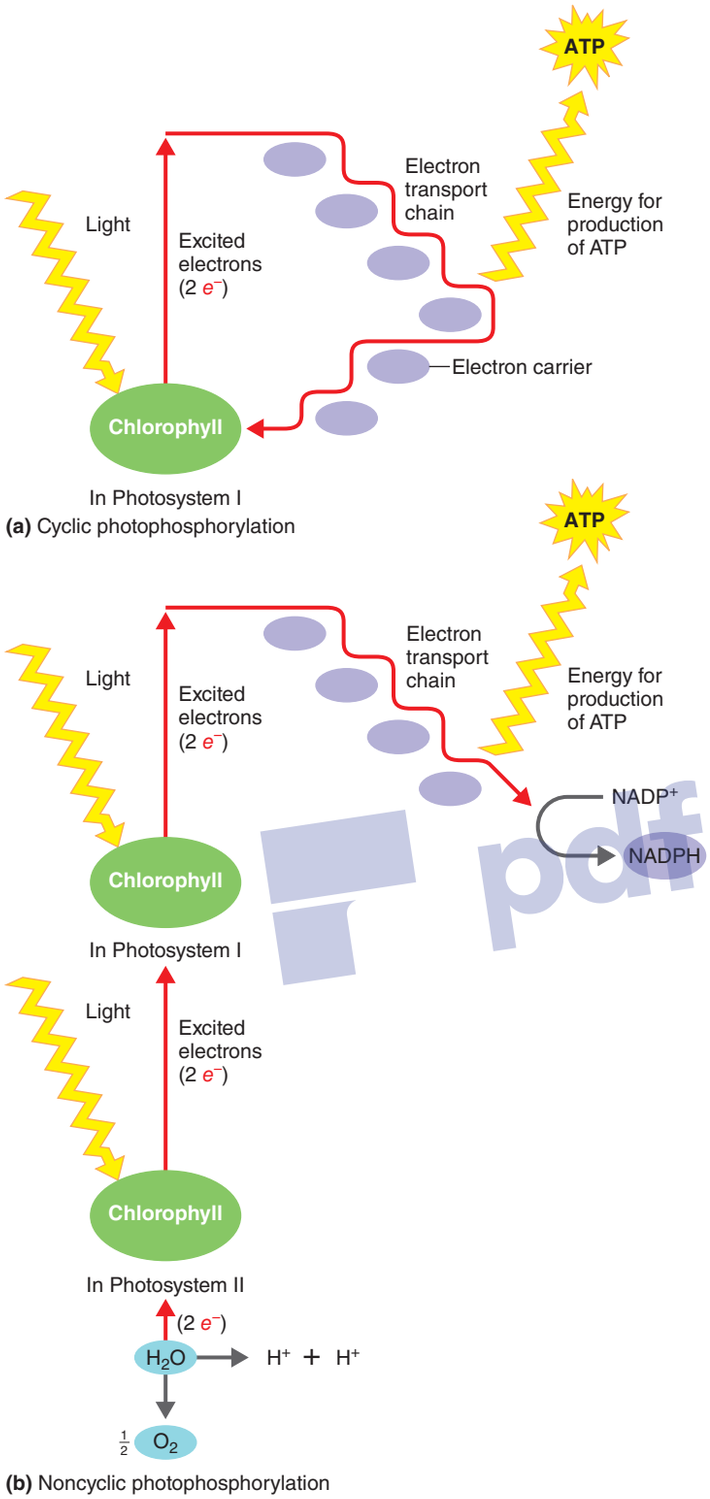


Figure 5.25 Photophosphorylation. (**a**) In cyclic photophosphorylation, electrons released from chlorophyll by light return to chlorophyll after passage along the electron transport chain. The energy from electron transfer is converted to ATP. (**b**) In noncyclic photophosphorylation, electrons released from chlorophyll in photosystem II are replaced by electrons from the hydrogen atoms in water. This process also releases hydrogen ions. Electrons from chlorophyll in photosystem I are passed along the electron transport chain to the electron acceptor $NADP^+$. $NADP^+$ combines with electrons and with hydrogen ions from water, forming NADPH.

Q How are oxidative phosphorylation and photophosphorylation similar?

used to synthesize sugars (**Figure 5.26**, see also Figure A.1 in Appendix A). **Animation** Light Independent Reactions

CHECK YOUR UNDERSTANDING

- How is photosynthesis important to catabolism? **5-19**
- What is made during the light-dependent reactions? **5-20**
- How are oxidative phosphorylation and photophosphorylation similar? **5-21**

A Summary of Energy Production Mechanisms

LEARNING OBJECTIVE

5-22 Write a sentence to summarize energy production in cells.

In the living world, energy passes from one organism to another in the form of the potential energy contained in the bonds of chemical compounds. Organisms obtain the energy from oxidation reactions. To obtain energy in a usable form, a cell must have an electron (or hydrogen) donor, which serves as an initial energy source within the cell. Electron donors are diverse and can include photosynthetic pigments, glucose or other organic compounds, elemental sulfur, ammonia, or hydrogen gas (**Figure 5.27**). Next, electrons removed from the chemical energy sources are transferred to electron carriers, such as the coenzymes NAD^+, $NADP^+$, and FAD. This transfer is an oxidation-reduction reaction; the initial energy source is oxidized as this first electron carrier is reduced. During this phase, some ATP is produced. In the third stage, electrons are transferred from electron carriers to their final electron acceptors in further oxidation-reduction reactions, producing more ATP.

In aerobic respiration, oxygen (O_2) serves as the final electron acceptor. In anaerobic respiration, inorganic substances other than oxygen, such as nitrate ions (NO_3^-) or sulfate ions (SO_4^{2-}), serve as the final electron acceptors. In fermentation, organic compounds serve as the final electron acceptors. In aerobic and anaerobic respiration, a series of electron carriers called an electron transport chain releases energy that is used by the mechanism of chemiosmosis to synthesize ATP. Regardless of their energy sources, all organisms use similar oxidation-reduction

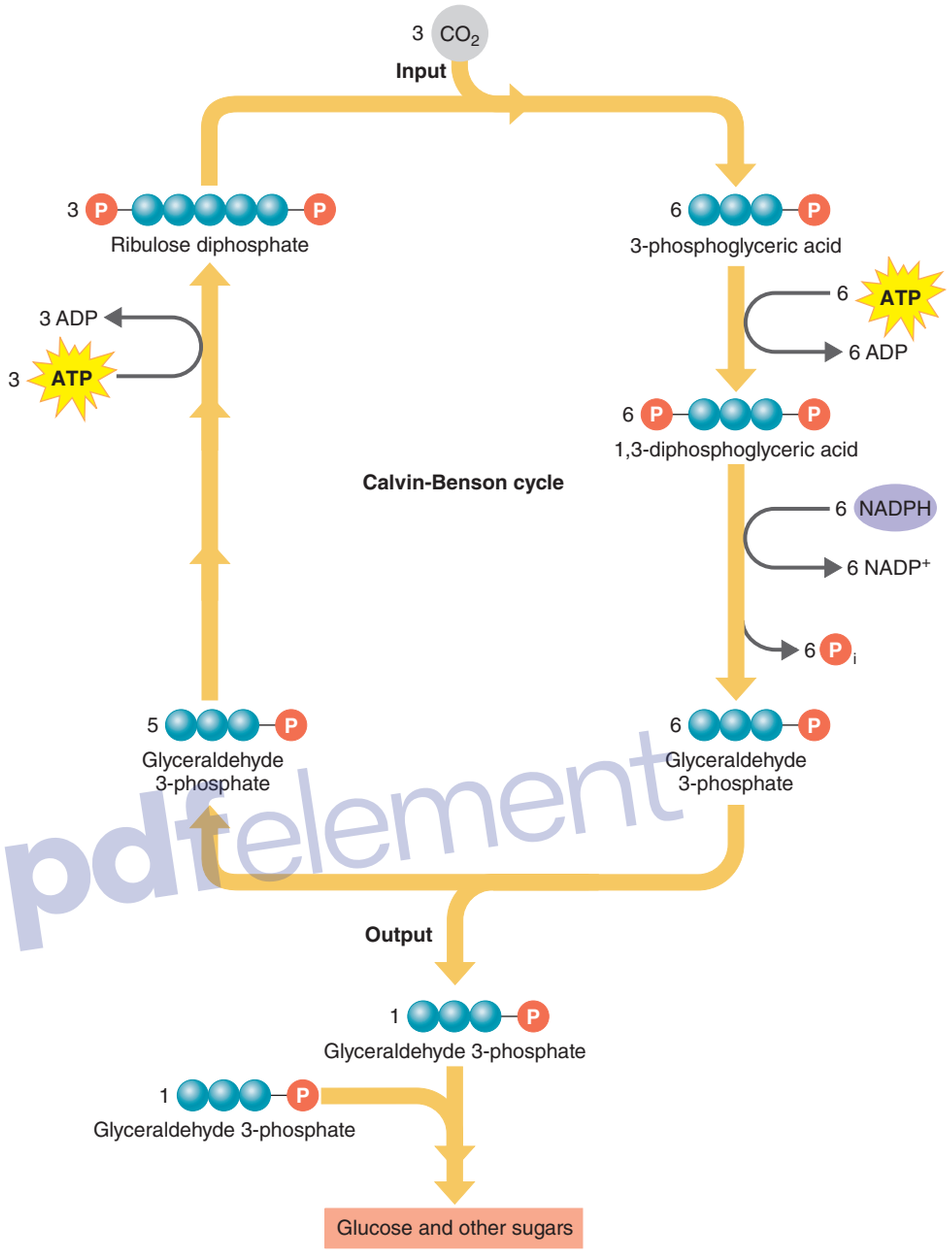


Figure 5.26 A simplified version of the Calvin-Benson cycle. This diagram shows three turns of the cycle, in which three molecules of CO_2 are fixed and one molecule of glyceraldehyde 3-phosphate is produced and leaves the cycle. Two molecules of glyceraldehyde 3-phosphate are needed to make one molecule of glucose. Therefore, the cycle must turn six times for each glucose molecule produced, requiring a total investment of 6 molecules of CO_2, 18 molecules of ATP, and 12 molecules of NADPH. A more detailed version of this cycle is presented in Figure A.1 in Appendix A.

Q In the Calvin-Benson cycle, which molecule is used to synthesize sugars?

reactions to transfer electrons and similar mechanisms to use the energy released to produce ATP.

CHECK YOUR UNDERSTANDING

✓ Summarize how oxidation enables organisms to get energy from glucose, sulfur, or sunlight. 5-22

Metabolic Diversity among Organisms

LEARNING OBJECTIVE

5-23 Categorize the various nutritional patterns among organisms according to carbon source and mechanisms of carbohydrate catabolism and ATP generation.

We have looked in detail at some of the energy-generating metabolic pathways that are used by animals and plants, as well as by many microbes. Microbes are distinguished by their great metabolic diversity, however, and some can sustain themselves on inorganic substances by using pathways that are unavailable to either plants or animals. All organisms, including microbes, can be classified metabolically according to their *nutritional pattern*—their source of energy and their source of carbon.

First considering the energy source, we can generally classify organisms as phototrophs or chemotrophs. **Phototrophs** use light as their primary energy source, whereas **chemotrophs** depend on oxidation-reduction reactions of inorganic or organic compounds for energy. For their principal carbon source, **autotrophs** (self-feeders) use carbon dioxide, and **heterotrophs**

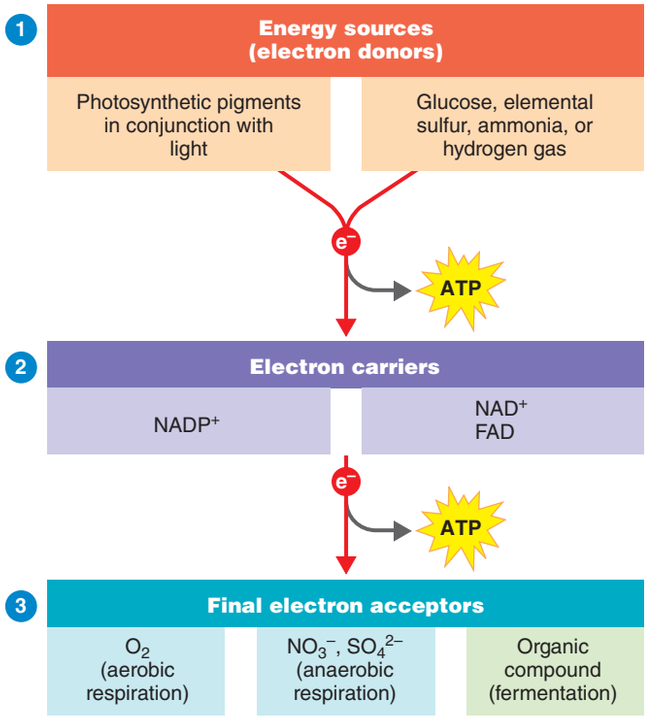


Figure 5.27 Requirements of ATP production. The production of ATP requires 1 an energy source (electron donor), 2 the transfer of electrons to an electron carrier during an oxidation-reduction reaction, and 3 the transfer of electrons to a final electron acceptor.

Q Are energy-generating reactions oxidations or reductions?

(feeders on others) require an organic carbon source. Autotrophs are also referred to as *lithotrophs* (rock eating), and heterotrophs are also referred to as *organotrophs*.

If we combine the energy and carbon sources, we derive the following nutritional classifications for organisms: *photoautotrophs, photoheterotrophs, chemoautotrophs,* and *chemoheterotrophs* (Figure 5.28). Almost all of the medically important microorganisms discussed in this book are chemoheterotrophs. Typically, infectious organisms catabolize substances obtained from the host.

Photoautotrophs

Photoautotrophs use light as a source of energy and carbon dioxide as their chief source of carbon. They include photosynthetic bacteria (green and purple bacteria and cyanobacteria), algae, and green plants. In the photosynthetic reactions of cyanobacteria, algae, and green plants, the hydrogen atoms of water are used to reduce carbon dioxide, and oxygen gas is given off. Because this photosynthetic process produces O_2, it is sometimes called **oxygenic.**

In addition to the cyanobacteria (see Figure 11.21, page 321), there are several other families of photosynthetic prokaryotes. Each is classified according to the way it reduces CO_2. These bacteria cannot use H_2O to reduce CO_2 and cannot carry on photosynthesis when oxygen is present (they must have an anaerobic environment). Consequently, their photosynthetic process does

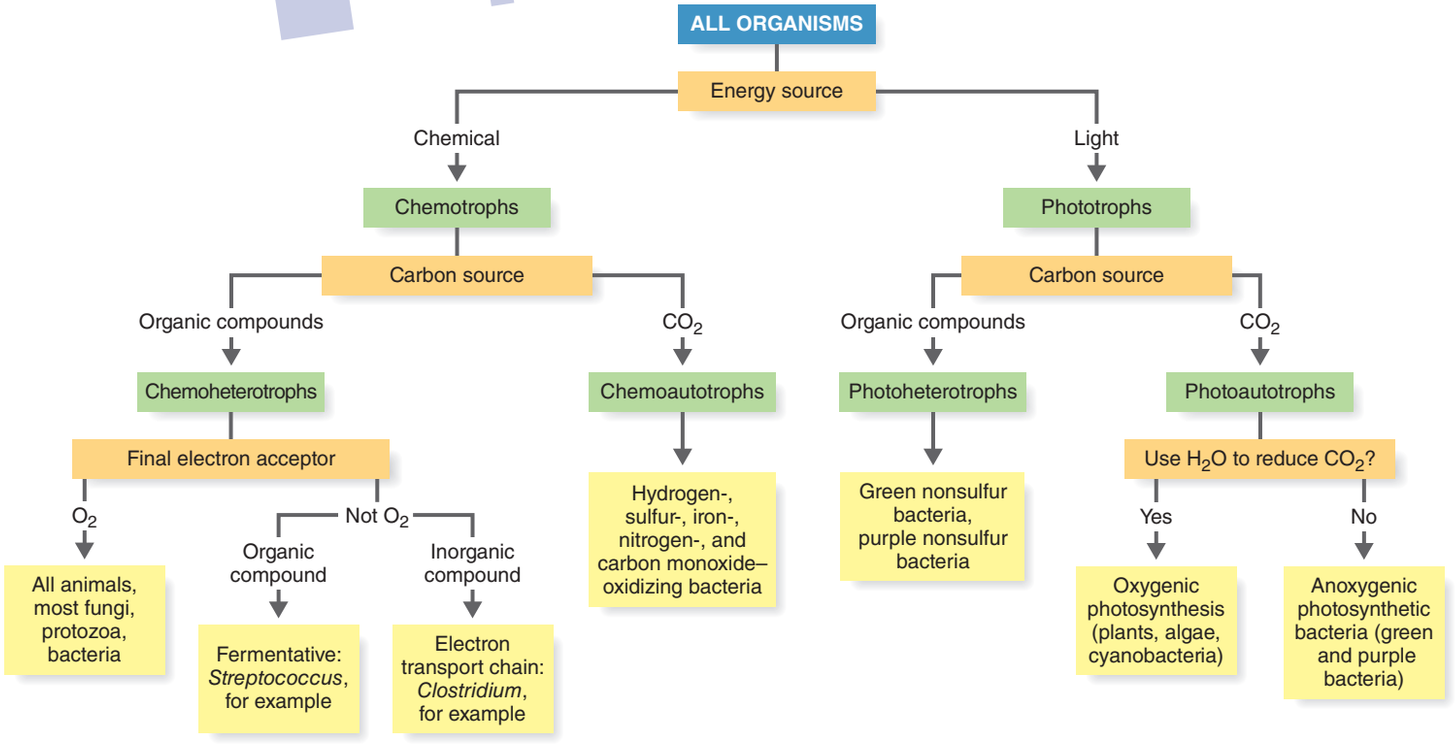


Figure 5.28 A nutritional classification of organisms.

Q What is the basic difference between chemotrophs and phototrophs?

Human Tuberculosis–Dallas, Texas

As you read through this box, you will encounter a series of questions that laboratory technicians ask themselves as they identify bacteria. Try to answer each question before going on to the next one.

1. Daria, a 12-month-old African American girl, is brought by her parents to the emergency department of a Dallas, Texas, hospital. She has a fever of 39°C, a distended abdomen, some abdominal pain, and watery diarrhea. Daria is admitted to the pediatric wing of the hospital, pending results of laboratory and radiologic tests. Test results suggest peritoneal tuberculosis. Caused by one of several closely related species in the *Mycobacterium tuberculosis* complex, TB is a reportable condition in the United States. Peritoneal TB is a disease of the intestines and abdominal cavity.
   What organ is usually associated with tuberculosis? How might someone get peritoneal TB?
2. Pulmonary TB is contracted by inhaling the bacteria; ingesting the bacteria can result in peritoneal TB. A laparoscopy reveals that nodules are present in Daria's abdominal cavity. A portion of a nodule is removed for biopsy so that it can be observed for the presence of acid-fast bacteria. Based on the presence of the abdominal nodules, Daria's physician begins conventional antituberculosis treatment. This long-term treatment can last up to 12 months.
   What is the next step?
3. The lab results confirm that acid-fast bacteria are indeed present in Daria's abdominal cavity. The laboratory now needs to identify the *Mycobacterium* species. Speciation of the *M. tuberculosis* complex is done by biochemical testing in reference laboratories (**Figure A**). The bacteria need to be grown in culture media. Slow-growing mycobacteria may take up to 6 weeks to form colonies.
   After colonies have been isolated, what is the next step?
4. Two weeks later, the laboratory results show that the bacteria are slow-growing. According to the identification scheme, the urease test should be performed.
   What is the result shown in Figure B?
5. Because the urease test is positive, the nitrate reduction test is performed. It shows that the bacteria do not produce the enzyme nitrate reductase. Daria's physician lets her parents know that they are very close to identifying the pathogen that is causing Daria's illness.
   What is the bacterium?
6. *M. bovis* is a pathogen that primarily infects cattle. However, humans can become infected by consuming unpasteurized dairy products or inhaling infectious droplets from cattle. Human-to-human transmission occurs only rarely. The clinical and pathologic characteristics of *M. bovis* TB are indistinguishable from *M. tuberculosis* TB, but identification of the bacterium is important for prevention and treatment. Children may be at higher risk. In one study, almost half of the culture-positive pediatric TB cases were caused by *M. bovis*.

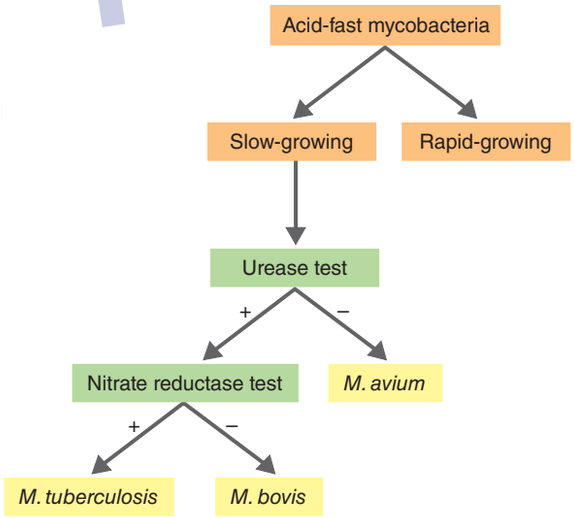


Figure A **An identification scheme for selected species of slow-growing mycobacteria.**

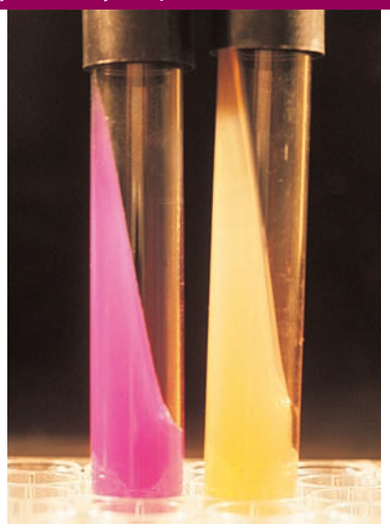


Figure B **The urease test. In a positive test, bacterial urease hydrolyzes urea, producing ammonia. The ammonia raises the pH, and the indicator in the medium turns to fuchsia.**

Unfortunately, Daria does not recover from her illness. Her cadiovascular system collapses, and she dies. The official cause of death is peritoneal tuberculosis caused by *M. bovis*. Everyone should avoid consuming products from unpasteurized cow's milk, which carry the risk of transmitting *M. bovis* if imported from countries where the bacterium is common in cattle.

Source: Adapted from Rodwell T.C., Moore M., Moser K.S., Brodine S.K., Strathdee S.A, "Mycobacterium bovis Tuberculosis in Binational Communities," Emerging Infectious Diseases, June 2008, Volume 14 (6), pp. 909–916. Available from http://www.cdc.gov/eid/content/14/6/909.htm.

not produce O_2 and is called **anoxygenic.** The anoxygenic photoautotrophs are the green and purple bacteria. The **green bacteria,** such as *Chlorobium* (klô-rō′bē-um), use sulfur (S), sulfur compounds (such as hydrogen sulfide, H_2S), or hydrogen gas (H_2) to reduce carbon dioxide and form organic compounds. Applying the energy from light and the appropriate enzymes, these bacteria oxidize sulfide (S^{2-}) or sulfur (S) to sulfate (SO_4^{2-}) or oxidize hydrogen gas to water (H_2O). The **purple bacteria,** such as *Chromatium* (krō-mā′tē-um), also use sulfur, sulfur compounds, or hydrogen gas to reduce carbon dioxide. They are distinguished from the green bacteria by their type of chlorophyll, location of stored sulfur, and ribosomal RNA.

TABLE 5.6 Photosynthesis Compared in Selected Eukaryotes and Prokaryotes

Characteristic	Eukaryotes	Prokaryotes		
	Algae, Plants	**Cyanobacteria**	**Green Bacteria**	**Purple Bacteria**
Substance That Reduces CO_2	H atoms of H_2O	H atoms of H_2O	Sulfur, sulfur compounds, H_2 gas	Sulfur, sulfur compounds, H_2 gas
Oxygen Production	Oxygenic	Oxygenic (and anoxygenic)	Anoxygenic	Anoxygenic
Type of Chlorophyll	Chlorophyll *a*	Chlorophyll *a*	Bacteriochlorophyll *a*	Bacteriochlorophyll *a* or *b*
Site of Photosynthesis	Chloroplasts with thylakoids	Thylakoids	Chlorosomes	Chromatophores
Environment	Aerobic	Aerobic (and anaerobic)	Anaerobic	Anaerobic

The chlorophylls used by these photosynthetic bacteria are called *bacteriochlorophylls,* and they absorb light at longer wavelengths than that absorbed by chlorophyll *a*. Bacteriochlorophylls of green sulfur bacteria are found in vesicles called *chlorosomes* (or *chlorobium vesicles*) underlying and attached to the plasma membrane. In the purple sulfur bacteria, the bacteriochlorophylls are located in invaginations of the plasma membrane (*chromatophores*).

Table 5.6 summarizes several characteristics that distinguish eukaryotic photosynthesis from prokaryotic photosynthesis.

Animation Comparing Prokaryotes and Eukaryotes

Photoheterotrophs

Photoheterotrophs use light as a source of energy but cannot convert carbon dioxide to sugar; rather, they use as sources of carbon organic compounds, such as alcohols, fatty acids, other organic acids, and carbohydrates. Photoheterotrophs are anoxygenic. The **green nonsulfur bacteria,** such as *Chloroflexus* (klô-rō-flex′us), and **purple nonsulfur bacteria,** such as *Rhodopseudomonas* (rō-dō-sū-dō-mō′nas), are photoheterotrophs (see also page 323).

Chemoautotrophs

Chemoautotrophs use the electrons from reduced inorganic compounds as a source of energy, and they use CO_2 as their principal source of carbon. They fix CO_2 in the Calvin-Benson Cycle (see Figure 5.26). Inorganic sources of energy for these organisms include hydrogen sulfide (H_2S) for *Beggiatoa* (bej-jē-ä-tō′ä); elemental sulfur (S) for *Thiobacillus thiooxidans;* ammonia (NH_3) for *Nitrosomonas* (nī-trō-sō-mō′näs); nitrite ions (NO_2^-) for *Nitrobacter* (nī-trō-bak′tėr); hydrogen gas (H_2) for *Cupriavidus* (kü′prē-ä-vid-us); ferrous iron (Fe^{2+}) for *Thiobacillus ferrooxidans;* and carbon monoxide (CO) for *Pseudomonas carboxydohydrogena* (kär′boks-i-dō-hi-drō-je-nä). The energy derived from the oxidation of these inorganic compounds is eventually stored in ATP, which is produced by oxidative phosphorylation.

Chemoheterotrophs

When we discuss photoautotrophs, photoheterotrophs, and chemoautotrophs, it is easy to categorize the energy source and carbon source because they occur as separate entities. However, in chemoheterotrophs, the distinction is not as clear because the energy source and carbon source are usually the same organic compound—glucose, for example. **Chemoheterotrophs** specifically use the electrons from hydrogen atoms in organic compounds as their energy source.

Heterotrophs are further classified according to their source of organic molecules. **Saprophytes** live on dead organic matter, and **parasites** derive nutrients from a living host. Most bacteria, and all fungi, protozoa, and animals, are chemoheterotrophs.

Bacteria and fungi can use a wide variety of organic compounds for carbon and energy sources. This is why they can live in diverse environments. Understanding microbial diversity is scientifically interesting and economically important. In some situations microbial growth is undesirable, such as when rubber-degrading bacteria destroy a gasket or shoe sole. However, these same bacteria might be beneficial if they decomposed discarded rubber products, such as used tires. *Rhodococcus erythropolis* (rō-dō-kok′kus er-i-throp′ō-lis) is widely distributed in soil and can cause disease in humans and other animals. However, this same species is able to replace sulfur atoms in petroleum with atoms of oxygen. A Texas company is currently using *R. erythropolis* to produce desulfurized oil.

CHECK YOUR UNDERSTANDING

Almost all medically important microbes belong to which of the four aforementioned groups? **5-23**

* * *

We will next consider how cells use ATP pathways for the synthesis of organic compounds such as carbohydrates, lipids, proteins, and nucleic acids.

Metabolic Pathways of Energy Use

LEARNING OBJECTIVE

5-24 Describe the major types of anabolism and their relationship to catabolism.

Up to now we have been considering energy production. Through the oxidation of organic molecules, organisms produce energy by aerobic respiration, anaerobic respiration, and fermentation. Much of this energy is given off as heat. The complete metabolic oxidation of glucose to carbon dioxide and water is considered a very efficient process, but about 45% of the energy of glucose is lost as heat. Cells use the remaining energy, which is trapped in the bonds of ATP, in a variety of ways. Microbes use ATP to provide energy for the transport of substances across plasma membranes—the process called active transport that we discussed in Chapter 4. Microbes also use some of their energy for flagellar motion (also discussed in Chapter 4). Most of the ATP, however, is used in the production of new cellular components. This production is a continuous process in cells and, in general, is faster in prokaryotic cells than in eukaryotic cells.

Autotrophs build their organic compounds by fixing carbon dioxide in the Calvin-Benson cycle (see Figure 5.26). This requires both energy (ATP) and electrons (from the oxidation of NADPH). Heterotrophs, by contrast, must have a ready source of organic compounds for biosynthesis—the production of needed cellular components, usually from simpler molecules. The cells use these compounds as both the carbon source and the energy source. We will next consider the biosynthesis of a few representative classes of biological molecules: carbohydrates, lipids, amino acids, purines, and pyrimidines. As we do so, keep in mind that synthesis reactions require a net input of energy.

Polysaccharide Biosynthesis

Microorganisms synthesize sugars and polysaccharides. The carbon atoms required to synthesize glucose are derived from the intermediates produced during processes such as glycolysis and the Krebs cycle and from lipids or amino acids. After synthesizing glucose (or other simple sugars), bacteria may assemble it into more complex polysaccharides, such as glycogen. For bacteria to build glucose into glycogen, glucose units must be phosphorylated and linked. The product of glucose phosphorylation is glucose 6-phosphate. Such a process involves the expenditure of energy, usually in the form of ATP. In order for bacteria to synthesize glycogen, a molecule of ATP is added to glucose 6-phosphate to form *adenosine diphosphoglucose (ADPG)* (**Figure 5.29**). Once ADPG is synthesized, it is linked with similar units to form glycogen.

Using a nucleotide called uridine triphosphate (UTP) as a source of energy and glucose 6-phosphate, animals synthesize glycogen (and many other carbohydrates) from *uridine diphosphoglucose, UDPG* (see Figure 5.29). A compound related to UDPG, called *UDP-N-acetylglucosamine (UDPNAc),* is a key starting material in the biosynthesis of peptidoglycan, the substance that forms bacterial cell walls. UDPNAc is formed from fructose 6-phosphate, and the reaction also uses UTP.

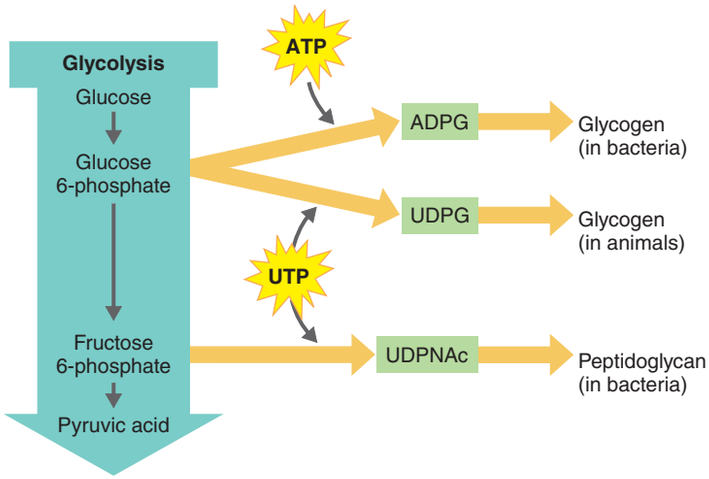


Figure 5.29 The biosynthesis of polysaccharides.

Q How are polysaccharides used in cells?

Lipid Biosynthesis

Because lipids vary considerably in chemical composition, they are synthesized by a variety of routes. Cells synthesize fats by joining glycerol and fatty acids. The glycerol portion of the fat is derived from dihydroxyacetone phosphate, an intermediate formed during glycolysis. Fatty acids, which are long-chain hydrocarbons (hydrogen linked to carbon), are built up when two-carbon fragments of acetyl CoA are successively added to each other (**Figure 5.30**). As with polysaccharide synthesis, the building units of fats and other lipids are linked via dehydration synthesis reactions that require energy, not always in the form of ATP.

The most important role of lipids is to serve as structural components of biological membranes, and most membrane lipids are phospholipids. A lipid of a very different structure, cholesterol, is also found in plasma membranes of eukaryotic cells. Waxes are lipids that are important components of the cell wall of acid-fast bacteria. Other lipids, such as carotenoids, provide the red, orange, and yellow pigments of some microorganisms. Some lipids form portions of chlorophyll molecules. Lipids also function in energy storage. Recall that the breakdown products of lipids after biological oxidation feed into the Krebs cycle.

Amino Acid and Protein Biosynthesis

Amino acids are required for protein biosynthesis. Some microbes, such as *E. coli,* contain the enzymes necessary to use starting materials, such as glucose and inorganic salts, for the synthesis of all the amino acids they need. Organisms with the necessary enzymes can synthesize all amino acids directly or indirectly from intermediates of carbohydrate metabolism (**Figure 5.31a**). Other microbes require that the environment provide some preformed amino acids.

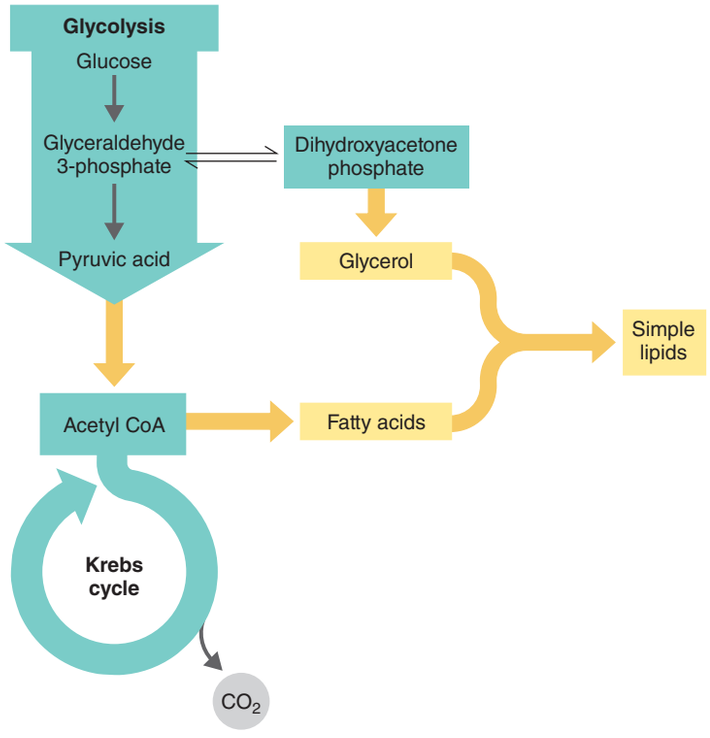


Figure 5.30 **The biosynthesis of simple lipids.**

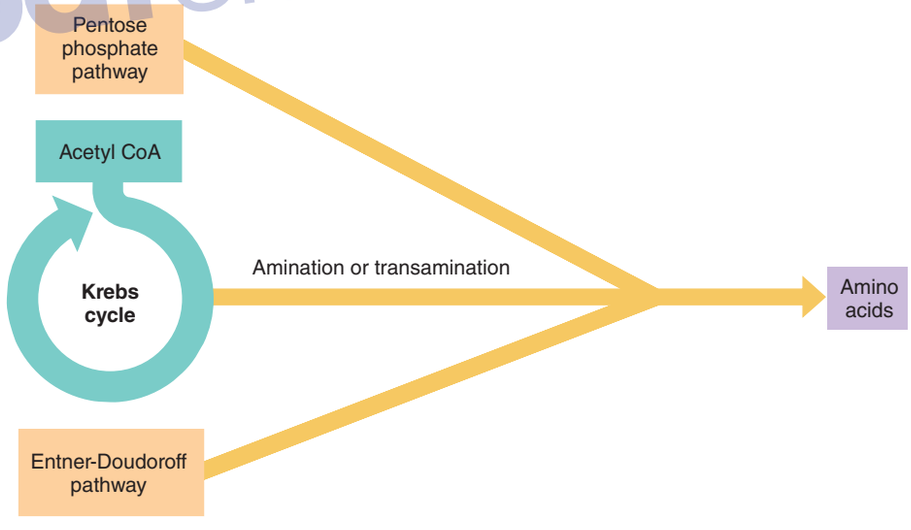
What is the primary use of lipids in cells?

One important source of the *precursors* (intermediates) used in amino acid synthesis is the Krebs cycle. Adding an amine group to pyruvic acid or to an appropriate organic acid of the Krebs cycle converts the acid into an amino acid. This process is called **amination.** If the amine group comes from a preexisting amino acid, the process is called **transamination** (Figure 5.31b).

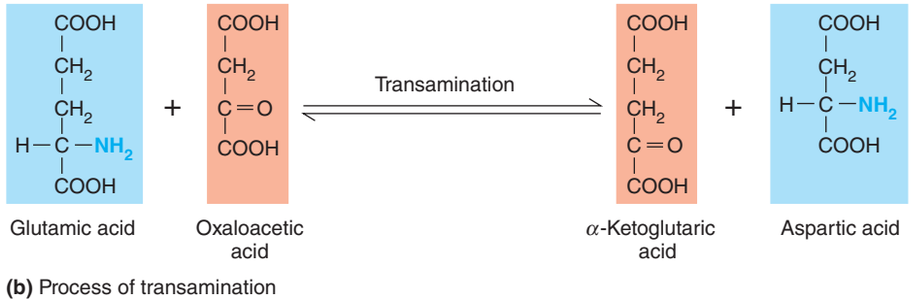
Most amino acids within cells are destined to be building blocks for protein synthesis. Proteins play major roles in the cell as enzymes, structural components, and toxins, to name just a few uses. The joining of amino acids to form proteins involves dehydration synthesis and requires energy in the form of ATP. The mechanism of protein synthesis involves genes and is discussed in Chapter 8.

Purine and Pyrimidine Biosynthesis

Recall from Chapter 2 that the informational molecules DNA and RNA consist of repeating units called *nucleotides,* each of which consists of a purine or pyrimidine, a pentose (five-carbon sugar), and a phosphate group. The five-carbon sugars of nucleotides are derived from either the pentose phosphate pathway or the Entner-Doudoroff pathway. Certain amino acids—aspartic acid, glycine, and glutamine—made from intermediates produced during glycolysis and in the Krebs cycle participate in the biosyntheses of purines and pyrimidines (Figure 5.32). The carbon and



(a) Amino acid biosynthesis



(b) Process of transamination

Figure 5.31 **The biosynthesis of amino acids.**
(**a**) Pathways of amino acid biosynthesis through amination or transamination of intermediates of carbohydrate metabolism from the Krebs cycle, pentose phosphate pathway, and Entner-Doudoroff pathway. (**b**) Transamination, a process by which new amino acids are made with the amine groups from old amino acids. Glutamic acid and aspartic acid are both amino acids; the other two compounds are intermediates in the Krebs cycle.

What is the function of amino acids in cells?

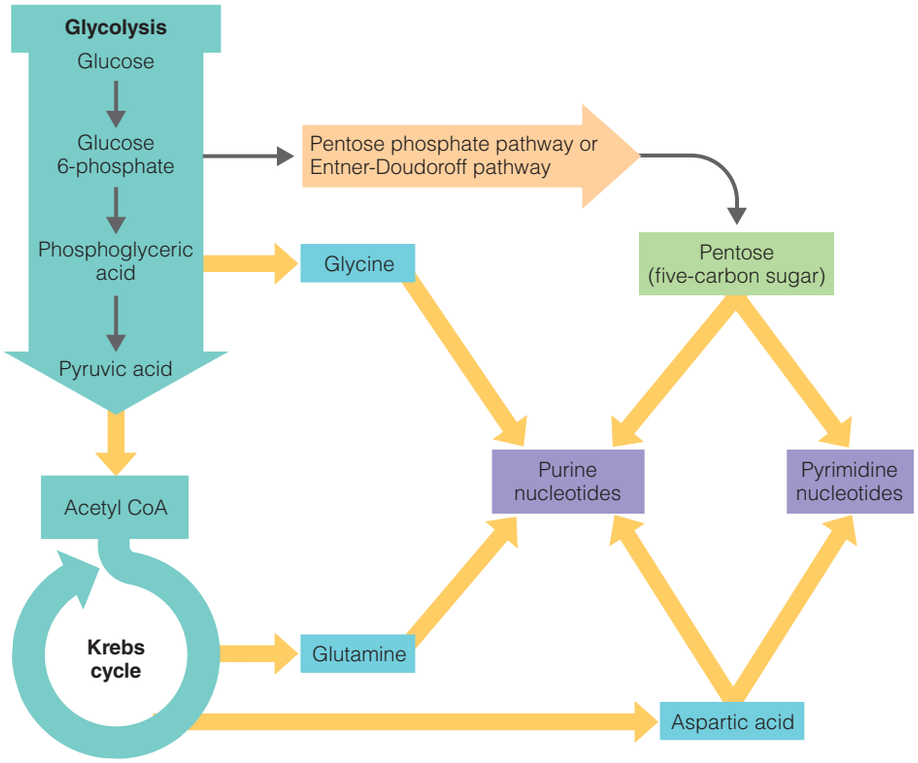


Figure 5.32 The biosynthesis of purine and pyrimidine nucleotides.

Q What are the functions of nucleotides in a cell?

nitrogen atoms derived from these amino acids form the purine and pyrimidine rings, and the energy for synthesis is provided by ATP. DNA contains all the information necessary to determine the specific structures and functions of cells. Both RNA and DNA are required for protein synthesis. In addition, such nucleotides as ATP, NAD^+, and $NADP^+$ assume roles in stimulating and inhibiting the rate of cellular metabolism. The synthesis of DNA and RNA from nucleotides will be discussed in Chapter 8.

CHECK YOUR UNDERSTANDING

✓ Where do amino acids required for protein synthesis come from? **5-24**

The Integration of Metabolism

LEARNING OBJECTIVE

5-25 Define *amphibolic pathways.*

We have seen thus far that the metabolic processes of microbes produce energy from light, inorganic compounds, and organic compounds. Reactions also occur in which energy is used for biosynthesis. With such a variety of activity, you might imagine that anabolic and catabolic reactions occur independently of each other in space and time. Actually, anabolic and catabolic reactions are joined through a group of common intermediates (identified as key intermediates in **Figure 5.33**). Both anabolic and catabolic reactions also share some metabolic pathways, such as the Krebs cycle. For example, reactions in the Krebs cycle not only participate in the oxidation of glucose but also produce intermediates that can be converted to amino acids. Metabolic pathways that function in both anabolism and catabolism are called **amphibolic pathways,** meaning that they are dual-purpose.

Amphibolic pathways bridge the reactions that lead to the breakdown and synthesis of carbohydrates, lipids, proteins, and nucleotides. Such pathways enable simultaneous reactions to occur in which the breakdown product formed in one reaction is used in another reaction to synthesize a different compound, and vice versa. Because various intermediates are common to both anabolic and catabolic reactions, mechanisms exist that regulate synthesis and breakdown pathways and allow these reactions to occur simultaneously. One such mechanism involves the use of different coenzymes for opposite pathways. For example, NAD^+ is involved in catabolic reactions, whereas $NADP^+$ is involved in anabolic reactions. Enzymes can also coordinate anabolic and catabolic reactions by accelerating or inhibiting the rates of biochemical reactions.

The energy stores of a cell can also affect the rates of biochemical reactions. For example, if ATP begins to accumulate, an enzyme shuts down glycolysis; this control helps to synchronize the rates of glycolysis and the Krebs cycle. Thus, if citric acid consumption increases, either because of a demand for more ATP or because anabolic pathways are draining off intermediates of the citric acid cycle, glycolysis accelerates and meets the demand. MM **Animation** Metabolism: The Big picture

CHECK YOUR UNDERSTANDING

✓ Summarize the integration of metabolic pathways using peptidoglycan synthesis as an example. **5-25**

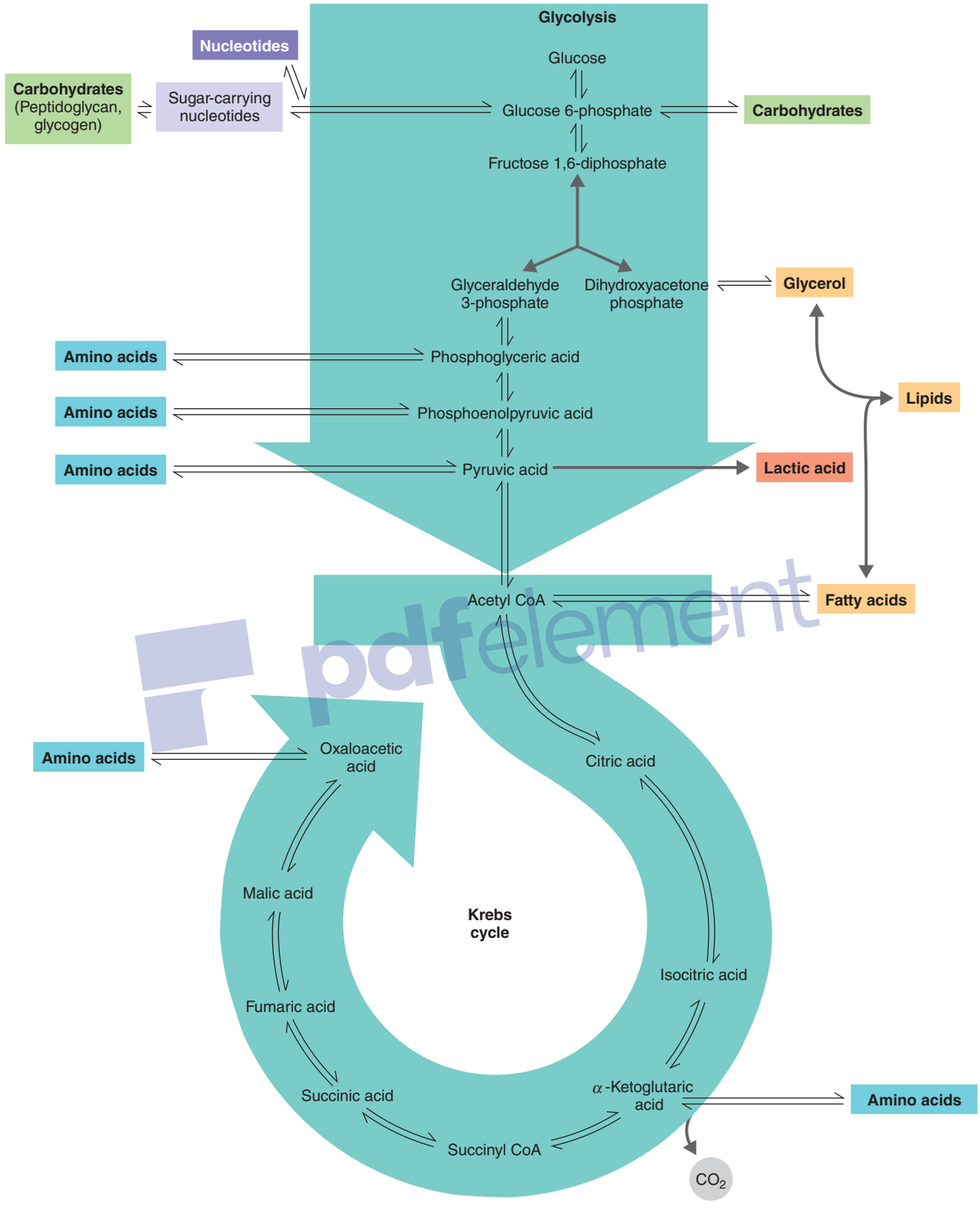


Figure 5.33 **The integration of metabolism.** Key intermediates are shown. Although not indicated in the figure, amino acids and ribose are used in the synthesis of purine and pyrimidine nucleotides (see Figure 5.32). The double arrows indicate amphibolic pathways.

Q What is the purpose of an amphibolic pathway?

Study Outline

MasteringMICROBIOLOGY™

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Catabolic and Anabolic Reactions (pp. 112–113)

1. The sum of all chemical reactions within a living organism is known as metabolism.
2. Catabolism refers to chemical reactions that result in the breakdown of more complex organic molecules into simpler substances. Catabolic reactions usually release energy.
3. Anabolism refers to chemical reactions in which simpler substances are combined to form more complex molecules. Anabolic reactions usually require energy.
4. The energy of catabolic reactions is used to drive anabolic reactions.
5. The energy for chemical reactions is stored in ATP.

Enzymes (pp. 113–119)

1. Enzymes are proteins, produced by living cells, that catalyze chemical reactions by lowering the activation energy.
2. Enzymes are generally globular proteins with characteristic three-dimensional shapes.
3. Enzymes are efficient, can operate at relatively low temperatures, and are subject to various cellular controls.

Naming Enzymes (p. 114)

4. Enzyme names usually end in *-ase*.
5. The six classes of enzymes are defined on the basis of the types of reactions they catalyze.

Enzyme Components (pp. 114–115)

6. Most enzymes are holoenzymes, consisting of a protein portion (apoenzyme) and a nonprotein portion (cofactor).
7. The cofactor can be a metal ion (iron, copper, magnesium, manganese, zinc, calcium, or cobalt) or a complex organic molecule known as a coenzyme (NAD^+, $NADP^+$, FMN, FAD, or coenzyme A).

The Mechanism of Enzymatic Action (pp. 115–116)

8. When an enzyme and substrate combine, the substrate is transformed, and the enzyme is recovered.
9. Enzymes are characterized by specificity, which is a function of their active sites.

Factors Influencing Enzymatic Activity (pp. 116–118)

10. At high temperatures, enzymes undergo denaturation and lose their catalytic properties; at low temperatures, the reaction rate decreases.
11. The pH at which enzymatic activity is maximal is known as the optimum pH.
12. Enzymatic activity increases as substrate concentration increases until the enzymes are saturated.
13. Competitive inhibitors compete with the normal substrate for the active site of the enzyme. Noncompetitive inhibitors act on other parts of the apoenzyme or on the cofactor and decrease the enzyme's ability to combine with the normal substrate.

Feedback Inhibition (pp. 118–119)

14. Feedback inhibition occurs when the end-product of a metabolic pathway inhibits an enzyme's activity near the start of the pathway.

Ribozymes (p. 119)

15. Ribozymes are enzymatic RNA molecules that cut and splice RNA in eukaryotic cells.

Energy Production (pp. 119–121)

Oxidation-Reduction Reactions (p. 120)

1. Oxidation is the removal of one or more electrons from a substrate. Protons (H^+) are often removed with the electrons.
2. Reduction of a substrate refers to its gain of one or more electrons.
3. Each time a substance is oxidized, another is simultaneously reduced.
4. NAD^+ is the oxidized form; NADH is the reduced form.
5. Glucose is a reduced molecule; energy is released during a cell's oxidation of glucose.

The Generation of ATP (pp. 120–121)

6. Energy released during certain metabolic reactions can be trapped to form ATP from ADP and P_i (phosphate). Addition of a P_i to a molecule is called phosphorylation.
7. During substrate-level phosphorylation, a high-energy P from an intermediate in catabolism is added to ADP.
8. During oxidative phosphorylation, energy is released as electrons are passed to a series of electron acceptors (an electron transport chain) and finally to O_2 or another inorganic compound.
9. During photophosphorylation, energy from light is trapped by chlorophyll, and electrons are passed through a series of electron acceptors. The electron transfer releases energy used for the synthesis of ATP.

Metabolic Pathways of Energy Production (p. 121)

10. A series of enzymatically catalyzed chemical reactions called metabolic pathways store energy in and release energy from organic molecules.

Carbohydrate Catabolism (pp. 122–133)

1. Most of a cell's energy is produced from the oxidation of carbohydrates.
2. Glucose is the most commonly used carbohydrate.
3. The two major types of glucose catabolism are respiration, in which glucose is completely broken down, and fermentation, in which it is partially broken down.

Glycolysis (pp. 122–123)

4. The most common pathway for the oxidation of glucose is glycolysis. Pyruvic acid is the end-product.

5. Two ATP and two NADH molecules are produced from one glucose molecule.

Alternatives to Glycolysis (pp. 123, 125)

6. The pentose phosphate pathway is used to metabolize five-carbon sugars; one ATP and 12 NADPH molecules are produced from one glucose molecule.
7. The Entner-Doudoroff pathway yields one ATP and two NADPH molecules from one glucose molecule.

Cellular Respiration (pp. 125–130)

8. During respiration, organic molecules are oxidized. Energy is generated from the electron transport chain.
9. In aerobic respiration, O_2 functions as the final electron acceptor.
10. In anaerobic respiration, the final electron acceptor is usually an inorganic molecule other than O_2.
11. Decarboxylation of pyruvic acid produces one CO_2 molecule and one acetyl group.
12. Two-carbon acetyl groups are oxidized in the Krebs cycle. Electrons are picked up by NAD^+ and FAD for the electron transport chain.
13. From one molecule of glucose, oxidation produces six molecules of NADH, two molecules of $FADH_2$, and two molecules of ATP.
14. Decarboxylation produces six molecules of CO_2.
15. Electrons are brought to the electron transport chain by NADH.
16. The electron transport chain consists of carriers, including flavoproteins, cytochromes, and ubiquinones.
17. Protons being pumped across the membrane generate a proton motive force as electrons move through a series of acceptors or carriers.
18. Energy produced from movement of the protons back across the membrane is used by ATP synthase to make ATP from ADP and P_i.
19. In eukaryotes, electron carriers are located in the inner mitochondrial membrane; in prokaryotes, electron carriers are in the plasma membrane.
20. In aerobic prokaryotes, 38 ATP molecules can be produced from complete oxidation of a glucose molecule in glycolysis, the Krebs cycle, and the electron transport chain.
21. In eukaryotes, 36 ATP molecules are produced from complete oxidation of a glucose molecule.
22. The final electron acceptors in anaerobic respiration include NO_3^-, SO_4^{2-}, and CO_3^{2-}.
23. The total ATP yield is less than in aerobic respiration because only part of the Krebs cycle operates under anaerobic conditions.

Fermentation (pp. 130–133)

24. Fermentation releases energy from sugars or other organic molecules by oxidation.
25. O_2 is not required in fermentation.
26. Two ATP molecules are produced by substrate-level phosphorylation.
27. Electrons removed from the substrate reduce NAD^+.
28. The final electron acceptor is an organic molecule.
29. In lactic acid fermentation, pyruvic acid is reduced by NADH to lactic acid.
30. In alcohol fermentation, acetaldehyde is reduced by NADH to produce ethanol.
31. Heterolactic fermenters can use the pentose phosphate pathway to produce lactic acid and ethanol.

Lipid and Protein Catabolism (pp. 133–135)

1. Lipases hydrolyze lipids into glycerol and fatty acids.
2. Fatty acids and other hydrocarbons are catabolized by beta-oxidation.
3. Catabolic products can be further broken down in glycolysis and the Krebs cycle.
4. Before amino acids can be catabolized, they must be converted to various substances that enter the Krebs cycle.
5. Transamination, decarboxylation, and dehydrogenation reactions convert the amino acids to be catabolized.

Biochemical Tests and Bacterial Identification (pp. 135–137)

1. Bacteria and yeast can be identified by detecting action of their enzymes.
2. Fermentation tests are used to determine whether an organism can ferment a carbohydrate to produce acid and gas.

Photosynthesis (pp. 137–139)

1. Photosynthesis is the conversion of light energy from the sun into chemical energy; the chemical energy is used for carbon fixation.

The Light-Dependent Reactions: Photophosphorylation (p. 138)

2. Chlorophyll *a* is used by green plants, algae, and cyanobacteria; it is found in thylakoid membranes.
3. Electrons from chlorophyll pass through an electron transport chain, from which ATP is produced by chemiosmosis.
4. Photosystems are made up of chlorophyll and other pigments packed into thylakoid membranes.
5. In cyclic photophosphorylation, the electrons return to the chlorophyll.
6. In noncyclic photophosphorylation, the electrons are used to reduce $NADP^+$. The electrons from H_2O or H_2S replace those lost from chlorophyll.
7. When H_2O is oxidized by green plants, algae, and cyanobacteria, O_2 is produced; when H_2S is oxidized by the sulfur bacteria, S^0 granules are produced.

The Light-Independent Reactions: The Calvin-Benson Cycle (pp. 138–139)

8. CO_2 is used to synthesize sugars in the Calvin-Benson cycle.

A Summary of Energy Production Mechanisms (pp. 139–140)

1. Sunlight is converted to chemical energy in oxidation-reduction reactions carried on by phototrophs. Chemotrophs can use this chemical energy.
2. In oxidation-reduction reactions, energy is derived from the transfer of electrons.
3. To produce energy, a cell needs an electron donor (organic or inorganic), a system of electron carriers, and a final electron acceptor (organic or inorganic).

Metabolic Diversity among Organisms

(pp. 140–143)

1. Photoautotrophs obtain energy by photophosphorylation and fix carbon from CO_2 via the Calvin-Benson cycle to synthesize organic compounds.
2. Cyanobacteria are oxygenic phototrophs. Green bacteria and purple bacteria are anoxygenic phototrophs.
3. Photoheterotrophs use light as an energy source and an organic compound for their carbon source and electron donor.
4. Chemoautotrophs use inorganic compounds as their energy source and carbon dioxide as their carbon source.
5. Chemoheterotrophs use complex organic molecules as their carbon and energy sources.

Metabolic Pathways of Energy Use (pp. 144–145)

Polysaccharide Biosynthesis (p. 144)

1. Glycogen is formed from ADPG.
2. UDPNAc is the starting material for the biosynthesis of peptidoglycan.

Lipid Biosynthesis (p. 144)

3. Lipids are synthesized from fatty acids and glycerol.
4. Glycerol is derived from dihydroxyacetone phosphate, and fatty acids are built from acetyl CoA.

Amino Acid and Protein Biosynthesis (pp. 144–145)

5. Amino acids are required for protein biosynthesis.
6. All amino acids can be synthesized either directly or indirectly from intermediates of carbohydrate metabolism, particularly from the Krebs cycle.

Purine and Pyrimidine Biosynthesis (p. 146)

7. The sugars composing nucleotides are derived from either the pentose phosphate pathway or the Entner-Doudoroff pathway.
8. Carbon and nitrogen atoms from certain amino acids form the backbones of the purines and pyrimidines.

The Integration of Metabolism (pp. 146–147)

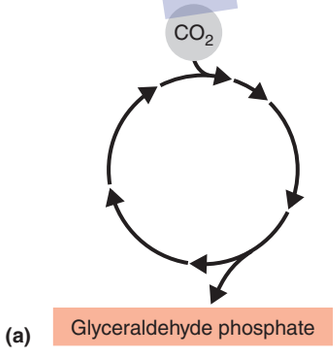
1. Anabolic and catabolic reactions are integrated through a group of common intermediates.
2. Such integrated metabolic pathways are referred to as amphibolic pathways.

Study Questions

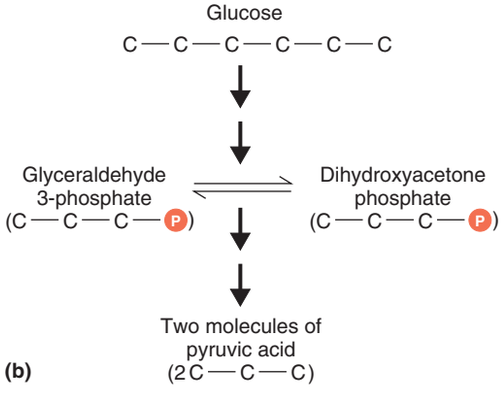
Answers to the Review and Multiple Choice questions can be found by turning to the Answers tab at the back of the textbook.

Review

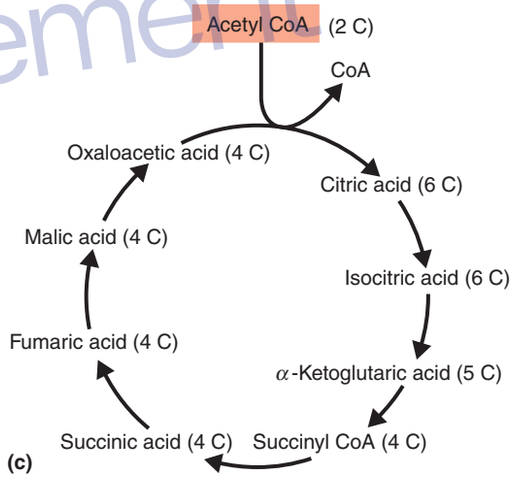
Use the following diagrams (a), (b), and (c) for question 1.



(a)

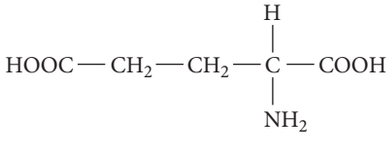


(b)



(c)

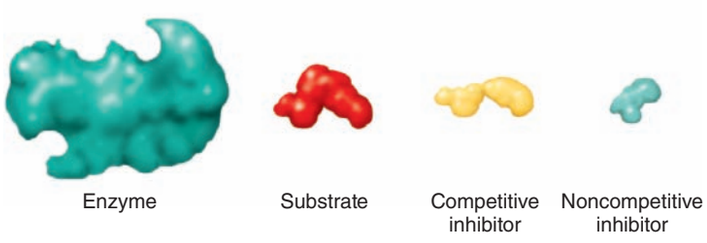
1. Name pathways diagrammed in parts (a), (b), and (c) of the figure.
   - **a.** Show where glycerol is catabolized and where fatty acids are catabolized.
   - **b.** Show where glutamic acid (an amino acid) is catabolized:



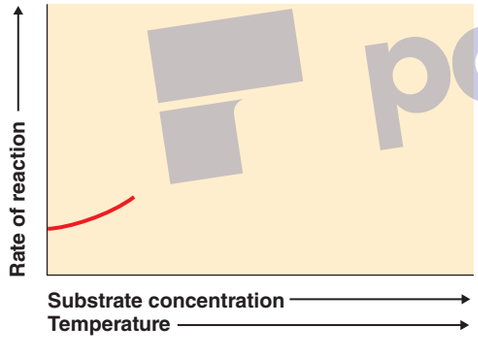
   - **c.** Show how these pathways are related.
   - **d.** Where is ATP required in pathways (a) and (b)?
   - **e.** Where is CO_2 released in pathways (b) and (c)?

f. Show where a long-chain hydrocarbon such as petroleum is catabolized.
g. Where is NADH (or $FADH_2$ or NADPH) used and produced in these pathways?
h. Identify four places where anabolic and catabolic pathways are integrated.

2. DRAW IT Using the diagrams below, show each of the following:
a. where the substrate will bind
b. where the competitive inhibitor will bind
c. where the noncompetitive inhibitor will bind
d. which of the four elements could be the inhibitor in feedback inhibition
e. What effect will the reactions in (a), (b), and (c) have?

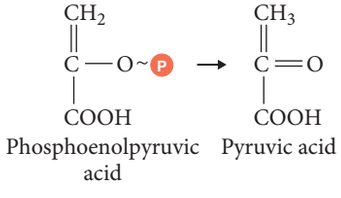


3. DRAW IT An enzyme and substrate are combined. The rate of reaction begins as shown in the following graph. To complete the graph, show the effect of increasing substrate concentration on a constant enzyme concentration. Show the effect of increasing temperature.



4. Define *oxidation-reduction*, and differentiate the following terms:
a. aerobic and anaerobic respiration
b. respiration and fermentation
c. cyclic and noncyclic photophosphorylation

5. There are three mechanisms for the phosphorylation of ADP to produce ATP. Write the name of the mechanism that describes each of the reactions in the following table.

ATP Generated by	Reaction
a. _____	An electron, liberated from chlorophyll by light, is passed down an electron transport chain.
b. _____	Cytochrome *c* passes two electrons to cytochrome *a*.
c. _____	

Phosphoenolpyruvic acid Pyruvic acid

6. All of the energy-producing biochemical reactions that occur in cells, such as photophosphorylation and glycolysis, are _____ reactions.

7. Fill in the following table with the carbon source and energy source of each type of organism.

Organism	Carbon Source	Energy Source
Photoautotroph	a. _____	b. _____
Photoheterotroph	c. _____	d. _____
Chemoautotroph	e. _____	f. _____
Chemoheterotroph	g. _____	h. _____

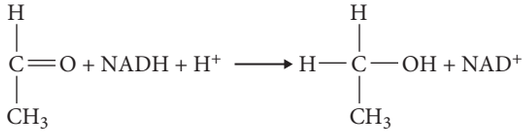
8. Write your own definition of the chemiosmotic mechanism of ATP generation. On Figure 5.16, mark the following using the appropriate letter:
a. the acidic side of the membrane
b. the side with a positive electrical charge
c. potential energy
d. kinetic energy

9. Why must NADH be reoxidized? How does this happen in an organism that uses respiration? Fermentation?

10. NAME IT What nutritional type is a colorless microbe that uses the Calvin cycle, uses H_2 as the electron donor to its ETC, and uses elemental S as the final electron acceptor in the ETC?

Multiple Choice

1. Which substance in the following reaction is being reduced?



Acetaldehyde Ethanol

a. acetaldehyde
b. NADH
c. ethanol
d. NAD^+

2. Which of the following reactions produces the most molecules of ATP during aerobic metabolism?
a. glucose → glucose 6-phosphate
b. phosphoenolpyruvic acid → pyruvic acid
c. glucose → pyruvic acid
d. acetyl CoA → CO_2 + H_2O
e. succinic acid → fumaric acid

3. Which of the following processes does *not* generate ATP?
a. photophosphorylation
b. the Calvin-Benson cycle
c. oxidative phosphorylation
d. substrate-level phosphorylation
e. none of the above

4. Which of the following compounds has the greatest amount of energy for a cell?
a. CO_2
b. ATP
c. glucose
d. O_2
e. lactic acid

5. Which of the following is the best definition of the Krebs cycle?
   a. the oxidation of pyruvic acid
   b. the way cells produce CO_2
   c. a series of chemical reactions in which NADH is produced from the oxidation of pyruvic acid
   d. a method of producing ATP by phosphorylating ADP
   e. a series of chemical reactions in which ATP is produced from the oxidation of pyruvic acid
6. Which of the following is the best definition of *respiration*?
   a. a sequence of carrier molecules with O_2 as the final electron acceptor
   b. a sequence of carrier molecules with an inorganic molecule as the final electron acceptor
   c. a method of generating ATP
   d. the complete oxidation of glucose to CO_2 and H_2O
   e. a series of reactions in which pyruvic acid is oxidized to CO_2 and H_2O

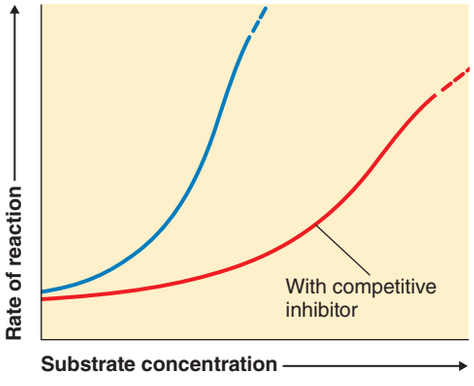
Use the following choices to answer questions 7–10.

a. *E. coli* growing in glucose broth at 35°C with O_2 for 5 days
b. *E. coli* growing in glucose broth at 35°C without O_2 for 5 days
c. both a and b
d. neither a nor b

7. Which culture produces the most lactic acid?
8. Which culture produces the most ATP?
9. Which culture uses NAD^+?
10. Which culture uses the most glucose?

Critical Thinking

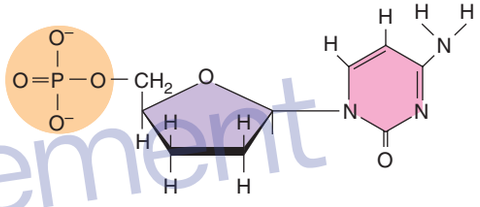
1. Explain why, even under ideal conditions, *Streptococcus* grows slowly.
2. The following graph shows the normal rate of reaction of an enzyme and its substrate (blue) and the rate when an excess of competitive inhibitor is present (red). Explain why the graph appears as it does.



3. Compare and contrast carbohydrate catabolism and energy production in the following bacteria:
   a. *Pseudomonas,* an aerobic chemoheterotroph
   b. *Spirulina,* an oxygenic photoautotroph
   c. *Ectothiorhodospira,* an anoxygenic photoautotroph
4. How much ATP could be obtained from the complete oxidation of one molecule of glucose? From one molecule of butterfat containing one glycerol and three 12-carbon chains?
5. The chemoautotroph *Thiobacillus* can obtain energy from the oxidation of arsenic ($As^{3+} \rightarrow As^{5+}$). How does this reaction provide energy? How can humans put this bacterium to use?

Clinical Applications

1. *Haemophilus influenzae* requires hemin (X factor) to synthesize cytochromes and NAD^+ (V factor) from other cells. For what does it use these two growth factors? What diseases does *H. influenzae* cause?
2. The drug Hivid, also called ddC, inhibits DNA synthesis. It is used to treat HIV infection and AIDS. Compare the following illustration of ddC to Figure 2.16 on page 46. How does this drug work?



3. The bacterial enzyme streptokinase is used to digest fibrin (blood clots) in patients with atherosclerosis. Why doesn't injection of streptokinase cause a streptococcal infection? How do we know the streptokinase will digest fibrin only and not good tissues?