Cell Chemistry and Biosynthesis

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It is at first sight difficult to accept the idea that each of the living creatures described in Chapter 1 is merely a chemical system. The incredible diversity of living forms, their seemingly purposeful behavior, and their ability to grow and reproduce appear to set them apart from the world of solids, liquids, and gases that chemistry normally describes. Indeed, until the nineteenth century animals were believed to contain a Vital Force—an "animus"—that was responsible for their distinctive properties.

We now know there is nothing in living organisms that disobeys chemical and physical laws. However, the chemistry of life is special. First, it is based overwhelmingly on carbon compounds, whose study is therefore known as *organic chemistry*. Second, cells are 70 percent water, and life depends largely on chemical reactions that take place in aqueous solution. Third, and most important, cell chemistry is enormously complex: even the simplest cell is vastly more complicated in its chemistry than any other chemical system known. Although cells contain a variety of small carbon-containing molecules, most of the carbon atoms in cells are incorporated into enormous *polymeric molecules*—chains of chemical subunits linked end-to-end. It is the unique properties of these macromolecules that enable cells and organisms to grow and reproduce—as well as to do all the other things that are characteristic of life.

THE CHEMICAL COMPONENTS OF A CELL

Matter is made of combinations of *elements*—substances such as hydrogen or carbon that cannot be broken down or converted into other substances by chemical means. The smallest particle of an element that still retains its distinctive chemical properties is an *atom* (**Figure 2–1**). However, the characteristics of substances other than pure elements—including the materials from which living cells are made—depend on the way their atoms are linked together in groups to form *molecules*. In order to understand how living organisms are built from inanimate matter, therefore, it is crucial to know how all of the chemical bonds that hold atoms together in molecules are formed.

Cells Are Made From a Few Types of Atoms

The **atomic weight** of an atom, or the **molecular weight** of a molecule, is its mass relative to that of a hydrogen atom. This is essentially equal to the number of protons plus neutrons that the atom or molecule contains, since the electrons are much lighter and contribute almost nothing to the total. Thus the major isotope of carbon has an atomic weight of 12 and is symbolized as ^{12}C, whereas an unstable isotope of carbon has an atomic weight of 14 and is written as ^{14}C. The mass of an atom or a molecule is often specified in *daltons*, one dalton being an atomic mass unit approximately equal to the mass of a hydrogen atom.

Atoms are so small that it is hard to imagine their size. An individual carbon atom is roughly 0.2 nm in diameter, so that it would take about 5 million of them, laid out in a straight line, to span a millimeter. One proton or neutron weighs

carbon atom
atomic number = 6
atomic weight = 12

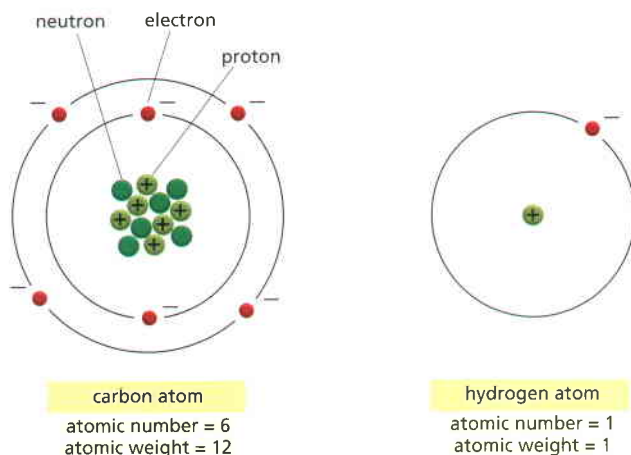hydrogen atom
atomic number = 1
atomic weight = 1

Figure 2–1 Highly schematic representations of an atom of carbon and an atom of hydrogen. The *nucleus* of every atom except hydrogen consists of both positively charged *protons* and electrically neutral *neutrons*. The number of electrons in an atom is equal to its number of protons (the *atomic number*), so that the atom has no net charge. Because it is the electrons that determine the chemical behavior of an atom, all of the atoms of a given element have the same atomic number.

Neutrons are uncharged subatomic particles of essentially the same mass as protons. They contribute to the structural stability of the nucleus—if there are too many or too few, the nucleus may disintegrate by radioactive decay—but they do not alter the chemical properties of the atom. Because of neutrons, an element can exist in several physically distinguishable but chemically identical forms, called *isotopes,* each isotope having a different number of neutrons but the same number of protons. Multiple isotopes of almost all the elements occur naturally, including some that are unstable. For example, while most carbon on Earth exists as the stable isotope carbon 12, with six protons and six neutrons, there are also small amounts of an unstable isotope, the radioactive carbon 14, whose atoms have six protons and eight neutrons. Carbon 14 undergoes radioactive decay at a slow but steady rate. This forms the basis for a technique known as carbon 14 dating, which is used in archaeology to determine the time of origin of organic materials.

The neutrons, protons, and electrons are in reality minute in relation to the atom as a whole; their size is greatly exaggerated here. In addition, the diameter of the nucleus is only about 10^{-4} that of the electron cloud. Finally, although the electrons are shown here as individual particles, in reality their behavior is governed by the laws of quantum mechanics, and there is no way of predicting exactly where an electron is at any given instant of time.

approximately $1/(6 \times 10^{23})$ gram, so one gram of hydrogen contains 6×10^{23} atoms. This huge number (6×10^{23}, called **Avogadro's number**) is the key scale factor describing the relationship between everyday quantities and quantities measured in terms of individual atoms or molecules. If a substance has a molecular weight of X, 6×10^{23} molecules of it will have a mass of X grams. This quantity is called one **mole** of the substance (**Figure 2–2**).

There are 89 naturally occurring elements, each differing from the others in the number of protons and electrons in its atoms. Living organisms, however, are made of only a small selection of these elements, four of which—carbon (C), hydrogen (H), nitrogen (N), and oxygen (O)—make up 96.5% of an organism's weight. This composition differs markedly from that of the nonliving inorganic environment (**Figure 2–3**) and is evidence of a distinctive type of chemistry.

The Outermost Electrons Determine How Atoms Interact

To understand how atoms bond together to form the molecules that make up living organisms, we focus on their electrons. Protons and neutrons are welded tightly to one another in the nucleus and change partners only under extreme conditions—during radioactive decay, for example, or in the interior of the sun or of a nuclear reactor. In living tissues, it is only the electrons of an atom that undergo rearrangements. They form the exterior of an atom and specify the rules of chemistry by which atoms combine to form molecules.

Electrons are in continuous motion around the nucleus, but motions on this submicroscopic scale obey very different laws from those familiar in everyday life. These laws dictate that electrons in an atom can exist only in certain discrete states, called orbitals, and that there is a strict limit to the number of electrons that can be accommodated in an orbital of a given type—a so-called *electron shell*. The electrons closest on average to the positive nucleus are attracted most strongly to it and occupy the innermost, most tightly bound shell. This shell holds a maximum of two electrons. The second shell is farther away from the nucleus, and its electrons are less tightly bound. This second shell holds up to eight electrons. The third shell contains electrons that are even less tightly bound; it also holds up to eight electrons. The fourth and fifth shells can hold 18 electrons each. Atoms with more than four shells are very rare in biological molecules.

The electron arrangement of an atom is most stable when all the electrons are in the most tightly bound states that are possible—that is, when they occupy the innermost shells. Therefore, with certain exceptions in the larger atoms, the electrons of an atom fill the orbitals in order—the first shell before the second, the second before the third, and so on. An atom whose outermost shell is entirely filled with electrons is especially stable and therefore chemically unreactive. Examples are helium with 2 electrons, neon with 2 + 8, and argon with 2 + 8 + 8; these are all inert gases. Hydrogen, by contrast, with only one electron

and only a half-filled shell, is highly reactive. Likewise, the other atoms found in living tissues have incomplete outer electron shells and can donate, accept, or share electrons with each other to form both molecules and ions (**Figure 2–4**).

Because an unfilled electron shell is less stable than a filled one, atoms with incomplete outer shells tend to interact with other atoms in a way that causes them to either gain or lose enough electrons to achieve a completed outermost shell. This electron exchange occurs either by transferring electrons from one atom to another or by sharing electrons between two atoms. These two strategies generate two types of **chemical bonds** between atoms: an *ionic bond* is formed when electrons are donated by one atom to another, whereas a *covalent bond* is formed when two atoms share a pair of electrons (**Figure 2–5**). Often, the pair of electrons is shared unequally, with a partial transfer between two atoms that attract electrons differently—one more *electronegative* than the other: this intermediate strategy results in a *polar covalent bond*, as we shall discuss later.

An H atom, which needs only one electron to fill its shell, generally acquires it by electron sharing, forming one covalent bond with another atom; often this bond is polar—meaning that the electrons are shared unequally. The other common elements in living cells—C, N, and O, with an incomplete second shell, and P and S, with an incomplete third shell (see Figure 2–4)—generally share electrons and achieve a filled outer shell of eight electrons by forming several covalent bonds. The number of electrons that an atom must acquire or lose (either by sharing or by transfer) to fill its outer shell is known as its *valence*.

The crucial role of the outer electron shell in determining the chemical properties of an element means that, when the elements are listed in order of their atomic number, there is a periodic recurrence of elements with similar properties: an element with, say, an incomplete second shell containing one electron will behave in much the same way as an element that has filled its second shell

A **mole** is X grams of a substance, where X is its relative molecular mass (molecular weight). A mole will contain 6×10^{23} molecules of the substance.

1 mole of carbon weighs 12 g
1 mole of glucose weighs 180 g
1 mole of sodium chloride weighs 58 g

Molar solutions have a concentration of 1 mole of the substance in 1 liter of solution. A molar solution (denoted as 1 M) of glucose, for example, has 180 g/l, while a millimolar solution (1 mM) has 180 mg/l.

The standard abbreviation for gram is g; the abbreviation for liter is l.

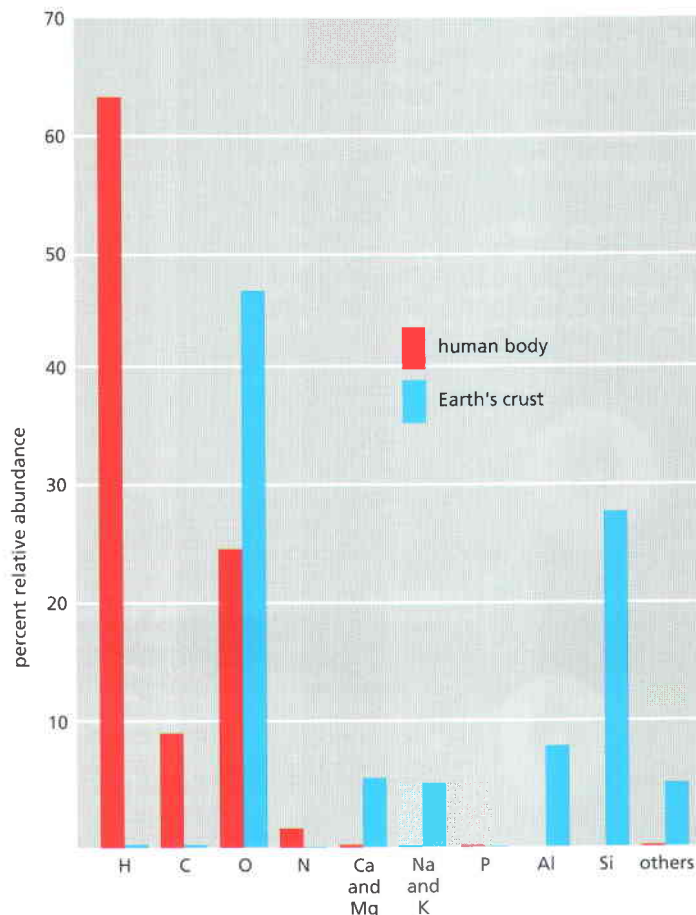**Figure 2–2 Moles and molar solutions.**

Figure 2–3 The abundances of some chemical elements in the nonliving world (the Earth's crust) compared with their abundances in the tissues of an animal. The abundance of each element is expressed as a percentage of the total number of atoms present including water. Thus, because of the abundance of water, more than 60% of the atoms in a living organism are hydrogen atoms. The relative abundance of elements is similar in all living things.

atomic number

	element	I	II	III	IV
1	Hydrogen	●			
2	Helium	●●			
6	Carbon	●●	●●●●		
7	Nitrogen	●●	●●●●●		
8	Oxygen	●●	●●●●●●		
10	Neon	●●	●●●●●●●●		
11	Sodium	●●	●●●●●●●●	●	
12	Magnesium	●●	●●●●●●●●	●●	
15	Phosphorus	●●	●●●●●●●●	●●●●●	
16	Sulfur	●●	●●●●●●●●	●●●●●●	
17	Chlorine	●●	●●●●●●●●	●●●●●●●	
18	Argon	●●	●●●●●●●●	●●●●●●●●	
19	Potassium	●●	●●●●●●●●	●●●●●●●●	●
20	Calcium	●●	●●●●●●●●	●●●●●●●●	●●

Figure 2–4 Filled and unfilled electron shells in some common elements. All the elements commonly found in living organisms have unfilled outermost shells *(red)* and can thus participate in chemical reactions with other atoms. For comparison, some elements that have only filled shells *(yellow)* are shown; these are chemically unreactive.

and has an incomplete third shell containing one electron. The metals, for example, have incomplete outer shells with just one or a few electrons, whereas, as we have just seen, the inert gases have full outer shells. This pattern gives rise to the famous *periodic table* of the elements, presented in **Figure 2–6** with the elements found in living organisms highlighted.

Covalent Bonds Form by the Sharing of Electrons

All the characteristics of a cell depend on the molecules it contains. A **molecule** is defined as a cluster of atoms held together by **covalent bonds**; here electrons are shared between atoms to complete the outer shells, rather than being transferred between them. In the simplest possible molecule—a molecule of hydrogen (H_2)—two H atoms, each with a single electron, share two electrons, which is the number required to fill the first shell. These shared electrons form a cloud of negative charge that is densest between the two positively charged nuclei and helps to hold them together, in opposition to the mutual repulsion between like charges that would otherwise force them apart. The attractive and repulsive forces are in balance when the nuclei are separated by a characteristic distance, called the *bond length*.

Another property of any bond—covalent or noncovalent—is its *bond strength*, which is measured by the amount of energy that must be supplied to break that bond. This is often expressed in units of kilocalories per mole (kcal/mole), where a kilocalorie is the amount of energy needed to raise the temperature of one liter
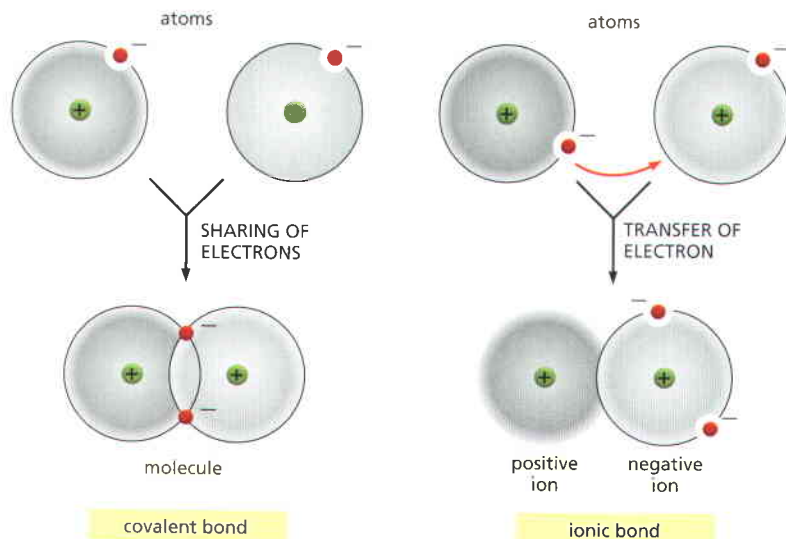

atoms

SHARING OF ELECTRONS

molecule

covalent bond

atoms

TRANSFER OF ELECTRON

positive   negative
ion   ion

ionic bond

Figure 2–5 Comparison of covalent and ionic bonds. Atoms can attain a more stable arrangement of electrons in their outermost shell by interacting with one another. An ionic bond is formed when electrons are transferred from one atom to the other. A covalent bond is formed when electrons are shared between atoms. The two cases shown represent extremes; often, covalent bonds form with a partial transfer (unequal sharing of electrons), resulting in a polar covalent bond (see Figure 2–43).

atomic number

Elements fall into groups that show similar properties based on the number of electrons each element possesses in its outer shell. For example, Mg and Ca tend to give away the two electrons in their outer shells; C, N, and O complete their second shells by sharing electrons. The four elements highlighted in *red* constitute 99% of the total number of atoms present in the human body. An additional seven elements, highlighted in *blue,* together represent about 0.9% of the total. Other elements, shown in *green,* are required in trace amounts by humans. It remains unclear whether those elements shown in *yellow* are essential in humans or not. The chemistry of life, it seems, is therefore predominantly the chemistry of lighter elements.

Atomic weights, given by the sum of the protons and neutrons in the atomic nucleus, will vary with the particular isotope of the element. The atomic weights shown here are those of the most common isotope of each element.

of water by one degree Celsius (centigrade). Thus if 1 kilocalorie must be supplied to break 6×10^{23} bonds of a specific type (that is, 1 mole of these bonds), then the strength of that bond is 1 kcal/mole. An equivalent, widely used measure of energy is the kilojoule, which is equal to 0.239 kilocalories.

To understand bond strengths, it is helpful to compare them with the average energies of the impacts that molecules are constantly experiencing from collisions with other molecules in their environment (their thermal, or heat, energy), as well as with other sources of biological energy such as light and glucose oxidation (**Figure 2–7**). Typical covalent bonds are stronger than the thermal energies by a factor of 100, so they resist being pulled apart by thermal motions and are normally broken only during specific chemical reactions with other atoms and molecules. The making and breaking of covalent bonds are violent events, and in living cells they are carefully controlled by highly specific catalysts, called *enzymes*. Noncovalent bonds as a rule are much weaker; we shall see later that they are important in the cell in the many situations where molecules have to associate and dissociate readily to carry out their functions.

Whereas an H atom can form only a single covalent bond, the other common atoms that form covalent bonds in cells—O, N, S, and P, as well as the all-important C atom—can form more than one. The outermost shell of these atoms, as we have seen, can accommodate up to eight electrons, and they form covalent bonds with as many other atoms as necessary to reach this number. Oxygen, with six electrons in its outer shell, is most stable when it acquires an extra two electrons by sharing with other atoms and therefore forms up to two covalent bonds. Nitrogen, with five outer electrons, forms a maximum of three covalent bonds, while carbon, with four outer electrons, forms up to four covalent bonds—thus sharing four pairs of electrons (see Figure 2–4).

When one atom forms covalent bonds with several others, these multiple bonds have definite arrangements in space relative to one another, reflecting the orientations of the orbits of the shared electrons. The covalent bonds of such an atom are therefore characterized by specific bond angles as well as by bond lengths and bond energies (**Figure 2–8**). The four covalent bonds that can form around a carbon atom, for example, are arranged as if pointing to the four corners of a regular tetrahedron. The precise orientation of covalent bonds forms the basis for the three-dimensional geometry of organic molecules.
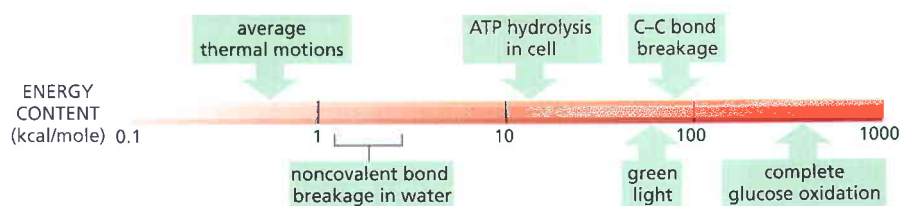

Figure 2–7 **Some energies important for cells.** Note that these energies are compared on a logarithmic scale.
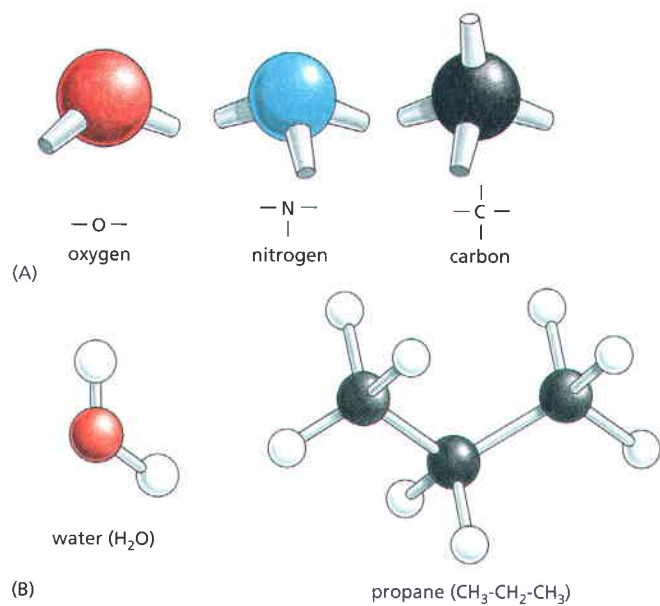
(A) —O— oxygen   —N— nitrogen   —C— carbon

(B) water (H_2O)   propane (CH_3-CH_2-CH_3)

There Are Different Types of Covalent Bonds

Most covalent bonds involve the sharing of two electrons, one donated by each participating atom; these are called *single bonds*. Some covalent bonds, however, involve the sharing of more than one pair of electrons. Four electrons can be shared, for example, two coming from each participating atom; such a bond is called a *double bond*. Double bonds are shorter and stronger than single bonds and have a characteristic effect on the three-dimensional geometry of molecules containing them. A single covalent bond between two atoms generally allows the rotation of one part of a molecule relative to the other around the bond axis. A double bond prevents such rotation, producing a more rigid and less flexible arrangement of atoms (**Figure 2–9** and Panel 2–1, pp. 106–107).

In some molecules, electrons are shared among three or more atoms, producing bonds that have a hybrid character intermediate between single and double bonds. The highly stable benzene molecule, for example, consists of a ring of six carbon atoms in which the bonding electrons are evenly distributed (although usually depicted as an alternating sequence of single and double bonds, as shown in Panel 2–1).

When the atoms joined by a single covalent bond belong to different elements, the two atoms usually attract the shared electrons to different degrees. Compared with a C atom, for example, O and N atoms attract electrons relatively strongly, whereas an H atom attracts electrons more weakly. By definition, a **polar** structure (in the electrical sense) is one with positive charge concentrated toward one end (the positive pole) and negative charge concentrated toward the other (the negative pole). Covalent bonds in which the electrons are shared unequally in this way are therefore known as *polar covalent bonds* (**Figure 2–10**). For example, the covalent bond between oxygen and hydrogen, –O–H, or between nitrogen and hydrogen, –N–H, is polar, whereas that between carbon and hydrogen, –C–H, has the electrons attracted much more equally by both atoms and is relatively nonpolar.

Polar covalent bonds are extremely important in biology because they create *permanent dipoles* that allow molecules to interact through electrical forces. Any large molecule with many polar groups will have a pattern of partial positive and negative charges on its surface. When such a molecule encounters a second molecule with a complementary set of charges, the two molecules will be attracted to each other by electrostatic interactions that resemble (but are weaker than) the ionic bonds discussed previously.
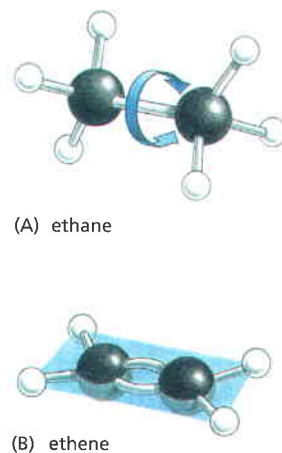

(A) ethane

(B) ethene

An Atom Often Behaves as if It Has a Fixed Radius

When a covalent bond forms between two atoms, the sharing of electrons brings the nuclei of these atoms unusually close together. But most of the atoms that are rapidly jostling each other in cells are located in separate molecules. What happens when two such atoms touch? RoshanKetab 021-66950639

For simplicity and clarity, atoms and molecules are usually represented schematically—either as a line drawing of the structural formula or as a ball-and-stick model. *Space-filling models,* however, give us a more accurate representation of molecular structure. In these models, a solid envelope represents the radius of the electron cloud at which strong repulsive forces prevent a closer approach of any second, non-bonded atom—the so-called *van der Waals radius* for an atom. This is possible because the amount of repulsion increases very steeply as two such atoms approach each other closely. At slightly greater distances, any two atoms will experience a weak attractive force, known as a *van der Waals attraction.* As a result, there is a distance at which repulsive and attractive forces precisely balance to produce an energy minimum in each atom's interaction with an atom of a second, non-bonded element (**Figure 2–11**).

Depending on the intended purpose, we shall represent small molecules as line drawings, ball-and-stick models, or space-filling models. For comparison, the water molecule is represented in all three ways in **Figure 2–12**. When representing very large molecules, such as proteins, we shall often need to further simplify the model used (see, for example, Panel 3–2, pp. 132–133).

Water Is the Most Abundant Substance in Cells

Water accounts for about 70% of a cell's weight, and most intracellular reactions occur in an aqueous environment. Life on Earth began in the ocean, and the conditions in that primeval environment put a permanent stamp on the chemistry of living things. Life therefore hinges on the properties of water.

In each water molecule (H_2O) the two H atoms are linked to the O atom by covalent bonds (see Figure 2–12). The two bonds are highly polar because the O is strongly attractive for electrons, whereas the H is only weakly attractive. Consequently, there is an unequal distribution of electrons in a water molecule, with a preponderance of positive charge on the two H atoms and of negative charge on the O (see Figure 2–10). When a positively charged region of one water molecule (that is, one of its H atoms) approaches a negatively charged region (that is, the O) of a second water molecule, the electrical attraction between them can result in a weak bond called a *hydrogen bond* (see Figure 2–15). These bonds are much weaker than covalent bonds and are easily broken by the random thermal motions due to the heat energy of the molecules, so each bond lasts only a short time. But the combined effect of many weak bonds can be profound. Each water molecule can form hydrogen bonds through its two H atoms to two other water molecules, producing a network in which hydrogen bonds are being continually broken and formed (Panel 2–2, pp. 108–109). It is only because of the
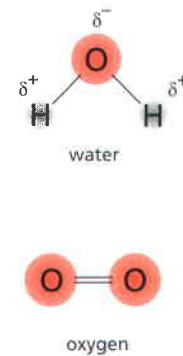
Figure 2–10 Polar and nonpolar covalent bonds. The electron distributions in the polar water molecule (H_2O) and the nonpolar oxygen molecule (O_2) are compared (δ^+, partial positive charge; δ^-, partial negative charge).
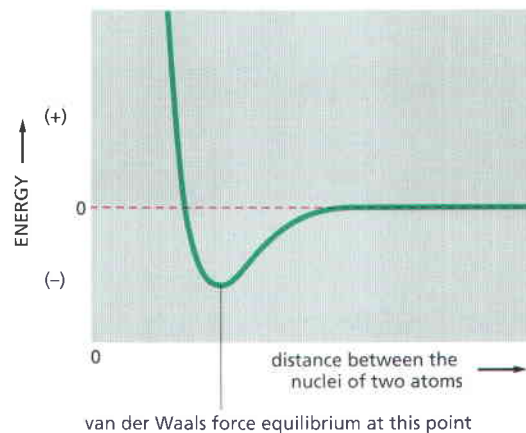
Figure 2–11 The balance of van der Waals forces between two atoms. As the nuclei of two atoms approach each other, they initially show a weak bonding interaction due to their fluctuating electric charges. However, the same atoms will strongly repel each other if they are brought too close together. The balance of these van der Waals attractive and repulsive forces occurs at the indicated energy minimum. This minimum determines the contact distance between any two noncovalently bonded atoms; this distance is the sum of their van der Waals radii. By definition, zero energy (indicated by the dotted *red* line) is the energy when the two nuclei are at infinite separation.
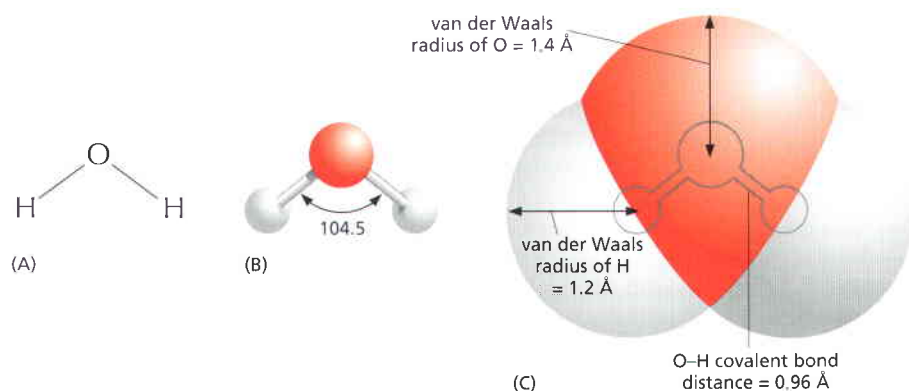
hydrogen bonds that link water molecules together that water is a liquid at room temperature, with a high boiling point and high surface tension—rather than a gas.

Molecules, such as alcohols, that contain polar bonds and that can form hydrogen bonds with water dissolve readily in water. Molecules carrying plus or minus charges (ions) likewise interact favorably with water. Such molecules are termed **hydrophilic**, meaning that they are water-loving. A large proportion of the molecules in the aqueous environment of a cell necessarily fall into this category, including sugars, DNA, RNA, and most proteins. **Hydrophobic** (water-hating) molecules, by contrast, are uncharged and form few or no hydrogen bonds, and so do not dissolve in water. Hydrocarbons are an important example (see Panel 2–1, pp. 106–107). In these molecules the H atoms are covalently linked to C atoms by a largely nonpolar bond. Because the H atoms have almost no net positive charge, they cannot form effective hydrogen bonds to other molecules. This makes the hydrocarbon as a whole hydrophobic—a property that is exploited in cells, whose membranes are constructed from molecules that have long hydrocarbon tails, as we shall see in Chapter 10.

Some Polar Molecules Are Acids and Bases

One of the simplest kinds of chemical reaction, and one that has profound significance in cells, takes place when a molecule containing a highly polar covalent bond between a hydrogen and a second atom dissolves in water. The hydrogen atom in such a molecule has largely given up its electron to the companion atom and so resembles an almost naked positively charged hydrogen nucleus—in other words, a **proton (H^+)**. When water molecules surround the polar molecule, the proton is attracted to the partial negative charge on the O atom of an adjacent water molecule and can dissociate from its original partner to associate instead with the oxygen atoms of the water molecule to generate a **hydronium ion (H_3O^+)** (**Figure 2–13**A). The reverse reaction also takes place very readily, so one has to imagine an equilibrium state in which billions of protons are constantly flitting to and fro from one molecule in the solution to another.

The same type of reaction takes place in a solution of pure water itself. As illustrated in Figure 2–13B, water molecules are constantly exchanging protons with each other. As a result, pure water contains an equal, very low concentration of H_3O^+ and OH^- ions, both being present at 10^{-7} M. (The concentration of H_2O in pure water is 55.5 M.)

Substances that release protons to form H_3O^+ when they dissolve in water are termed **acids**. The higher the concentration of H_3O^+, the more acidic the solution. As H_3O^+ rises, the concentration of OH^- falls, according to the equilibrium equation for water: $[H_3O^+][OH^-] = 1.0 \times 10^{-14}$, where square brackets denote molar concentrations to be multiplied. By tradition, the H_3O^+ concentration is usually referred to as the H^+ concentration, even though nearly all H^+ in an aqueous solution is present as H_3O^+. To avoid the use of unwieldy numbers, the concentration of H^+ is expressed using a logarithmic scale called the **pH scale**, as illustrated in Panel 2–2 (pp. 108–109). Pure water has a pH of 7.0, and is neutral—that is, neither acidic (pH < 7.0) nor basic (pH > 7.0).
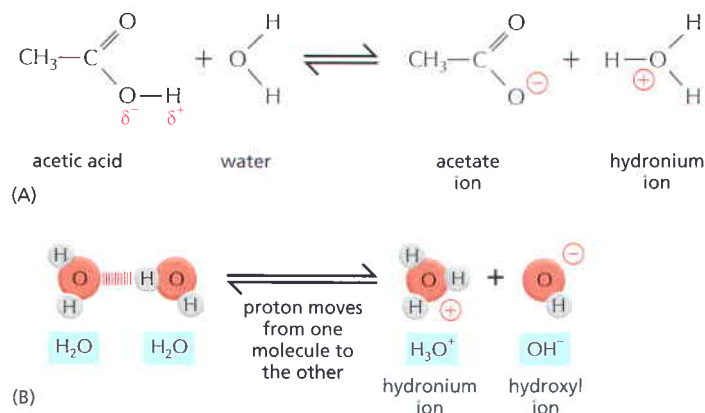
(A)

(B)

Figure 2–13 **Acids in water.** (A) The reaction that takes place when a molecule of acetic acid dissolves in water. (B) Water molecules are continuously exchanging protons with each other to form hydronium and hydroxyl ions. These ions in turn rapidly recombine to form water molecules.

Because the proton of a hydronium ion can be passed readily to many types of molecules in cells, altering their character, the concentration of H_3O^+ inside a cell (the acidity) must be closely regulated. The interior of a cell is kept close to neutrality, and it is buffered by the presence of many chemical groups that can take up and release protons near pH 7.

The opposite of an acid is a **base**. Just as the defining property of an acid is that it donates protons to a water molecule so as to raise the concentration of H_3O^+ ions, the defining property of a base is that it accepts protons so as to lower the concentration of H_3O^+ ions, and thereby raise the concentration of hydroxyl ions (OH^-). A base can either combine with protons directly or form hydroxyl ions that immediately combine with protons to produce H_2O. Thus sodium hydroxide (NaOH) is basic (or *alkaline*) because it dissociates in aqueous solution to form Na^+ ions and OH^- ions. Other bases, especially important in living cells, contain NH_2 groups. These groups directly take up a proton from water: $-NH_2 + H_2O \rightarrow -NH_3^+ + OH^-$.

All molecules that accept protons from water will do so most readily when the concentration of H_3O^+ is high (acidic solutions). Likewise, molecules that can give up protons do so more readily if the concentration of H_3O^+ in solution is low (basic solutions), and they will tend to receive them back if this concentration is high.

Four Types of Noncovalent Attractions Help Bring Molecules Together in Cells

In aqueous solutions, covalent bonds are 10–100 times stronger than the other attractive forces between atoms, allowing their connections to define the boundaries of one molecule from another. But much of biology depends on the specific binding of different molecules to each other. This binding is mediated by a group of noncovalent attractions that are individually quite weak, but whose energies can sum to create an effective force between two separate molecules. We have previously introduced three of these attractive forces: electrostatic attractions (ionic bonds), hydrogen bonds, and van der Waals attractions. **Table 2–1** compares the strengths of these three types of *noncovalent bonds* with that of a typical covalent bond, both in the presence and in the

Table 2–1 Covalent and Noncovalent Chemical Bonds

BOND TYPE		LENGTH (nm)	STRENGTH (kcal/mole)	
			IN VACUUM	IN WATER
Covalent		0.15	90	90
Noncovalent:	ionic*	0.25	80	3
	hydrogen	0.30	4	1
	van der Waals attraction (per atom)	0.35	0.1	0.1

*An ionic bond is an electrostatic attraction between two fully charged atoms.

absence of water. Because of their fundamental importance in all biological systems, we summarize their properties here:

- **Electrostatic attractions**. These result from the attractive forces between oppositely charged atoms. Electrostatic attractions are quite strong in the absence of water. They readily form between permanent dipoles, but are greatest when the two atoms involved are fully charged (*ionic bonds*). However, the polar water molecules cluster around both fully charged ions and polar molecules that contain permanent dipoles (**Figure 2–14**). This greatly reduces the attractiveness of these charged species for each other in most biological settings.
- **Hydrogen bonds**. The structure of a typical hydrogen bond is illustrated in **Figure 2–15**. This bond represents a special form of polar interaction in which an electropositive hydrogen atom is partially shared by two electronegative atoms. Its hydrogen can be viewed as a proton that has partially dissociated from a donor atom, allowing it to be shared by a second acceptor atom. Unlike a typical electrostatic interaction, this bond is highly directional—being strongest when a straight line can be drawn between all three of the involved atoms. As already discussed, water weakens these bonds by forming competing hydrogen-bond interactions with the involved molecules.
- **van der Waals attractions**. The electron cloud around any nonpolar atom will fluctuate, producing a flickering dipole. Such dipoles will transiently induce an oppositely polarized flickering dipole in a nearby atom. This interaction generates a very weak attraction between atoms. But since many atoms can be simultaneously in contact when two surfaces fit closely, the net result is often significant. Water does not weaken these so-called van der Waals attractions.

The fourth effect that often brings molecules together in water is not, strictly speaking, a bond at all. However, a very important **hydrophobic force** is caused by a pushing of nonpolar surfaces out of the hydrogen-bonded water network, where they would otherwise physically interfere with the highly favorable interactions between water molecules. Bringing any two nonpolar surfaces together reduces their contact with water; in this sense, the force is nonspecific. Nevertheless, we shall see in Chapter 3 that hydrophobic forces are central to the proper folding of protein molecules.

Panel 2–3 provides an overview of the four types of attractions just described. And **Figure 2–16** illustrates schematically how many such interactions can sum to hold together the matching surfaces of two macromolecules, even though each interaction by itself would be much too weak to be effective in the face of thermal motions.

A Cell Is Formed from Carbon Compounds

Having looked at the ways atoms combine into small molecules and how these molecules behave in an aqueous environment, we now examine the main classes of small molecules found in cells and their biological roles. We shall see that a few basic categories of molecules, formed from a handful of different elements, give rise to all the extraordinary richness of form and behavior shown by living things.

If we disregard water and inorganic ions such as potassium, nearly all the molecules in a cell are based on carbon. Carbon is outstanding among all the elements in its ability to form large molecules; silicon is a poor second. Because it is small and has four electrons and four vacancies in its outermost shell, a carbon atom can form four covalent bonds with other atoms. Most important, one carbon atom can join to other carbon atoms through highly stable covalent C–C
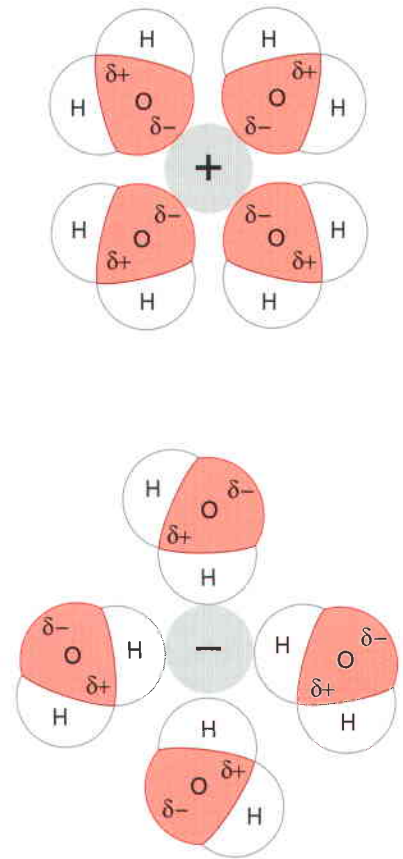

Figure 2–14 How the dipoles on water molecules orient to reduce the affinity of oppositely charged ions or polar groups for each other.
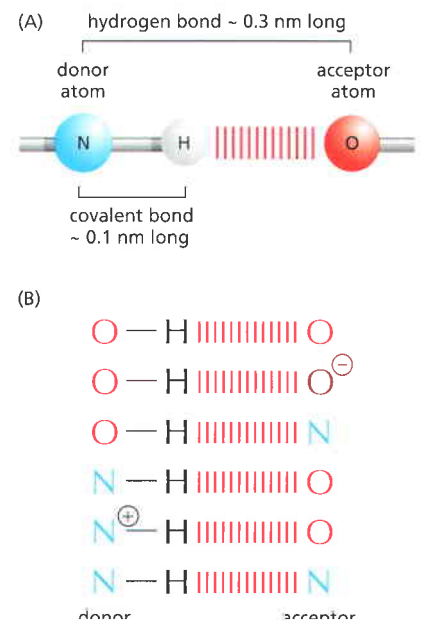

Figure 2–15 Hydrogen bonds. (A) Ball-and-stick model of a typical hydrogen bond. The distance between the hydrogen and the oxygen atom here is less than the sum of their van der Waals radii, indicating a partial sharing of electrons. (B) The most common hydrogen bonds in cells.

bonds to form chains and rings and hence generate large and complex molecules with no obvious upper limit to their size (see Panel 2–1, pp. 106–107). The small and large carbon compounds made by cells are called *organic molecules.*

Certain combinations of atoms, such as the methyl (–CH$_3$), hydroxyl (–OH), carboxyl (–COOH), carbonyl (–C=O), phosphate (–PO$_3{}^{2-}$), sulfhydryl (–SH), and amino (–NH$_2$) groups, occur repeatedly in organic molecules. Each such **chemical group** has distinct chemical and physical properties that influence the behavior of the molecule in which the group occurs. The most common chemical groups and some of their properties are summarized in Panel 2–1, pp. 106–107.

Cells Contain Four Major Families of Small Organic Molecules

The small organic molecules of the cell are carbon-based compounds that have molecular weights in the range 100–1000 and contain up to 30 or so carbon atoms. They are usually found free in solution and have many different fates. Some are used as *monomer* subunits to construct the giant polymeric *macromolecules*—the proteins, nucleic acids, and large polysaccharides—of the cell. Others act as energy sources and are broken down and transformed into other small molecules in a maze of intracellular metabolic pathways. Many small molecules have more than one role in the cell—for example, acting both as a potential subunit for a macromolecule and as an energy source. Small organic molecules are much less abundant than the organic macromolecules, accounting for only about one-tenth of the total mass of organic matter in a cell (**Table 2–2**). As a rough guess, there may be a thousand different kinds of these small molecules in a typical cell.

All organic molecules are synthesized from and are broken down into the same set of simple compounds. Both their synthesis and their breakdown occur through sequences of limited chemical changes that follow definite rules. As a consequence, the compounds in a cell are chemically related and most can be classified into a few distinct families. Broadly speaking, cells contain four major families of small organic molecules: the *sugars*, the *fatty acids*, the *amino acids*, and the *nucleotides* (**Figure 2–17**). Although many compounds present in cells do not fit into these categories, these four families of small organic molecules, together with the macromolecules made by linking them into long chains, account for a large fraction of cell mass (see Table 2–2).
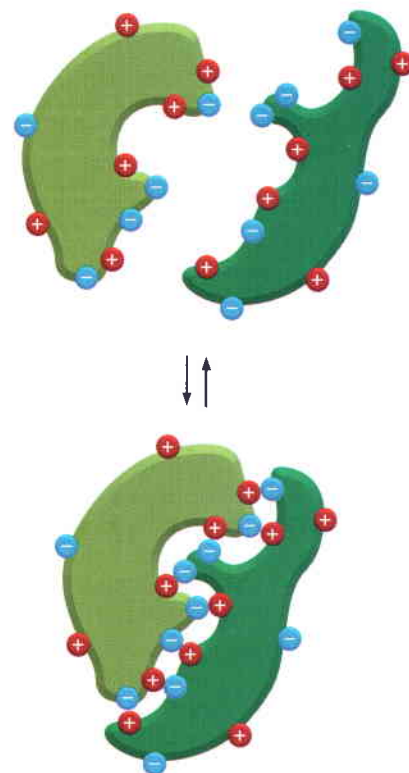

Figure 2–16 **Schematic indicating how two macromolecules with complementary surfaces can bind tightly to one another through noncovalent interactions.**

Sugars Provide an Energy Source for Cells and Are the Subunits of Polysaccharides

The simplest **sugars**—the *monosaccharides*—are compounds with the general formula (CH$_2$O)$_n$, where n is usually 3, 4, 5, 6, 7, or 8. Sugars, and the molecules made from them, are also called *carbohydrates* because of this simple formula. Glucose, for example, has the formula C$_6$H$_{12}$O$_6$ (**Figure 2–18**). The formula, however, does not fully define the molecule: the same set of carbons, hydrogens, and

Table 2–2 **The Types of Molecules That Form a Bacterial Cell**

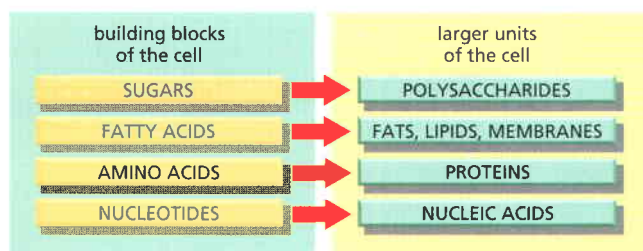|	PERCENT OF TOTAL CELL WEIGHT	NUMBER OF TYPES OF EACH MOLECULE
Water	70	1
Inorganic ions	1	20
Sugars and precursors	1	250
Amino acids and precursors	0.4	100
Nucleotides and precursors	0.4	100
Fatty acids and precursors	1	50
Other small molecules	0.2	~300
Macromolecules (proteins, nucleic acids, and polysaccharides)	26	~3000

oxygens can be joined together by covalent bonds in a variety of ways, creating structures with different shapes. As shown in Panel 2–4 (pp. 112–113), for example, glucose can be converted into a different sugar—mannose or galactose—simply by switching the orientations of specific OH groups relative to the rest of the molecule. Each of these sugars, moreover, can exist in either of two forms, called the D-form and the L-form, which are mirror images of each other. Sets of molecules with the same chemical formula but different structures are called *isomers*, and the subset of such molecules that are mirror-image pairs are called *optical isomers*. Isomers are widespread among organic molecules in general, and they play a major part in generating the enormous variety of sugars.

Panel 2–4 presents an outline of sugar structure and chemistry. Sugars can exist as rings or as open chains. In their open-chain form, sugars contain a number of hydroxyl groups and either one aldehyde ($_H{>}C{=}O$) or one ketone (${>}C{=}O$) group. The aldehyde or ketone group plays a special role. First, it can react with a hydroxyl group in the same molecule to convert the molecule into a ring; in the ring form the carbon of the original aldehyde or ketone group can be recognized as the only one that is bonded to two oxygens. Second, once the ring is formed, this same carbon can become further linked, via oxygen, to one of the carbons bearing a hydroxyl group on another sugar molecule. This creates a *disaccharide* such as sucrose, which is composed of a glucose and a fructose unit. Larger sugar polymers range from the *oligosaccharides* (trisaccharides, tetrasaccharides, and so on) up to giant *polysaccharides*, which can contain thousands of monosaccharide units.

The way that sugars are linked together to form polymers illustrates some common features of biochemical bond formation. A bond is formed between an –OH group on one sugar and an –OH group on another by a **condensation reaction,** in which a molecule of water is expelled as the bond is formed (**Figure 2–19**). Subunits in other biological polymers, such as nucleic acids and proteins, are also linked by condensation reactions in which water is expelled. The bonds created by all of these condensation reactions can be broken by the reverse process of **hydrolysis,** in which a molecule of water is consumed (see Figure 2–19).
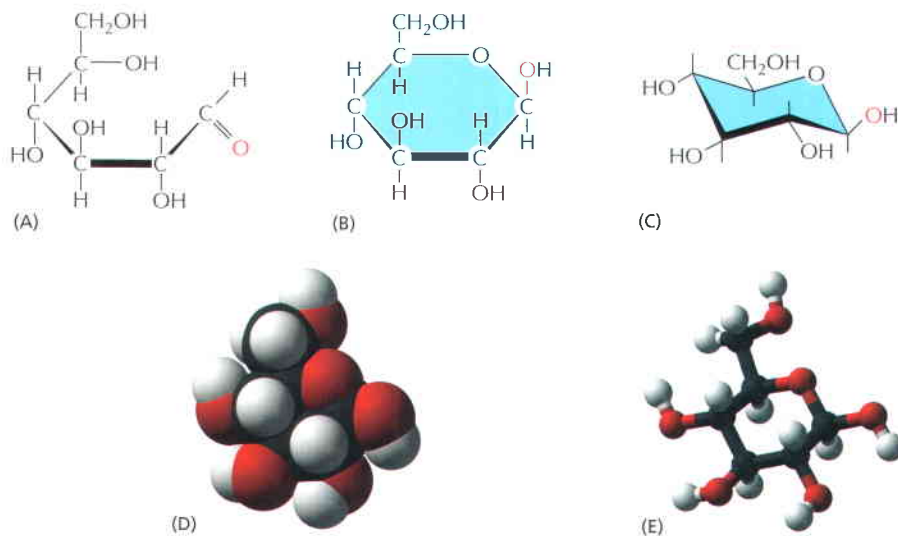
Figure 2–18 **The structure of glucose, a simple sugar.** As illustrated previously for water (see Figure 2–12), any molecule can be represented in several ways. In the structural formulas shown in (A), (B) and (C), the atoms are shown as chemical symbols linked together by lines representing the covalent bonds. The *thickened lines* here are used to indicate the plane of the sugar ring, in an attempt to emphasize that the –H and –OH groups are not in the same plane as the ring. (A) The open-chain form of this sugar, which is in equilibrium with the more stable cyclic or ring form in (B). (C) The chair form is an alternative way to draw the cyclic molecule that reflects the geometry more accurately than the structural formula in (B). (D) A space-filling model, which, as well as depicting the three-dimensional arrangement of the atoms, also uses the van der Waals radii to represent the surface contours of the molecule. (E) A ball-and-stick model in which the three-dimensional arrangement of the atoms in space is shown. (H, *white*; C, *black*; O, *red*; N, *blue*.)
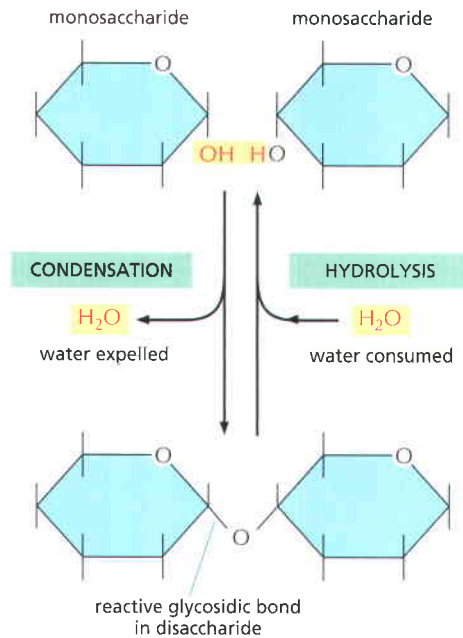
Figure 2–19 The reaction of two
monosaccharides to form a
disaccharide. This reaction belongs to a
general category of reactions termed
condensation reactions, in which two
molecules join together as a result of the
loss of a water molecule. The reverse
reaction (in which water is added) is
termed *hydrolysis*. Note that the reactive
carbon at which the new bond is formed
(on the monosaccharide on the *left* here)
is the carbon joined to two oxygens as a
result of sugar ring formation (see Figure
2–18). As indicated, this common type of
covalent bond between two sugar
molecules is known as a *glycosidic bond*
(see also Figure 2–20).

Because each monosaccharide has several free hydroxyl groups that can
form a link to another monosaccharide (or to some other compound), sugar
polymers can be branched, and the number of possible polysaccharide struc-
tures is extremely large. Even a simple disaccharide consisting of two glucose
units can exist in eleven different varieties (**Figure 2–20**), while three different
hexoses ($C_6H_{12}O_6$) can join together to make several thousand trisaccharides.
For this reason it is a much more complex task to determine the arrangement of
sugars in a polysaccharide than to determine the nucleotide sequence of a DNA
molecule, where each unit is joined to the next in exactly the same way.

The monosaccharide *glucose* is a key energy source for cells. In a series of
reactions, it is broken down to smaller molecules, releasing energy that the cell
can harness to do useful work, as we shall explain later. Cells use simple polysac-
charides composed only of glucose units—principally *glycogen* in animals and
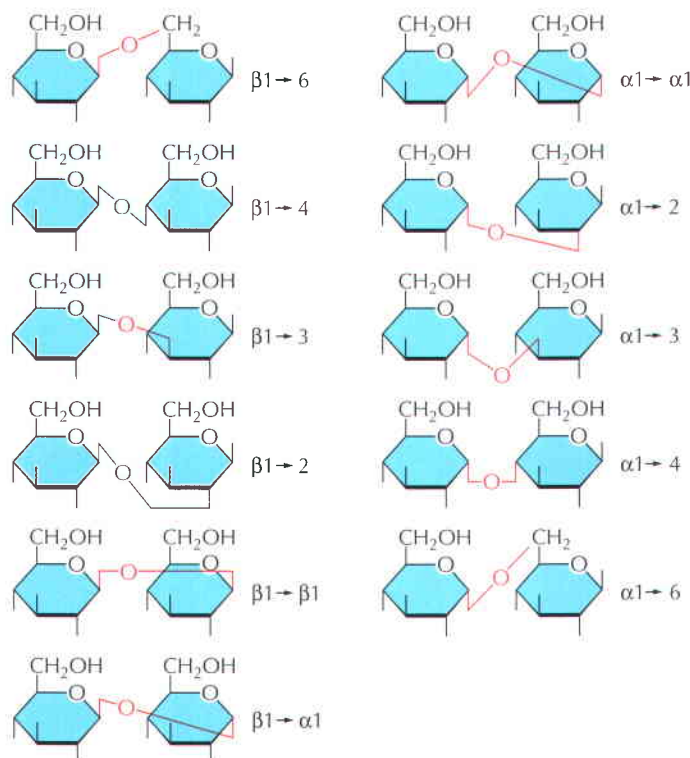*starch* in plants—as energy stores.

Figure 2–20 Eleven disaccharides
consisting of two D-glucose units.
Although these differ only in the type of
linkage between the two glucose units,
they are chemically distinct. Since the
oligosaccharides associated with proteins
and lipids may have six or more different
kinds of sugar joined in both linear and
branched arrangements through
glycosidic bonds such as those illustrated
here, the number of distinct types of
oligosaccharides that can be used in cells
is extremely large. For an explanation
of α and β linkages, see Panel 2–4
(pp. 112–113). Short *black* lines ending
"blind" indicate OH positions. (*Red* lines
merely indicate disaccharide bond
orientations and "corners" do not imply
extra atoms.)

Sugars do not function only in the production and storage of energy. They can also be used, for example, to make mechanical supports. Thus, the most abundant organic chemical on Earth—the *cellulose* of plant cell walls—is a polysaccharide of glucose. Because the glucose–glucose linkages in cellulose differ from those in starch and glycogen, however, humans cannot digest cellulose and use its glucose. Another extraordinarily abundant organic substance, the *chitin* of insect exoskeletons and fungal cell walls, is also an indigestible polysaccharide—in this case a linear polymer of a sugar derivative called *N*-acetylglucosamine (see Panel 2–4). Other polysaccharides are the main components of slime, mucus, and gristle.

Smaller oligosaccharides can be covalently linked to proteins to form glycoproteins and to lipids to form *glycolipids*, both of which are found in cell membranes. As described in Chapter 10, most cell surfaces are clothed and decorated with glycoproteins and glycolipids in the cell membrane. The sugar side chains on these molecules are often recognized selectively by other cells. And differences between people in the details of their cell-surface sugars are the molecular basis for the different major human blood groups, termed A, B, AB, and O.

Fatty Acids Are Components of Cell Membranes, as Well as a Source of Energy

A fatty acid molecule, such as *palmitic acid*, has two chemically distinct regions (**Figure 2–21**). One is a long hydrocarbon chain, which is hydrophobic and not very reactive chemically. The other is a carboxyl (–COOH) group, which behaves as an acid (carboxylic acid): it is ionized in solution (–COO⁻), extremely hydrophilic, and chemically reactive. Almost all the fatty acid molecules in a cell are covalently linked to other molecules by their carboxylic acid group.

The hydrocarbon tail of palmitic acid is *saturated*: it has no double bonds between carbon atoms and contains the maximum possible number of hydrogens. Stearic acid, another one of the common fatty acids in animal fat, is also saturated. Some other fatty acids, such as oleic acid, have *unsaturated* tails, with one or more double bonds along their length. The double bonds create kinks in the molecules, interfering with their ability to pack together in a solid mass. It is this that accounts for the difference between hard margarine (saturated) and liquid vegetable oils (polyunsaturated). The many different fatty acids found in cells differ only in the length of their hydrocarbon chains and the number and position of the carbon–carbon double bonds (see Panel 2–5, pp. 114–115).

Fatty acids are stored in the cytoplasm of many cells in the form of droplets of *triacylglycerol* molecules, which consist of three fatty acid chains joined to a glycerol molecule (see Panel 2–5); these molecules are the animal fats found in meat, butter, and cream, and the plant oils such as corn oil and olive oil. When required to provide energy, the fatty acid chains are released from triacylglycerols and broken down into two-carbon units. These two-carbon units are identical to those derived from the breakdown of glucose and they enter the same energy-yielding reaction pathways, as will be described later in this chapter. Triglycerides serve as a concentrated food reserve in cells, because they can be broken down to produce about six times as much usable energy, weight for weight, as glucose.

Fatty acids and their derivatives such as triacylglycerols are examples of **lipids**. Lipids comprise a loosely defined collection of biological molecules that are insoluble in water, while being soluble in fat and organic solvents such as benzene. They typically contain either long hydrocarbon chains, as in the fatty acids and isoprenes, or multiple linked rings, as in the *steroids*.

The most important function of fatty acids in cells is in the construction of cell membranes. These thin sheets enclose all cells and surround their internal organelles. They are composed largely of *phospholipids*, which are small molecules that, like triacylglycerols, are constructed mainly from fatty acids and glycerol. In phospholipids the glycerol is joined to two fatty acid chains, however, rather than to three as in triacylglycerols. The "third" site on the glycerol is linked to a hydrophilic phosphate group, which is in turn attached to a small hydrophilic compound such as choline (see Panel 2–5). Each phospholipid
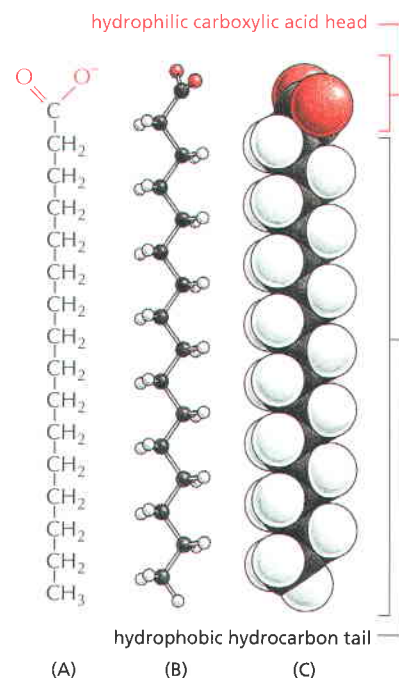

hydrophilic carboxylic acid head

hydrophobic hydrocarbon tail

(A)   (B)   (C)

Figure 2–21 A fatty acid. A fatty acid is composed of a hydrophobic hydrocarbon chain to which is attached a hydrophilic carboxylic acid group. Palmitic acid is shown here. Different fatty acids have different hydrocarbon tails. (A) Structural formula. The carboxylic acid group is shown in its ionized form. (B) Ball-and-stick model. (C) Space-filling model.
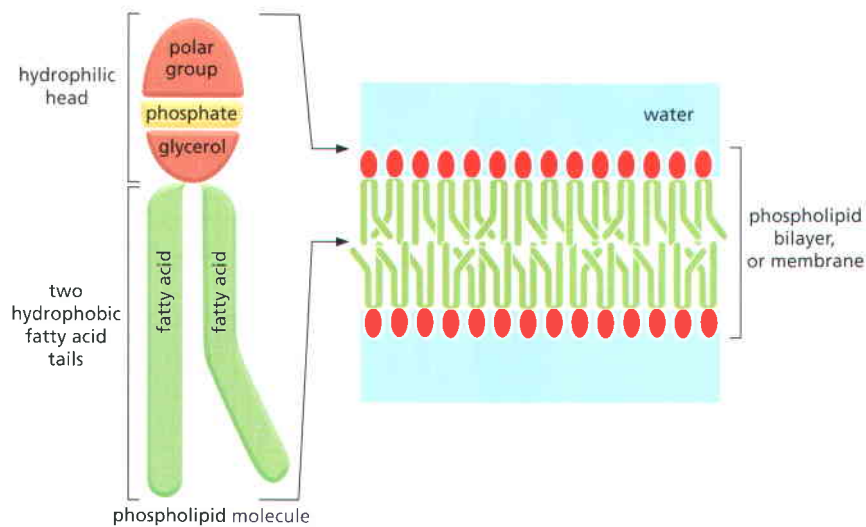
Figure 2–22 **Phospholipid structure and the orientation of phospholipids in membranes.** In an aqueous environment, the hydrophobic tails of phospholipids pack together to exclude water. Here they have formed a bilayer with the hydrophilic head of each phospholipid facing the water. Lipid bilayers are the basis for cell membranes, as discussed in detail in Chapter 10.

molecule, therefore, has a hydrophobic tail composed of the two fatty acid chains and a hydrophilic head, where the phosphate is located. This gives them different physical and chemical properties from triacylglycerols, which are predominantly hydrophobic. Molecules such as phospholipids, with both hydrophobic and hydrophilic regions, are termed *amphiphilic*.

The membrane-forming property of phospholipids results from their amphiphilic nature. Phospholipids will spread over the surface of water to form a monolayer of phospholipid molecules, with the hydrophobic tails facing the air and the hydrophilic heads in contact with the water. Two such molecular layers can readily combine tail-to-tail in water to make a phospholipid sandwich, or **lipid bilayer**. This bilayer is the structural basis of all cell membranes (**Figure 2–22**).

Amino Acids Are the Subunits of Proteins

Amino acids are a varied class of molecules with one defining property: they all possess a carboxylic acid group and an amino group, both linked to a single carbon atom called the α-carbon (**Figure 2–23**). Their chemical variety comes from the side chain that is also attached to the α-carbon. The importance of amino acids to the cell comes from their role in making **proteins**, which are polymers of amino acids joined head-to-tail in a long chain that is then folded into a three-dimensional structure unique to each type of protein. The covalent linkage between two adjacent amino acids in a protein chain forms an amide (see Panel 2–1), and it is called a **peptide bond**; the chain of amino acids is also known as a *polypeptide* (**Figure 2–24**). Regardless of the specific amino acids from which it is made, the polypeptide has an amino (NH_2) group at one end (its *N-terminus*) and a carboxyl (COOH) group at its other end (its *C-terminus*). This gives it a definite directionality—a structural (as opposed to an electrical) polarity.

Each of the 20 amino acids found commonly in proteins has a different side chain attached to the α-carbon atom (see Panel 3–1, pp. 128–129). All organisms,
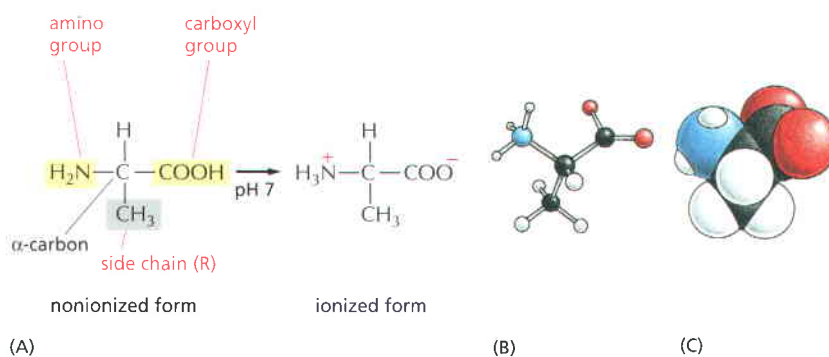

(A)

(B)   (C)

Figure 2–23 **The amino acid alanine.** (A) In the cell, where the pH is close to 7, the free amino acid exists in its ionized form; but when it is incorporated into a polypeptide chain, the charges on the amino and carboxyl groups disappear. (B) A ball-and-stick model and (C) a space-filling model of alanine (H, *white*; C, *black*; O, *red*; N, *blue*).

Figure 2–24 A small part of a protein molecule. The four amino acids shown are linked together by three peptide bonds, one of which is highlighted in *yellow*. One of the amino acids is shaded in *gray*. The amino acid side chains are shown in *red*. The two ends of a polypeptide chain are chemically distinct. One end, the N-terminus, terminates in an amino group, and the other, the C-terminus, in a carboxyl group. The sequence is always read from the N-terminal end; hence this sequence is Phe–Ser–Glu–Lys.
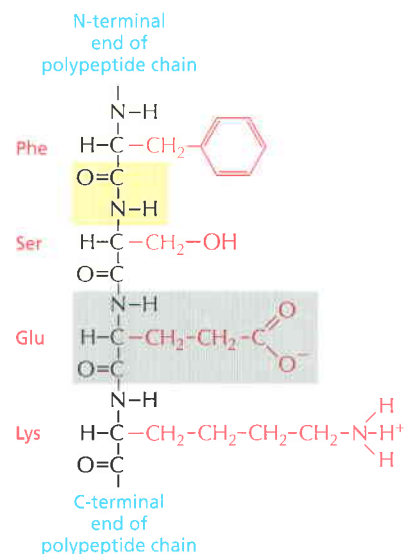
whether bacteria, archea, plants, or animals, have proteins made of the same 20 amino acids. How this precise set of 20 came to be chosen is one of the mysteries of the evolution of life; there is no obvious chemical reason why other amino acids could not have served just as well. But once the choice was established, it could not be changed; too much depended on it.

Like sugars, all amino acids, except glycine, exist as optical isomers in D- and L-forms (see Panel 3–1). But only L-forms are ever found in proteins (although D-amino acids occur as part of bacterial cell walls and in some antibiotics). The origin of this exclusive use of L-amino acids to make proteins is another evolutionary mystery.

The chemical versatility of the 20 amino acids is essential to the function of proteins. Five of the 20 amino acids have side chains that can form ions in neutral aqueous solution and thereby can carry a charge (**Figure 2–25**). The others are uncharged; some are polar and hydrophilic, and some are nonpolar and hydrophobic. As we discuss in Chapter 3, the properties of the amino acid side chains underlie the diverse and sophisticated functions of proteins.
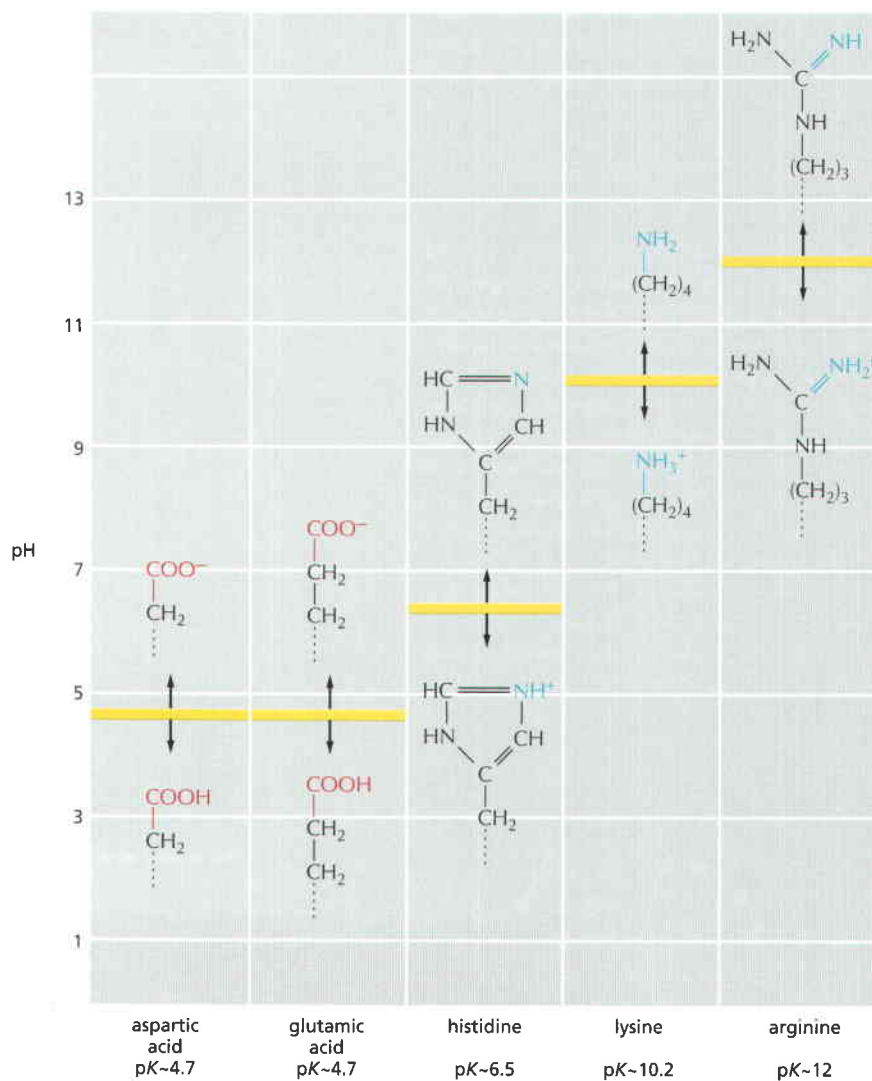

Figure 2–25 The charge on amino acid side chains depends on the pH. The five different side chains that can carry a charge are shown. Carboxylic acids can readily lose H+ in aqueous solution to form a negatively charged ion, which is denoted by the suffix "-ate," as in aspart*ate* or glutam*ate*. A comparable situation exists for amines, which in aqueous solution can take up H+ to form a positively charged ion (which does not have a special name). These reactions are rapidly reversible, and the amounts of the two forms, charged and uncharged, depend on the pH of the solution. At a high pH, carboxylic acids tend to be charged and amines uncharged. At a low pH, the opposite is true—the carboxylic acids are uncharged and amines are charged. The pH at which exactly *half* of the carboxylic acid or amine residues are charged is known as the p*K* of that amino acid side chain (indicated by *yellow* stripe).

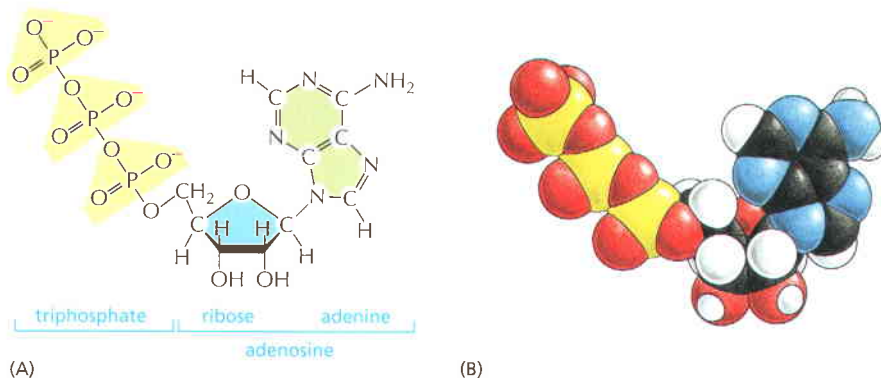In the cell the pH is close to 7, and almost all carboxylic acids and amines are in their fully charged form.

Figure 2–26 **Chemical structure of adenosine triphosphate (ATP).** (A) Structural formula. (B) Space-filling model. In (B) the colors of the atoms are C, *black*; N, *blue*; H, *white*; O, *red*; and P, *yellow*.

(A)   (B)

Nucleotides Are the Subunits of DNA and RNA

A **nucleotide** is a molecule made up of a nitrogen-containing ring compound linked to a five-carbon sugar, which in turn carries one or more phosphate groups (Panel 2–6, pp. 116–117). The five-carbon sugar can be either ribose or deoxyribose. Nucleotides containing ribose are known as ribonucleotides, and those containing deoxyribose as deoxyribonucleotides. The nitrogen-containing rings are generally referred to as *bases* for historical reasons: under acidic conditions they can each bind an H⁺ (proton) and thereby increase the concentration of OH⁻ ions in aqueous solution. There is a strong family resemblance between the different bases. *Cytosine (C), thymine (T),* and *uracil (U)* are called pyrimidines because they all derive from a six-membered pyrimidine ring; *guanine (G)* and *adenine (A)* are *purine* compounds, and they have a second, five-membered ring fused to the six-membered ring. Each nucleotide is named for the base it contains (see Panel 2–6).

Nucleotides can act as short-term carriers of chemical energy. Above all others, the ribonucleotide **adenosine triphosphate**, or **ATP** (**Figure 2–26**), transfers energy in hundreds of different cell reactions. ATP is formed through reactions that are driven by the energy released by the oxidative breakdown of foodstuffs. Its three phosphates are linked in series by two *phosphoanhydride bonds*, whose rupture releases large amounts of useful energy. The terminal phosphate group in particular is frequently split off by hydrolysis, often transferring a phosphate to other molecules and releasing energy that drives energy-requiring biosynthetic reactions (**Figure 2–27**). Other nucleotide derivatives are carriers for the transfer of other chemical groups, as will be described later.

The most fundamental role of nucleotides in the cell, however, is in the storage and retrieval of biological information. Nucleotides serve as building blocks for the construction of *nucleic acids*—long polymers in which nucleotide subunits are covalently linked by the formation of a **phosphodiester bond** between the
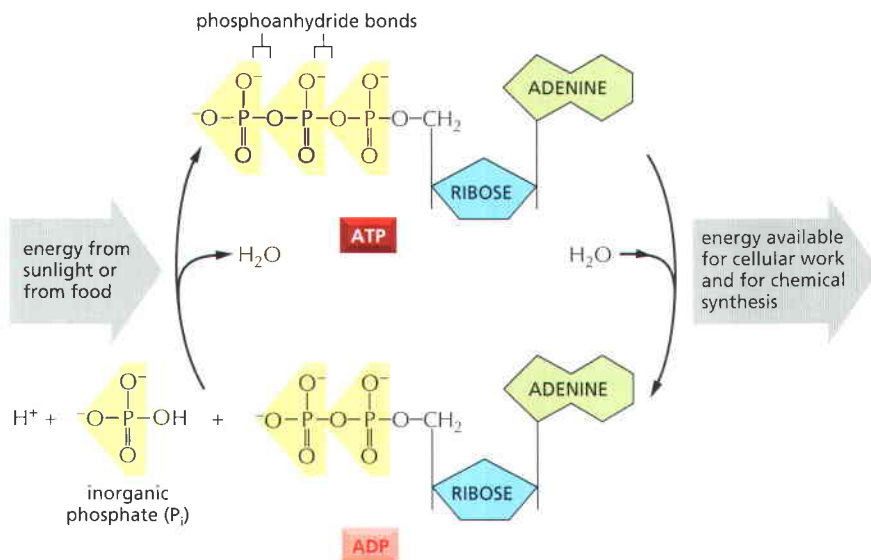

Figure 2–27 **The ATP molecule serves as an energy carrier in cells.** The energy-requiring formation of ATP from ADP and inorganic phosphate is coupled to the energy-yielding oxidation of foodstuffs (in animal cells, fungi, and some bacteria) or to the capture of light energy (in plant cells and some bacteria). The hydrolysis of this ATP back to ADP and inorganic phosphate in turn provides the energy to drive many cell reactions.

Figure 2–28 A small part of one chain of a deoxyribonucleic acid (DNA) molecule. Four nucleotides are shown. One of the phosphodiester bonds that links adjacent nucleotide residues is highlighted in *yellow,* and one of the nucleotides is shaded in *gray.* Nucleotides are linked together by a phosphodiester linkage between specific carbon atoms of the ribose, known as the 5′ and 3′ atoms. For this reason, one end of a polynucleotide chain, the *5′ end,* will have a free phosphate group and the other, the *3′ end,* a free hydroxyl group. The linear sequence of nucleotides in a polynucleotide chain is commonly abbreviated by a one-letter code, and the sequence is always read from the 5′ end. In the example illustrated the sequence is G–A–T–C.
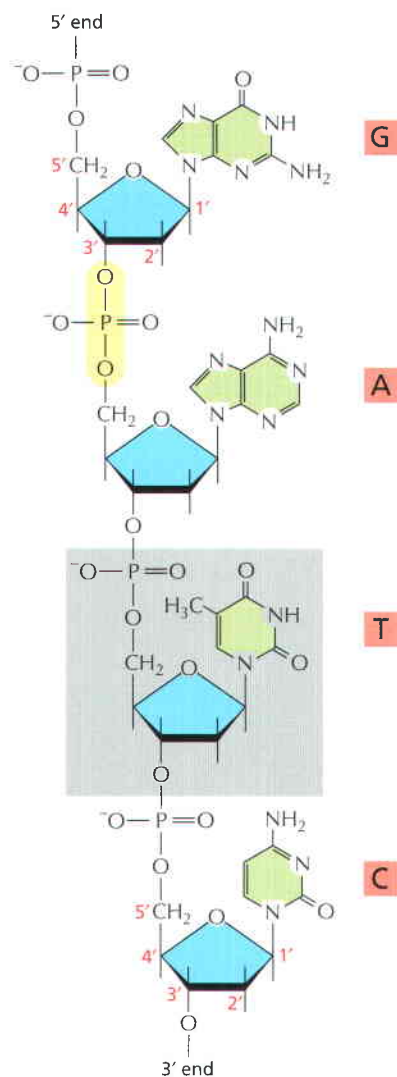
phosphate group attached to the sugar of one nucleotide and a hydroxyl group on the sugar of the next nucleotide (**Figure 2–28**). Nucleic acid chains are synthesized from energy-rich nucleoside triphosphates by a condensation reaction that releases inorganic pyrophosphate during phosphodiester bond formation.

There are two main types of nucleic acids, differing in the type of sugar in their sugar-phosphate backbone. Those based on the sugar *ribose* are known as **ribonucleic acids,** or **RNA,** and normally contain the bases A, G, C, and U. Those based on *deoxyribose* (in which the hydroxyl at the 2′ position of the ribose carbon ring is replaced by a hydrogen are known as **deoxyribonucleic acids,** or **DNA,** and contain the bases A, G, C, and T (T is chemically similar to the U in RNA, merely adding the methyl group on the pyrimidine ring; see Panel 2–6). RNA usually occurs in cells as a single polynucleotide chain, but DNA is virtually always a double-stranded molecule—a DNA double helix composed of two polynucleotide chains running antiparallel to each other and held together by hydrogen-bonding between the bases of the two chains.

The linear sequence of nucleotides in a DNA or an RNA encodes the genetic information of the cell. The ability of the bases in different nucleic acid molecules to recognize and pair with each other by hydrogen-bonding (called *base-pairing*)—G with C, and A with either T or U—underlies all of heredity and evolution, as explained in Chapter 4.

The Chemistry of Cells Is Dominated by Macromolecules with Remarkable Properties

By weight, macromolecules are the most abundant carbon-containing molecules in a living cell (**Figure 2–29** and **Table 2–3**). They are the principal building blocks from which a cell is constructed and also the components that confer the most distinctive properties of living things. The macromolecules in cells are polymers that are constructed by covalently linking small organic molecules (called *monomers)* into long chains (**Figure 2–30**). Yet they have remarkable properties that could not have been predicted from their simple constituents.

Proteins are especially abundant and versatile. They perform thousands of distinct functions in cells. Many proteins serve as *enzymes,* the catalysts that
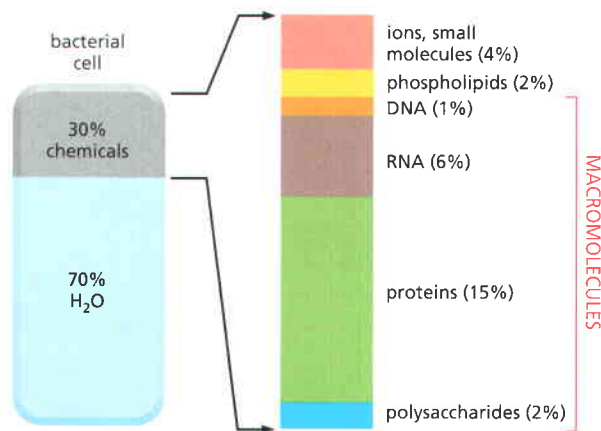

Figure 2–29 Macromolecules are abundant in cells. The approximate composition of a bacterial cell is shown by weight. The composition of an animal cell is similar (see Table 2–3).

Table 2–3 Approximate Chemical Compositions of a Typical Bacterium and a Typical Mammalian Cell

COMPONENT	PERCENT OF TOTAL CELL WEIGHT	
	E. COLI BACTERIUM	MAMMALIAN CELL
H_2O	70	70
Inorganic ions (Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, etc.)	1	1
Miscellaneous small metabolites	3	3
Proteins	15	18
RNA	6	1.1
DNA	1	0.25
Phospholipids	2	3
Other lipids	–	2
Polysaccharides	2	2
Total cell volume	2×10^{-12} cm^3	4×10^{-9} cm^3
Relative cell volume	1	2000

Proteins, polysaccharides, DNA, and RNA are macromolecules. Lipids are not generally classed as macromolecules even though they share some of their features; for example, most are synthesized as linear polymers of a smaller molecule (the acetyl group on acetyl CoA), and they self-assemble into larger structures (membranes). Note that water and protein comprise most of the mass of both mammalian and bacterial cells.

direct the many covalent bond-making and bond-breaking reactions that the cell needs. Enzymes catalyze all of the reactions whereby cells extract energy from food molecules, for example, and an enzyme called ribulose bisphosphate carboxylase helps to convert CO_2 to sugars in photosynthetic organisms, producing most of the organic matter needed for life on Earth. Other proteins are used to build structural components, such as tubulin, a protein that self-assembles to make the cell's long microtubules, or histones, proteins that compact the DNA in chromosomes. Yet other proteins act as molecular motors to produce force and movement, as in the case of myosin in muscle. Proteins perform many other functions, and we shall examine the molecular basis for many of them later in this book. Here we identify some general principles of macromolecular chemistry that make such functions possible.

Although the chemical reactions for adding subunits to each polymer are different in detail for proteins, nucleic acids, and polysaccharides, they share important features. Each polymer grows by the addition of a monomer onto the end of a growing polymer chain in a *condensation reaction*, in which a molecule of water is lost with each subunit added (see Figure 2–19). The stepwise polymerization of monomers into a long chain is a simple way to manufacture a large, complex molecule, since the subunits are added by the same reaction performed over and over again by the same set of enzymes. In a sense, the process resembles the repetitive operation of a machine in a factory—except in one crucial respect. Apart from some of the polysaccharides, most macromolecules are made from a set of monomers that are slightly different from one another—for example, the 20 different amino acids from which proteins are made. It is critical to life that the polymer chain is not assembled at random from these subunits; instead the subunits are added in a particular order, or *sequence*. The elaborate mechanisms that allow this to be accomplished by enzymes are described in detail in Chapters 5 and 6.

Noncovalent Bonds Specify Both the Precise Shape of a Macromolecule and its Binding to Other Molecules

Most of the covalent bonds in a macromolecule allow rotation of the atoms they join, giving the polymer chain great flexibility. In principle, this allows a macromolecule to adopt an almost unlimited number of shapes, or *conformations*, as
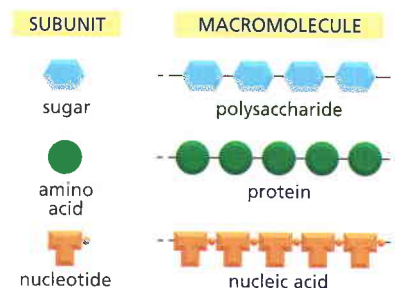
SUBUNIT	MACROMOLECULE
sugar	polysaccharide
amino acid	protein
nucleotide	nucleic acid

Figure 2–30 Three families of macromolecules. Each is a polymer formed from small molecules (called monomers) linked together by covalent bonds.
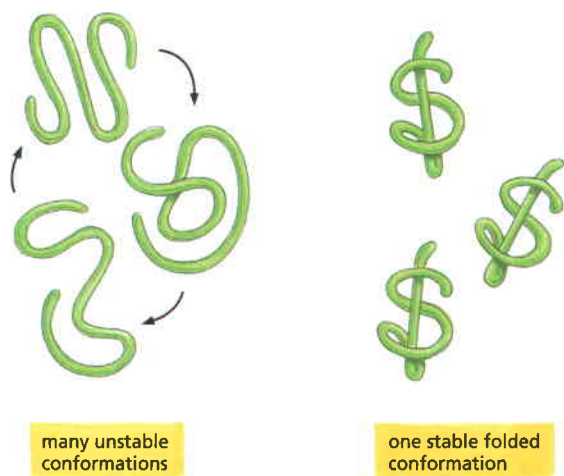
many unstable
conformations

one stable folded
conformation

Figure 2–31 **Most proteins and many RNA molecules fold into only one stable conformation.** If the noncovalent bonds maintaining this stable conformation are disrupted, the molecule becomes a flexible chain that usually has no biological value.

random thermal energy causes the polymer chain to writhe and rotate. However, the shapes of most biological macromolecules are highly constrained because of the many weak *noncovalent bonds* that form between different parts of the same molecule. If these noncovalent bonds are formed in sufficient numbers, the polymer chain can strongly prefer one particular conformation, determined by the linear sequence of monomers in its chain. Most protein molecules and many of the small RNA molecules found in cells fold tightly into one highly preferred conformation in this way (**Figure 2–31**).

The four types of noncovalent interactions important in biological molecules were described earlier, and they are reviewed in Panel 2–3 (pp. 110–111). Although individually very weak, these interactions cooperate to fold biological macromolecules into unique shapes. In addition, they can also add up to create a strong attraction between two different molecules when these molecules fit together very closely, like a hand in a glove. This form of molecular interaction provides for great specificity, inasmuch as the multipoint contacts required for strong binding make it possible for a macromolecule to select out—through binding—just one of the many thousands of other types of molecules present inside a cell. Moreover, because the strength of the binding depends on the number of noncovalent bonds that are formed, interactions of almost any affinity are possible—allowing rapid dissociation when necessary.

Binding of this type underlies all biological catalysis, making it possible for proteins to function as enzymes. Noncovalent interactions also allow macromolecules to be used as building blocks for the formation of larger structures. In cells, macromolecules often bind together into large complexes, thereby forming intricate machines with multiple moving parts that perform such complex tasks as DNA replication and protein synthesis (**Figure 2–32**).
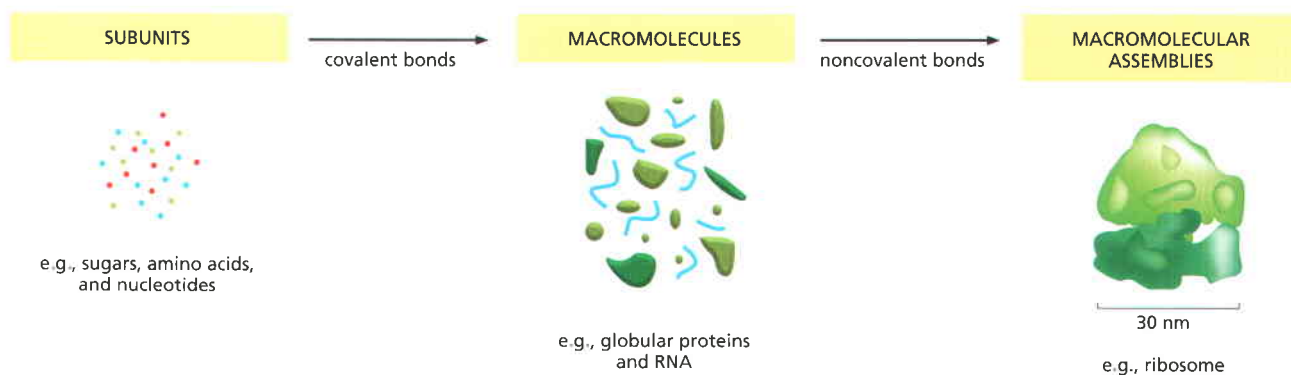

SUBUNITS	MACROMOLECULES	MACROMOLECULAR ASSEMBLIES

covalent bonds   noncovalent bonds

e.g., sugars, amino acids, and nucleotides

e.g., globular proteins and RNA

30 nm

e.g., ribosome

Figure 2–32 **Small molecules, proteins, and a ribosome drawn approximately to scale.** Ribosomes are a central part of the machinery that the cell uses to make proteins: each ribosome is formed as a complex of about 90 macromolecules (protein and RNA molecules).

Summary

Living organisms are autonomous, self-propagating chemical systems. They are made from a distinctive and restricted set of small carbon-based molecules that are essentially the same for every living species. Each of these molecules is composed of a small set of atoms linked to each other in a precise configuration through covalent bonds. The main categories are sugars, fatty acids, amino acids, and nucleotides. Sugars are a primary source of chemical energy for cells and can be incorporated into polysaccharides for energy storage. Fatty acids are also important for energy storage, but their most critical function is in the formation of cell membranes. Polymers consisting of amino acids constitute the remarkably diverse and versatile macromolecules known as proteins. Nucleotides play a central part in energy transfer. They are also the subunits for the informational macromolecules, RNA and DNA.

Most of the dry mass of a cell consists of macromolecules that have been produced as linear polymers of amino acids (proteins) or nucleotides (DNA and RNA), covalently linked to each other in an exact order. Most of the protein molecules and many of the RNAs fold into a unique conformation that depends on their sequence of subunits. This folding process creates unique surfaces, and it depends on a large set of weak attractions produced by noncovalent forces between atoms. These forces are of four types: electrostatic attractions, hydrogen bonds, van der Waals attractions, and an interaction between nonpolar groups caused by their hydrophobic expulsion from water. The same set of weak forces governs the specific binding of other molecules to macromolecules, making possible the myriad associations between biological molecules that produce the structure and the chemistry of a cell.

CATALYSIS AND THE USE OF ENERGY BY CELLS

One property of living things above all makes them seem almost miraculously different from nonliving matter: they create and maintain order, in a universe that is tending always to greater disorder (**Figure 2–33**). To create this order, the cells in a living organism must perform a never-ending stream of chemical reactions. In some of these reactions, small organic molecules—amino acids, sugars, nucleotides, and lipids—are being taken apart or modified to supply the many other small molecules that the cell requires. In other reactions, these small molecules are being used to construct an enormously diverse range of proteins, nucleic acids, and other macromolecules that endow living systems with all of their most distinctive properties. Each cell can be viewed as a tiny chemical factory, performing many millions of reactions every second.
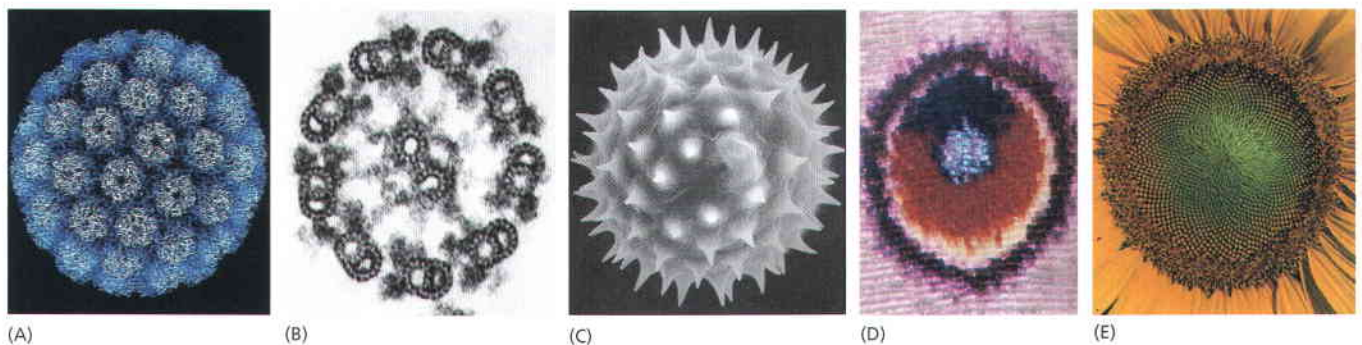

(A)   (B)   (C)   (D)   (E)

Figure 2–33 Order in biological structures. Well-defined, ornate, and beautiful spatial patterns can be found at every level of organization in living organisms. In order of increasing size: (A) protein molecules in the coat of a virus; (B) the regular array of microtubules seen in a cross section of a sperm tail; (C) surface contours of a pollen grain (a single cell); (D) close-up of the wing of a butterfly showing the pattern created by scales, each scale being the product of a single cell; (E) spiral array of seeds, made of millions of cells, in the head of a sunflower. (A, courtesy of R.A. Grant and J.M. Hogle; B, courtesy of Lewis Tilney; C, courtesy of Colin MacFarlane and Chris Jeffree; D and E, courtesy of Kjell B. Sandved.)
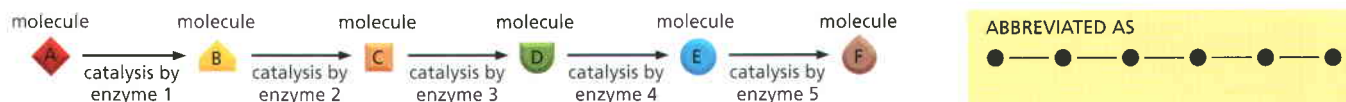
Figure 2–34 **How a set of enzyme-catalyzed reactions generates a metabolic pathway.** Each enzyme catalyzes a particular chemical reaction, leaving the enzyme unchanged. In this example, a set of enzymes acting in series converts molecule A to molecule F, forming a metabolic pathway.

Cell Metabolism Is Organized by Enzymes

The chemical reactions that a cell carries out would normally occur only at much higher temperatures than those existing inside cells. For this reason, each reaction requires a specific boost in chemical reactivity. This requirement is crucial, because it allows the cell to control each reaction. The control is exerted through the specialized proteins called *enzymes*, each of which accelerates, or *catalyzes*, just one of the many possible kinds of reactions that a particular molecule might undergo. Enzyme-catalyzed reactions are usually connected in series, so that the product of one reaction becomes the starting material, or *substrate*, for the next (**Figure 2–34**). These long linear reaction pathways are in turn linked to one another, forming a maze of interconnected reactions that enable the cell to survive, grow, and reproduce (**Figure 2–35**).

Two opposing streams of chemical reactions occur in cells: (1) the *catabolic* pathways break down foodstuffs into smaller molecules, thereby generating both a useful form of energy for the cell and some of the small molecules that the cell needs as building blocks, and (2) the *anabolic*, or *biosynthetic*, pathways use the energy harnessed by catabolism to drive the synthesis of the many other molecules that form the cell. Together these two sets of reactions constitute the **metabolism** of the cell (**Figure 2–36**).

Many of the details of cell metabolism form the traditional subject of *biochemistry* and need not concern us here. But the general principles by which cells obtain energy from their environment and use it to create order are central to cell biology. We begin with a discussion of why a constant input of energy is needed to sustain living organisms.

Biological Order Is Made Possible by the Release of Heat Energy from Cells

The universal tendency of things to become disordered is a fundamental law of physics—the *second law of thermodynamics*—which states that in the universe, or in any isolated system (a collection of matter that is completely isolated from the rest of the universe), the degree of disorder only increases. This law has such profound implications for all living things that we restate it in several ways.

For example, we can present the second law in terms of probability and state that systems will change spontaneously toward those arrangements that have the greatest probability. If we consider, for example, a box of 100 coins all lying heads up, a series of accidents that disturbs the box will tend to move the arrangement toward a mixture of 50 heads and 50 tails. The reason is simple: there is a huge number of possible arrangements of the individual coins in the mixture that can achieve the 50–50 result, but only one possible arrangement that keeps all of the coins oriented heads up. Because the 50–50 mixture is therefore the most probable, we say that it is more "disordered." For the same reason,

Figure 2–35 **Some of the metabolic pathways and their interconnections in a typical cell.** About 500 common metabolic reactions are shown diagrammatically, with each molecule in a metabolic pathway represented by a filled circle, as in the *yellow* box in Figure 2–34. The pathway that is highlighted in this diagram with larger circles and connecting lines is the central pathway of sugar metabolism, which will be discussed shortly.
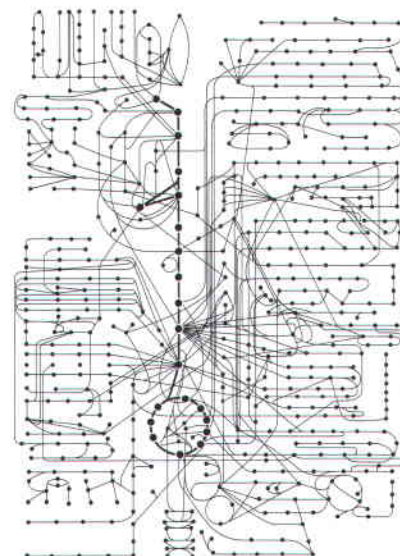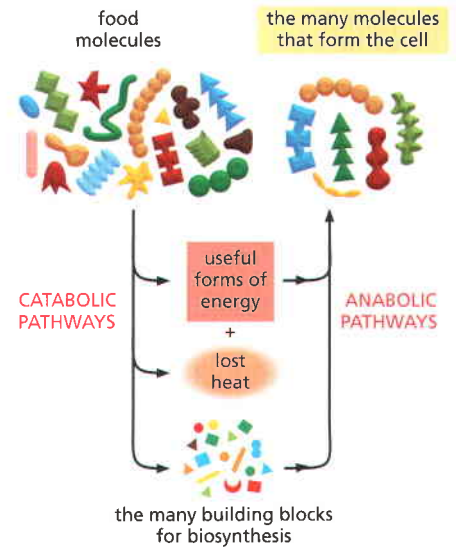
Figure 2–36 **Schematic representation of the relationship between catabolic and anabolic pathways in metabolism.** As suggested here, since a major portion of the energy stored in the chemical bonds of food molecules is dissipated as heat, the mass of food required by any organism that derives all of its energy from catabolism is much greater than the mass of the molecules that can be produced by anabolism.

food molecules

the many molecules that form the cell

CATABOLIC PATHWAYS

useful forms of energy

+

lost heat

ANABOLIC PATHWAYS

the many building blocks for biosynthesis

it is a common experience that one's living space will become increasingly disordered without intentional effort: the movement toward disorder is a *spontaneous process*, requiring a periodic effort to reverse it (**Figure 2–37**).

The amount of disorder in a system can be quantified and expressed as the **entropy** of the system: the greater the disorder, the greater the entropy. Thus, another way to express the second law of thermodynamics is to say that systems will change spontaneously toward arrangements with greater entropy.

Living cells—by surviving, growing, and forming complex organisms—are generating order and thus might appear to defy the second law of thermodynamics. How is this possible? The answer is that a cell is not an isolated system: it takes in energy from its environment in the form of food, or as photons from the sun (or even, as in some chemosynthetic bacteria, from inorganic molecules alone), and it then uses this energy to generate order within itself. In the course of the chemical reactions that generate order, the cell converts part of the energy it uses into heat. The heat is discharged into the cell's environment and disorders it, so that the total entropy—that of the cell plus its surroundings—increases, as demanded by the laws of thermodynamics.

To understand the principles governing these energy conversions, think of a cell surrounded by a sea of matter representing the rest of the universe. As the cell lives and grows, it creates internal order. But it constantly releases heat energy as it synthesizes molecules and assembles them into cell structures. Heat is energy in its most disordered form—the random jostling of molecules. When the cell releases heat to the sea, it increases the intensity of molecular motions there (thermal motion)—thereby increasing the randomness, or disorder, of the sea. The second law of thermodynamics is satisfied because the increase in the amount of order inside the cell is more than compensated for by an even greater decrease in order (increase in entropy) in the surrounding sea of matter (**Figure 2–38**).

Where does the heat that the cell releases come from? Here we encounter another important law of thermodynamics. The *first law of thermodynamics* states that energy can be converted from one form to another, but that it cannot
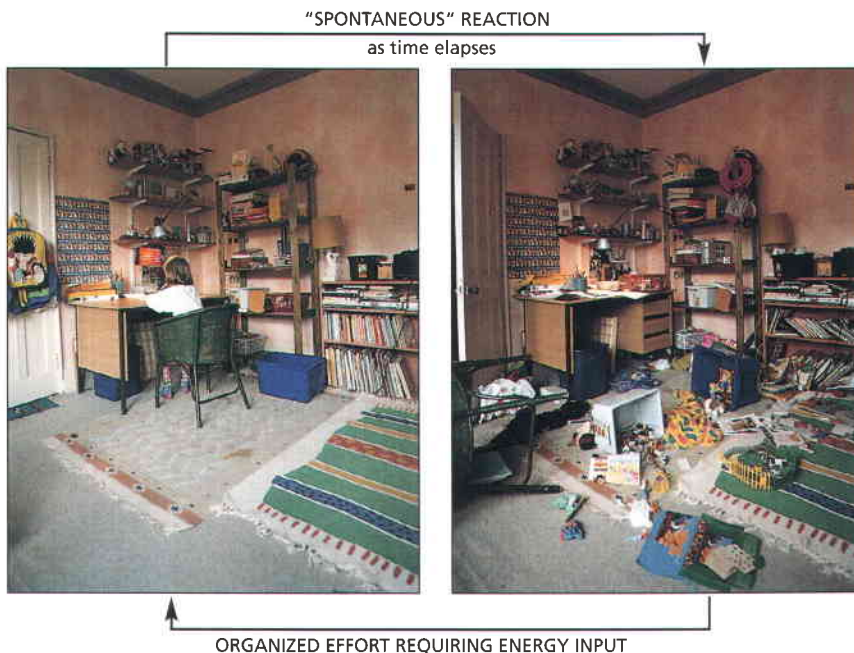
"SPONTANEOUS" REACTION

as time elapses

ORGANIZED EFFORT REQUIRING ENERGY INPUT

Figure 2–37 **An everyday illustration of the spontaneous drive toward disorder.** Reversing this tendency toward disorder requires an intentional effort and an input of energy: it is not spontaneous. In fact, from the second law of thermodynamics, we can be certain that the human intervention required will release enough heat to the environment to more than compensate for the reordering of the items in this room.
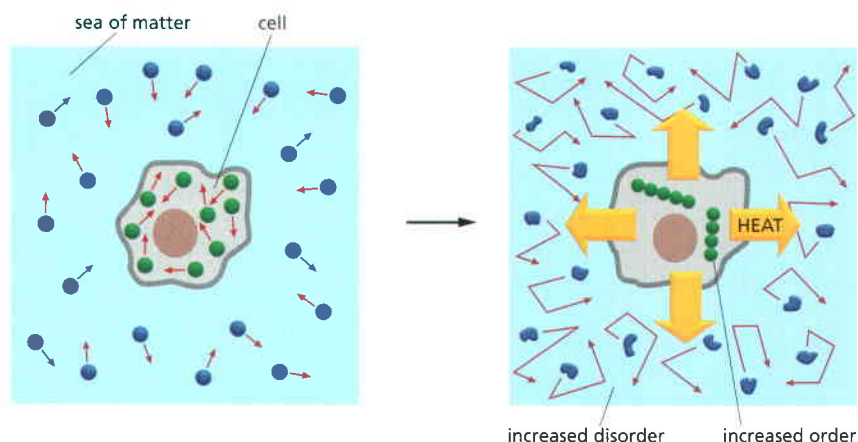
Figure 2–38 A simple thermodynamic analysis of a living cell. In the diagram on the left the molecules of both the cell and the rest of the universe (the sea of matter) are depicted in a relatively disordered state. In the diagram on the right the cell has taken in energy from food molecules and released heat by a reaction that orders the molecules the cell contains. Because the heat increases the disorder in the environment around the cell (depicted by the *jagged arrows* and distorted molecules, indicating the increased molecular motions caused by heat), the second law of thermodynamics—which states that the amount of disorder in the universe must always increase—is satisfied as the cell grows and divides. For a detailed discussion, see Panel 2–7 (pp. 118–119).

be created or destroyed. **Figure 2–39** illustrates some interconversions between different forms of energy. The amount of energy in different forms will change as a result of the chemical reactions inside the cell, but the first law tells us that the total amount of energy must always be the same. For example, an animal cell takes in foodstuffs and converts some of the energy present in the chemical bonds between the atoms of these food molecules (chemical bond energy) into the random thermal motion of molecules (heat energy). As described above, this conversion of chemical energy into heat energy is essential if the reactions that create order inside the cell are to cause the universe as a whole to become more disordered.

The cell cannot derive any benefit from the heat energy it releases unless the heat-generating reactions inside the cell are directly linked to the processes that generate molecular order. It is the tight *coupling* of heat production to an increase in order that distinguishes the metabolism of a cell from the wasteful burning of fuel in a fire. Later, we shall illustrate how this coupling occurs. For now, it is sufficient to recognize that a direct linkage of the "burning" of food molecules to the generation of biological order is required for cells to create and maintain an island of order in a universe tending toward chaos.

Photosynthetic Organisms Use Sunlight to Synthesize Organic Molecules

All animals live on energy stored in the chemical bonds of organic molecules made by other organisms, which they take in as food. The molecules in food also provide the atoms that animals need to construct new living matter. Some animals obtain their food by eating other animals. But at the bottom of the animal food chain are animals that eat plants. The plants, in turn, trap energy directly from sunlight. As a result, the sun is the ultimate source of the energy used by animal cells.

Solar energy enters the living world through **photosynthesis** in plants and photosynthetic bacteria. Photosynthesis converts the electromagnetic energy in sunlight into chemical bond energy in the cell. Plants obtain all the atoms they need from inorganic sources: carbon from atmospheric carbon dioxide, hydrogen and oxygen from water, nitrogen from ammonia and nitrates in the
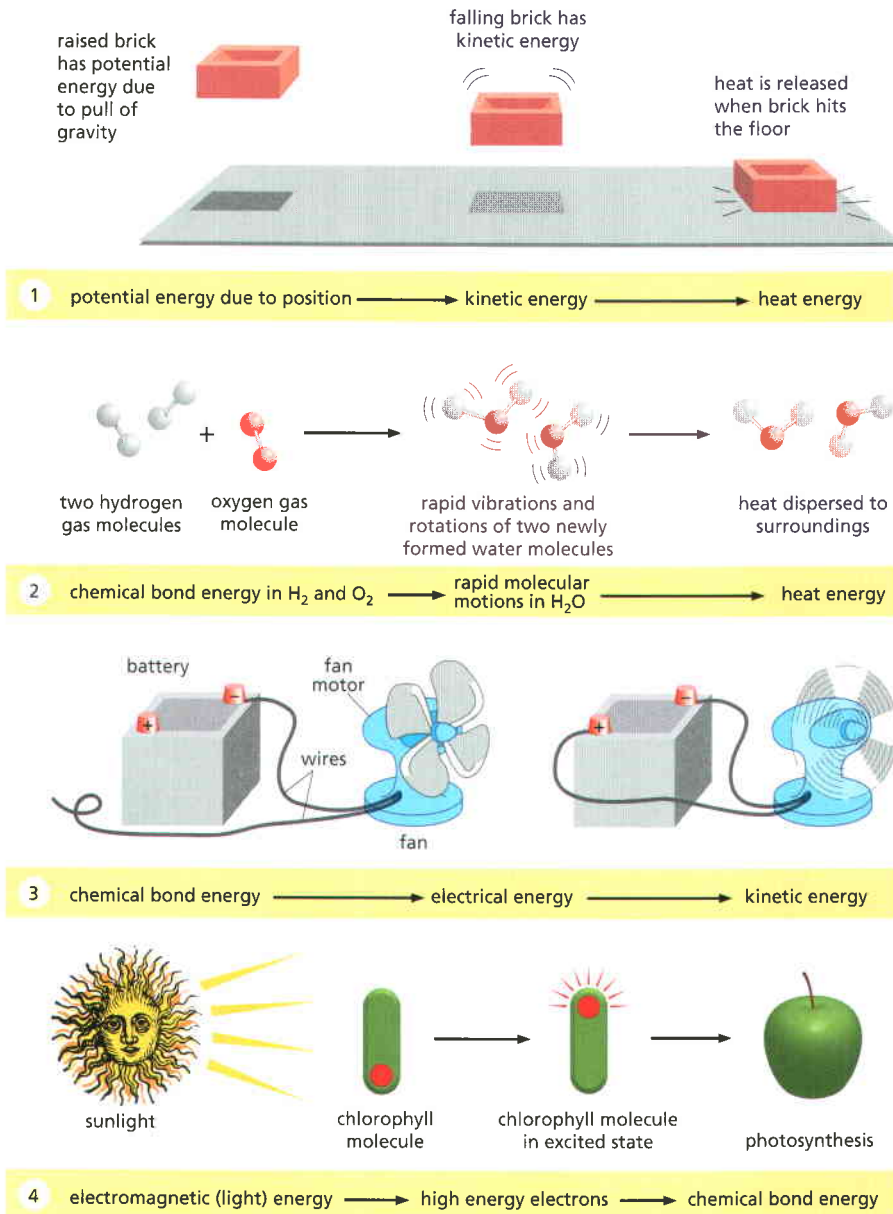
1 potential energy due to position ⟶ kinetic energy ⟶ heat energy

2 chemical bond energy in H_2 and O_2 ⟶ rapid molecular motions in H_2O ⟶ heat energy

3 chemical bond energy ⟶ electrical energy ⟶ kinetic energy

4 electromagnetic (light) energy ⟶ high energy electrons ⟶ chemical bond energy

Figure 2–39 Some interconversions between different forms of energy. All energy forms are, in principle, interconvertible. In all these processes the total amount of energy is conserved. Thus, for example, from the height and weight of the brick in (1), we can predict exactly how much heat will be released when it hits the floor. In (2), note that the large amount of chemical bond energy released when water is formed is initially converted to very rapid thermal motions in the two new water molecules; but collisions with other molecules almost instantaneously spread this kinetic energy evenly throughout the surroundings (heat transfer), making the new molecules indistinguishable from all the rest.

soil, and other elements needed in smaller amounts from inorganic salts in the soil. They use the energy they derive from sunlight to build these atoms into sugars, amino acids, nucleotides, and fatty acids. These small molecules in turn are converted into the proteins, nucleic acids, polysaccharides, and lipids that form the plant. All of these substances serve as food molecules for animals, if the plants are later eaten.

The reactions of photosynthesis take place in two stages (**Figure 2–40**). In the first stage, energy from sunlight is captured and transiently stored as chemical bond energy in specialized small molecules that act as carriers of energy and reactive chemical groups. (We discuss these "activated carrier" molecules later.) Molecular oxygen (O_2 gas) derived from the splitting of water by light is released as a waste product of this first stage.

In the second stage, the molecules that serve as energy carriers are used to help drive a *carbon fixation* process in which sugars are manufactured from carbon dioxide gas (CO_2) and water (H_2O), thereby providing a useful source of stored chemical bond energy and materials—both for the plant itself and for any animals that eat it. We describe the elegant mechanisms that underlie these two stages of photosynthesis in Chapter 14.
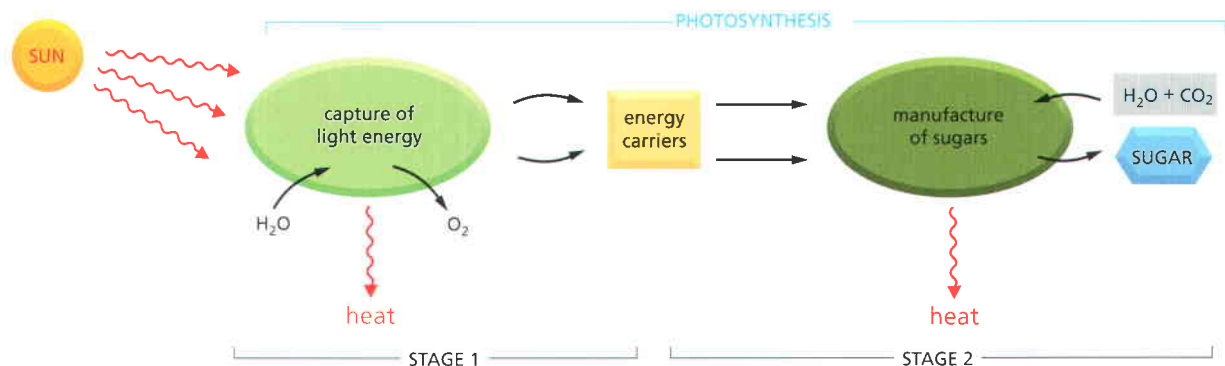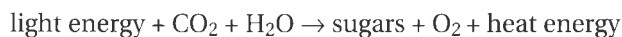
Figure 2–40 **Photosynthesis.** The two stages of photosynthesis. The energy carriers created in the first stage are two molecules that we discuss shortly—ATP and NADPH.

The net result of the entire process of photosynthesis, so far as the green plant is concerned, can be summarized simply in the equation

$$\text{light energy} + CO_2 + H_2O \rightarrow \text{sugars} + O_2 + \text{heat energy}$$

The sugars produced are then used both as a source of chemical bond energy and as a source of materials to make the many other small and large organic molecules that are essential to the plant cell.

Cells Obtain Energy by the Oxidation of Organic Molecules

All animal and plant cells are powered by energy stored in the chemical bonds of organic molecules, whether they are sugars that a plant has photosynthesized as food for itself or the mixture of large and small molecules that an animal has eaten. Organisms must extract this energy in usable form to live, grow, and reproduce. In both plants and animals, energy is extracted from food molecules by a process of gradual oxidation, or controlled burning.

The Earth's atmosphere contains a great deal of oxygen, and in the presence of oxygen the most energetically stable form of carbon is CO_2 and that of hydrogen is H_2O. A cell is therefore able to obtain energy from sugars or other organic molecules by allowing their carbon and hydrogen atoms to combine with oxygen to produce CO_2 and H_2O, respectively—a process called **respiration**.

Photosynthesis and respiration are complementary processes (**Figure 2–41**). This means that the transactions between plants and animals are not all one way. Plants, animals, and microorganisms have existed together on this planet for so long that many of them have become an essential part of the others' environments. The oxygen released by photosynthesis is consumed in the combustion of organic molecules by nearly all organisms. And some of the CO_2 molecules that are fixed today into organic molecules by photosynthesis in a green leaf were yesterday released into the atmosphere by the respiration of an animal—or by that of a fungus or bacterium decomposing dead organic matter. We therefore see that carbon utilization forms a huge cycle that involves the *biosphere* (all of the living organisms on Earth) as a whole, crossing boundaries
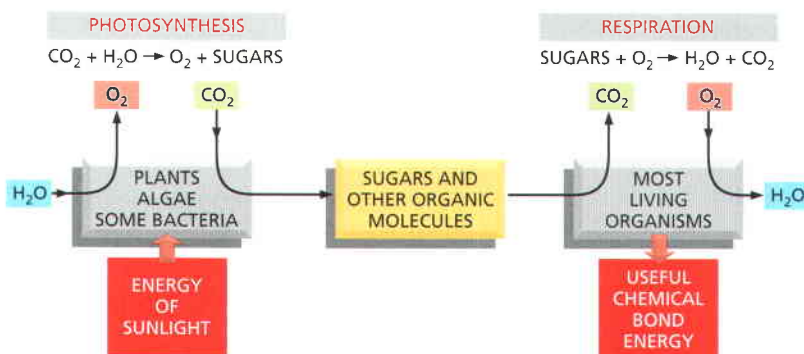
Figure 2–41 **Photosynthesis and respiration as complementary processes in the living world.** Photosynthesis uses the energy of sunlight to produce sugars and other organic molecules. These molecules in turn serve as food for other organisms. Many of these organisms carry out respiration, a process that uses O_2 to form CO_2 from the same carbon atoms that had been taken up as CO_2 and converted into sugars by photosynthesis. In the process, the organisms that respire obtain the chemical bond energy that they need to survive. The first cells on the Earth are thought to have been capable of neither photosynthesis nor respiration (discussed in Chapter 14). However, photosynthesis must have preceded respiration on the Earth, since there is strong evidence that billions of years of photosynthesis were required before O_2 had been released in sufficient quantity to create an atmosphere rich in this gas. (The Earth's atmosphere currently contains 20% O_2.)
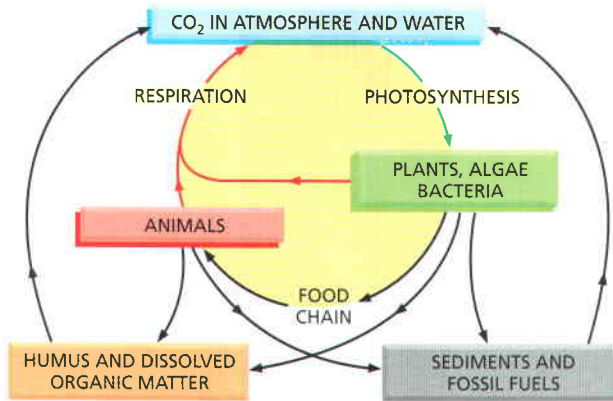
Figure 2–42 **The carbon cycle.** Individual carbon atoms are incorporated into organic molecules of the living world by the photosynthetic activity of bacteria and plants (including algae). They pass to animals, microorganisms, and organic material in soil and oceans in cyclic paths. CO_2 is restored to the atmosphere when organic molecules are oxidized by cells or burned by humans as fuels.

between individual organisms (**Figure 2–42**). Similarly, atoms of nitrogen, phosphorus, and sulfur move between the living and nonliving worlds in cycles that involve plants, animals, fungi, and bacteria.

Oxidation and Reduction Involve Electron Transfers

The cell does not oxidize organic molecules in one step, as occurs when organic material is burned in a fire. Through the use of enzyme catalysts, metabolism takes the molecules through a large number of reactions that only rarely involve the direct addition of oxygen. Before we consider some of these reactions and their purpose, we discuss what is meant by the process of oxidation.

Oxidation does not mean only the addition of oxygen atoms; rather, it applies more generally to any reaction in which electrons are transferred from one atom to another. Oxidation in this sense refers to the removal of electrons, and **reduction**—the converse of oxidation—means the addition of electrons. Thus, Fe^{2+} is oxidized if it loses an electron to become Fe^{3+}, and a chlorine atom is reduced if it gains an electron to become Cl^-. Since the number of electrons is conserved (no loss or gain) in a chemical reaction, oxidation and reduction always occur simultaneously: that is, if one molecule gains an electron in a reaction (reduction), a second molecule loses the electron (oxidation). When a sugar molecule is oxidized to CO_2 and H_2O, for example, the O_2 molecules involved in forming H_2O gain electrons and thus are said to have been reduced.

The terms "oxidation" and "reduction" apply even when there is only a partial shift of electrons between atoms linked by a covalent bond (**Figure 2–43**).
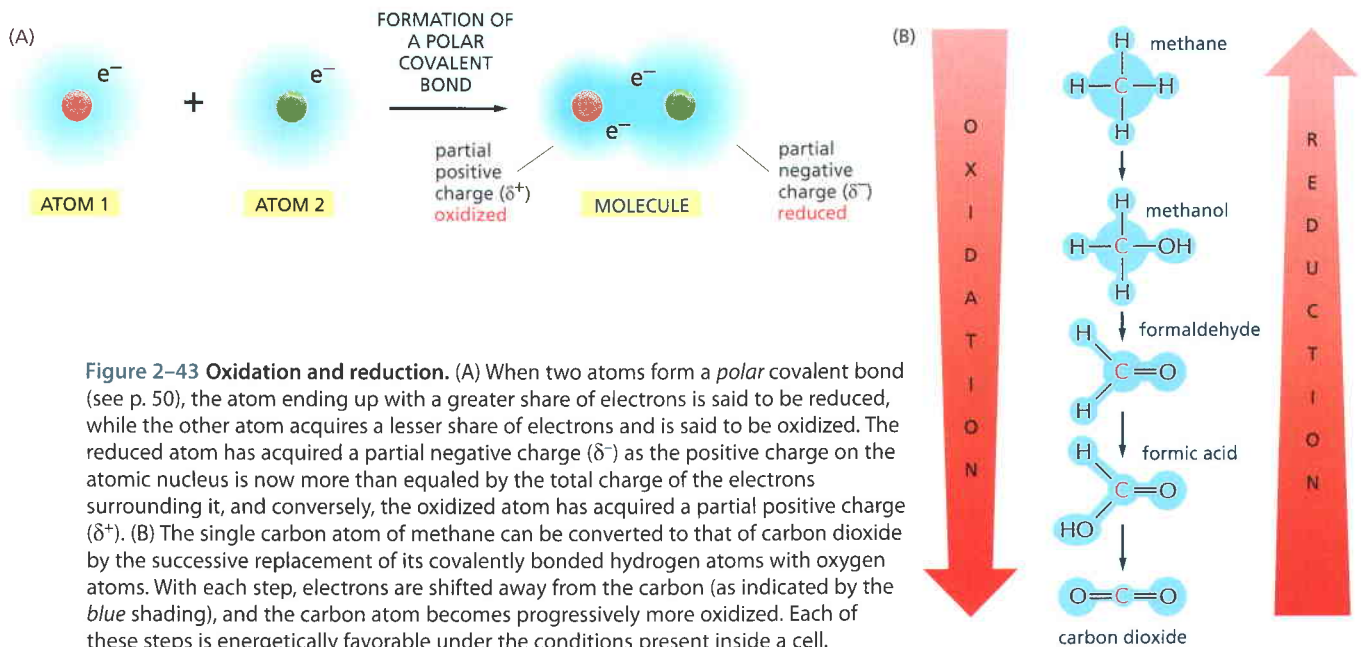

Figure 2–43 **Oxidation and reduction.** (A) When two atoms form a *polar* covalent bond (see p. 50), the atom ending up with a greater share of electrons is said to be reduced, while the other atom acquires a lesser share of electrons and is said to be oxidized. The reduced atom has acquired a partial negative charge (δ^-) as the positive charge on the atomic nucleus is now more than equaled by the total charge of the electrons surrounding it, and conversely, the oxidized atom has acquired a partial positive charge (δ^+). (B) The single carbon atom of methane can be converted to that of carbon dioxide by the successive replacement of its covalently bonded hydrogen atoms with oxygen atoms. With each step, electrons are shifted away from the carbon (as indicated by the *blue* shading), and the carbon atom becomes progressively more oxidized. Each of these steps is energetically favorable under the conditions present inside a cell.

When a carbon atom becomes covalently bonded to an atom with a strong affinity for electrons, such as oxygen, chlorine, or sulfur, for example, it gives up more than its equal share of electrons and forms a *polar* covalent bond: the positive charge of the carbon nucleus is now somewhat greater than the negative charge of its electrons, and the atom therefore acquires a partial positive charge and is said to be oxidized. Conversely, a carbon atom in a C–H linkage has slightly more than its share of electrons, and so it is said to be reduced (see Figure 2–43).

When a molecule in a cell picks up an electron (e^-), it often picks up a proton (H^+) at the same time (protons being freely available in water). The net effect in this case is to add a hydrogen atom to the molecule

$$A + e^- + H^+ \rightarrow AH$$

Even though a proton plus an electron is involved (instead of just an electron), such *hydrogenation* reactions are reductions, and the reverse, *dehydrogenation* reactions, are oxidations. It is especially easy to tell whether an organic molecule is being oxidized or reduced: reduction is occurring if its number of C–H bonds increases, whereas oxidation is occurring if its number of C–H bonds decreases (see Figure 2–43B).

Cells use enzymes to catalyze the oxidation of organic molecules in small steps, through a sequence of reactions that allows useful energy to be harvested. We now need to explain how enzymes work and some of the constraints under which they operate.

Enzymes Lower the Barriers That Block Chemical Reactions

Consider the reaction

$$paper + O_2 \rightarrow smoke + ashes + heat + CO_2 + H_2O$$
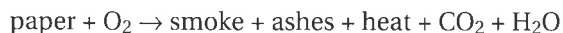
The paper burns readily, releasing to the atmosphere both energy as heat and water and carbon dioxide as gases, but the smoke and ashes never spontaneously retrieve these entities from the heated atmosphere and reconstitute themselves into paper. When the paper burns, its chemical energy is dissipated as heat—not lost from the universe, since energy can never be created or destroyed, but irretrievably dispersed in the chaotic random thermal motions of molecules. At the same time, the atoms and molecules of the paper become dispersed and disordered. In the language of thermodynamics, there has been a loss of *free energy*, that is, of energy that can be harnessed to do work or drive chemical reactions. This loss reflects a loss of orderliness in the way the energy and molecules were stored in the paper. We shall discuss free energy in more detail shortly, but the general principle is clear enough intuitively: chemical reactions proceed spontaneously only in the direction that leads to a loss of free energy; in other words, the spontaneous direction for any reaction is the direction that goes "downhill." A "downhill" reaction in this sense is often said to be *energetically favorable*.

Although the most energetically favorable form of carbon under ordinary conditions is CO_2, and that of hydrogen is H_2O, a living organism does not disappear in a puff of smoke, and the book in your hands does not burst into flames. This is because the molecules both in the living organism and in the book are in a relatively stable state, and they cannot be changed to a state of lower energy without an input of energy: in other words, a molecule requires **activation energy**—a kick over an energy barrier—before it can undergo a chemical reaction that leaves it in a more stable state (**Figure 2–44**). In the case of a burning book, the activation energy is provided by the heat of a lighted match. For the molecules in the watery solution inside a cell, the kick is delivered by an unusually energetic random collision with surrounding molecules—collisions that become more violent as the temperature is raised.

In a living cell, the kick over the energy barrier is greatly aided by a specialized class of proteins—the **enzymes**. Each enzyme binds tightly to one or more molecules, called **substrates**, and holds them in a way that greatly reduces the activation energy of a particular chemical reaction that the bound substrates can undergo. A substance that can lower the activation energy of a reaction is
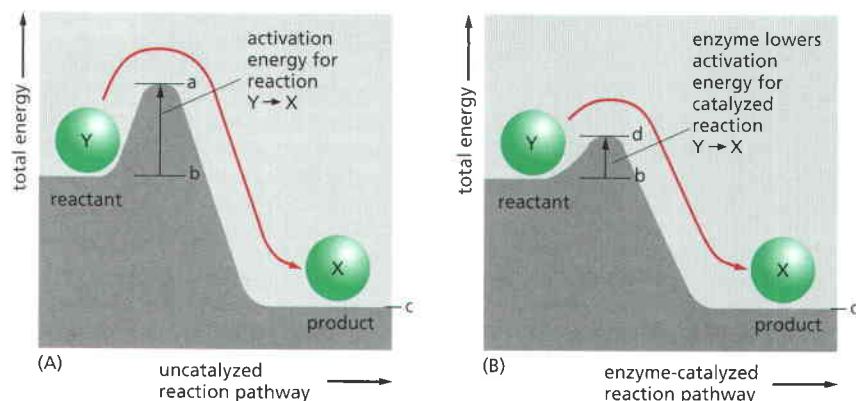
Figure 2–44 **The important principle of activation energy.** (A) Compound Y (a reactant) is in a relatively stable state, and energy is required to convert it to compound X (a product), even though X is at a lower overall energy level than Y. This conversion will not take place, therefore, unless compound Y can acquire enough activation energy *(energy a minus energy b)* from its surroundings to undergo the reaction that converts it into compound X. This energy may be provided by means of an unusually energetic collision with other molecules. For the reverse reaction, X → Y, the activation energy will be much larger *(energy a minus energy c);* this reaction will therefore occur much more rarely. Activation energies are always positive; note, however, that the total energy change for the energetically favorable reaction Y → X is *energy c minus energy b,* a negative number. (B) Energy barriers for specific reactions can be lowered by catalysts, as indicated by the line marked *d*. Enzymes are particularly effective catalysts because they greatly reduce the activation energy for the reactions they perform.

termed a **catalyst**; catalysts increase the rate of chemical reactions because they allow a much larger proportion of the random collisions with surrounding molecules to kick the substrates over the energy barrier, as illustrated in **Figure 2–45**. Enzymes are among the most effective catalysts known, capable of speeding up reactions by factors of 10^{14} or more. They thereby allow reactions that would not otherwise occur to proceed rapidly at normal temperatures.

Enzymes are also highly selective. Each enzyme usually catalyzes only one particular reaction: in other words, it selectively lowers the activation energy of only one of the several possible chemical reactions that its bound substrate molecules could undergo. In this way, enzymes direct each of the many different molecules in a cell along specific reaction pathways (**Figure 2–46**).

The success of living organisms is attributable to a cell's ability to make enzymes of many types, each with precisely specified properties. Each enzyme has a unique shape containing an *active site*, a pocket or groove in the enzyme into which only particular substrates will fit (**Figure 2–47**). Like all other catalysts, enzyme molecules themselves remain unchanged after participating in a reaction and therefore can function over and over again. In Chapter 3, we discuss further how enzymes work.
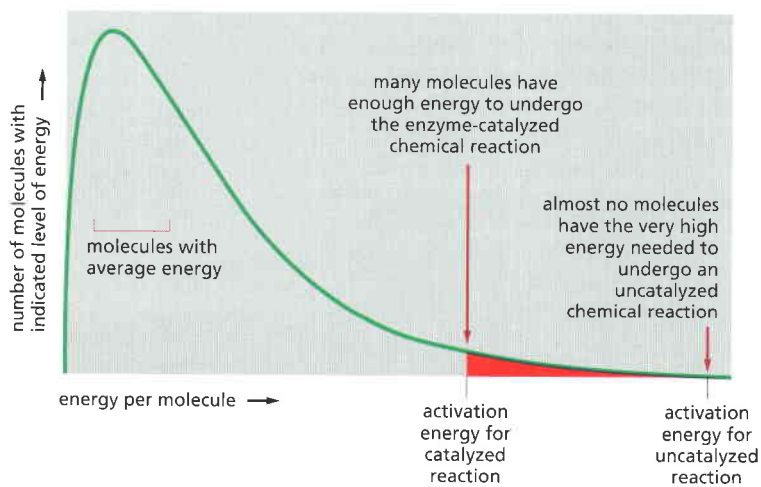
Figure 2–45 **Lowering the activation energy greatly increases the probability of reaction.** At any given instant, a population of identical substrate molecules will have a range of energies, distributed as shown on the graph. The varying energies come from collisions with surrounding molecules, which make the substrate molecules jiggle, vibrate, and spin. For a molecule to undergo a chemical reaction, the energy of the molecule must exceed the activation energy barrier for that reaction; for most biological reactions, this almost never happens without enzyme catalysis. Even with enzyme catalysis, the substrate molecules must experience a particularly energetic collision to react *(red shaded area)*. Raising the temperature can also increase the number of molecules with sufficient energy to overcome the activation energy needed for a reaction; but in contrast to enzyme catalysis, this effect is nonselective, speeding up all reactions.
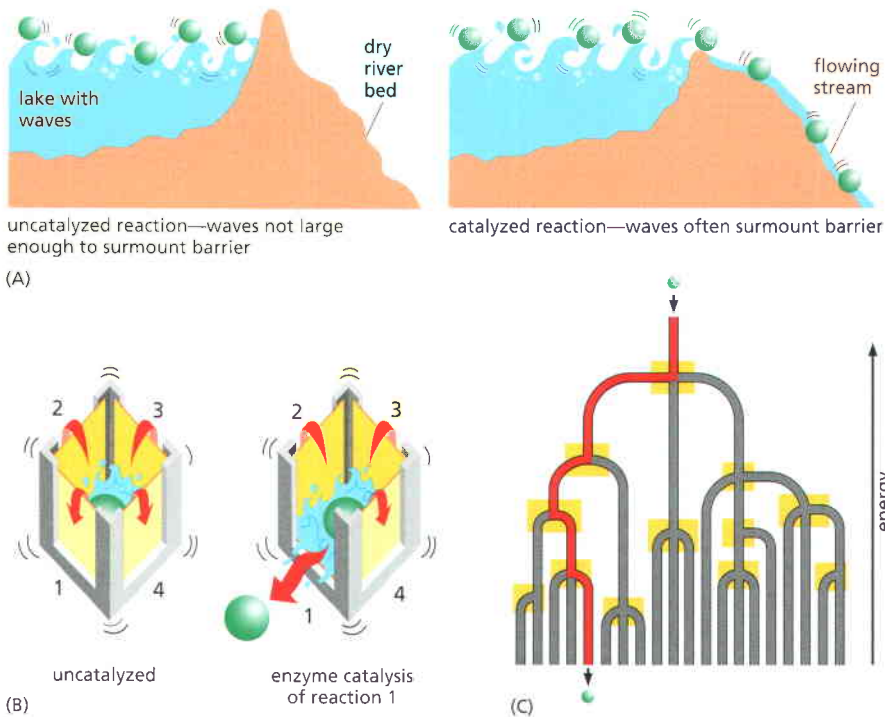
(A)

(B) uncatalyzed   enzyme catalysis
                       of reaction 1

(C)

energy

Figure 2–46 **Floating ball analogies for enzyme catalysis.** <TAAA> (A) A barrier dam is lowered to represent enzyme catalysis. The *green ball* represents a potential reactant (compound Y) that is bouncing up and down in energy level due to constant encounters with waves (an analogy for the thermal bombardment of the reactant molecule with the surrounding water molecules). When the barrier (activation energy) is lowered significantly, it allows the energetically favorable movement of the ball (the reactant) downhill. (B) The four walls of the box represent the activation energy barriers for four different chemical reactions that are all energetically favorable, in the sense that the products are at lower energy levels than the reactants. In the *left-hand* box, none of these reactions occurs because even the largest waves are not large enough to surmount any of the energy barriers. In the *right-hand* box, enzyme catalysis lowers the activation energy for reaction number 1 only; now the jostling of the waves allows passage of the reactant molecule over this energy barrier, inducing reaction 1. (C) A branching river with a set of barrier dams *(yellow boxes)* serves to illustrate how a series of enzyme-catalyzed reactions determines the exact reaction pathway followed by each molecule inside the cell.

How Enzymes Find Their Substrates: The Enormous Rapidity of Molecular Motions

An enzyme will often catalyze the reaction of thousands of substrate molecules every second. This means that it must be able to bind a new substrate molecule in a fraction of a millisecond. But both enzymes and their substrates are present in relatively small numbers in a cell. How do they find each other so fast? Rapid binding is possible because the motions caused by heat energy are enormously fast at the molecular level. These molecular motions can be classified broadly into three kinds: (1) the movement of a molecule from one place to another (*translational motion*), (2) the rapid back-and-forth movement of covalently linked atoms with respect to one another (vibrations), and (3) rotations. All of these motions help to bring the surfaces of interacting molecules together.

The rates of molecular motions can be measured by a variety of spectroscopic techniques. A large globular protein is constantly tumbling, rotating about its axis about a million times per second. Molecules are also in constant translational motion, which causes them to explore the space inside the cell very efficiently by wandering through it—a process called **diffusion**. In this way, every molecule in a cell collides with a huge number of other molecules each second. As the molecules in a liquid collide and bounce off one another, an individual molecule moves first one way and then another, its path constituting a *random walk* (**Figure 2–48**). In such a walk, the average net distance that each molecule travels (as the crow flies) from its starting point is proportional to the square root of the time involved: that is, if it takes a molecule 1 second on average to travel 1 μm, it takes 4 seconds to travel 2 μm, 100 seconds to travel 10 μm, and so on.

The inside of a cell is very crowded (**Figure 2–49**). Nevertheless, experiments in which fluorescent dyes and other labeled molecules are injected into cells
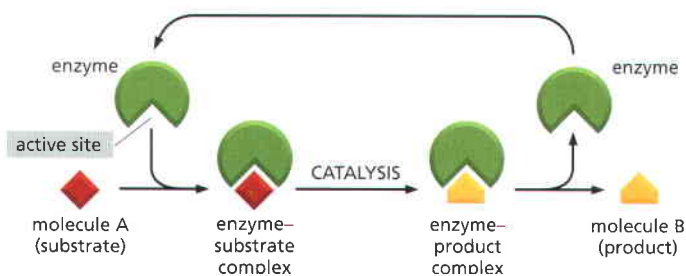

Figure 2–47 **How enzymes work.** Each enzyme has an active site to which one or more *substrate* molecules bind, forming an enzyme–substrate complex. A reaction occurs at the active site, producing an enzyme–product complex. The *product* is then released, allowing the enzyme to bind further substrate molecules.

show that small organic molecules diffuse through the watery gel of the cytosol nearly as rapidly as they do through water. A small organic molecule, for example, takes only about one-fifth of a second on average to diffuse a distance of 10 μm. Diffusion is therefore an efficient way for small molecules to move the limited distances in the cell (a typical animal cell is 15 μm in diameter).

Since enzymes move more slowly than substrates in cells, we can think of them as sitting still. The rate of encounter of each enzyme molecule with its substrate will depend on the concentration of the substrate molecule. For example, some abundant substrates are present at a concentration of 0.5 mM. Since pure water is 55.5 M, there is only about one such substrate molecule in the cell for every 10^5 water molecules. Nevertheless, the active site on an enzyme molecule that binds this substrate will be bombarded by about 500,000 random collisions with the substrate molecule per second. (For a substrate concentration tenfold lower, the number of collisions drops to 50,000 per second, and so on.) A random encounter between the surface of an enzyme and the matching surface of its substrate molecule often leads immediately to the formation of an enzyme–substrate complex that is ready to react. A reaction in which a covalent bond is broken or formed can now occur extremely rapidly. When one appreciates how quickly molecules move and react, the observed rates of enzymatic catalysis do not seem so amazing.

Once an enzyme and substrate have collided and snuggled together properly at the active site, they form multiple weak bonds with each other that persist until random thermal motion causes the molecules to dissociate again. In general, the stronger the binding of the enzyme and substrate, the slower their rate of dissociation. However, when two colliding molecules have poorly matching surfaces, they form few noncovalent bonds and their total energy is negligible compared with that of thermal motion. In this case the two molecules dissociate as rapidly as they come together, preventing incorrect and unwanted associations between mismatched molecules, such as between an enzyme and the wrong substrate.
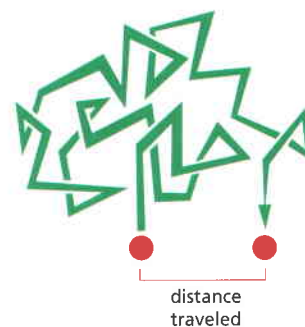

Figure 2–48 A random walk. <GGTA> Molecules in solution move in a random fashion as a result of the continual buffeting they receive in collisions with other molecules. This movement allows small molecules to diffuse rapidly from one part of the cell to another, as described in the text.

The Free-Energy Change for a Reaction Determines Whether It Can Occur

We must now digress briefly to introduce some fundamental chemistry. Cells are chemical systems that must obey all chemical and physical laws. Although enzymes speed up reactions, they cannot by themselves force energetically unfavorable reactions to occur. In terms of a water analogy, enzymes by themselves cannot make water run uphill. Cells, however, must do just that in order to grow and divide: they must build highly ordered and energy-rich molecules from small and simple ones. We shall see that this is done through enzymes that directly *couple* energetically favorable reactions, which release energy and produce heat, to energetically unfavorable reactions, which produce biological order.

Before examining how such coupling is achieved, we must consider more carefully the term "energetically favorable." According to the second law of thermodynamics, a chemical reaction can proceed spontaneously only if it results in a net increase in the disorder of the universe (see Figure 2–38). The criterion for an increase in disorder of the universe can be expressed most conveniently in terms of a quantity called the **free energy**, G, of a system. The value of G is of interest only when a system undergoes a *change*, and the change in G, denoted ΔG (delta G), is critical. Suppose that the system being considered is a collection of molecules. As explained in Panel 2–7 (pp. 118–119), free energy has been defined such that ΔG directly measures the amount of disorder created in the universe when a reaction takes place that involves these molecules. *Energetically favorable reactions*, by definition, are those that decrease free energy; in other words, they have a *negative* ΔG and disorder the universe (**Figure 2–50**).

An example of an energetically favorable reaction on a macroscopic scale is the "reaction" by which a compressed spring relaxes to an expanded state, releasing its stored elastic energy as heat to its surroundings; an example on a microscopic scale is salt dissolving in water. Conversely, *energetically unfavorable reactions*, with a *positive* ΔG—such as the joining of two amino acids to

100 nm
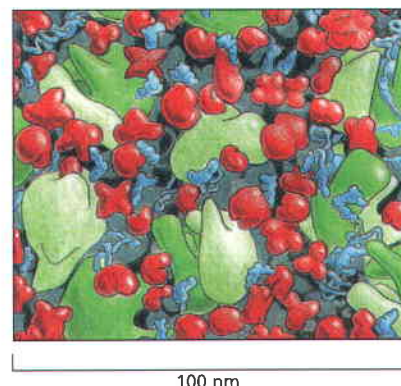
Figure 2–49 The structure of the cytoplasm. The drawing is approximately to scale and emphasizes the crowding in the cytoplasm. Only the macromolecules are shown: RNAs are shown in *blue*, ribosomes in *green*, and proteins in *red*. Enzymes and other macromolecules diffuse relatively slowly in the cytoplasm, in part because they interact with many other macromolecules; small molecules, by contrast, diffuse nearly as rapidly as they do in water. (Adapted from D.S. Goodsell, *Trends Biochem. Sci.* 16:203–206, 1991. With permission from Elsevier.)

form a peptide bond—by themselves create order in the universe. Therefore, these reactions can take place only if they are coupled to a second reaction with a negative ΔG so large that the ΔG of the entire process is negative (**Figure 2–51**).

The Concentration of Reactants Influences the Free-Energy Change and a Reaction's Direction

As we have just described, a reaction $Y \rightleftharpoons X$ will go in the direction $Y \rightarrow X$ when the associated free-energy change, ΔG, is negative, just as a tensed spring left to itself will relax and lose its stored energy to its surroundings as heat. For a chemical reaction, however, ΔG depends not only on the energy stored in each individual molecule, but also on the concentrations of the molecules in the reaction mixture. Remember that ΔG reflects the degree to which a reaction creates a more disordered—in other words, a more probable—state of the universe. Recalling our coin analogy, it is very likely that a coin will flip from a head to a tail orientation if a jiggling box contains 90 heads and 10 tails, but this is a less probable event if the box has 10 heads and 90 tails.

The same is true for a chemical reaction. For a reversible reaction $Y \rightleftharpoons X$, a large excess of Y over X will tend to drive the reaction in the direction $Y \rightarrow X$; that is, there will be a tendency for there to be more molecules making the transition $Y \rightarrow X$ than there are molecules making the transition $X \rightarrow Y$. If the ratio of Y to X increases, the ΔG becomes more negative for the transition $Y \rightarrow X$ (and more positive for the transition $X \rightarrow Y$).

How much of a concentration difference is needed to compensate for a given decrease in chemical bond energy (and accompanying heat release)? The answer is not intuitively obvious, but it can be determined from a thermodynamic analysis that makes it possible to separate the concentration-dependent and the concentration-independent parts of the free-energy change. The ΔG for a given reaction can thereby be written as the sum of two parts: the first, called the *standard free-energy change*, $\Delta G°$, depends on the intrinsic characters of the reacting molecules; the second depends on their concentrations. For the simple reaction $Y \rightarrow X$ at 37°C,

$$\Delta G = \Delta G° + 0.616 \ln \frac{[X]}{[Y]} = \Delta G° + 1.42 \log \frac{[X]}{[Y]}$$

where ΔG is in kilocalories per mole, [Y] and [X] denote the concentrations of Y and X, ln is the natural logarithm, and the constant 0.616 is equal to RT: the product of the gas constant, R, and the absolute temperature, T.

Note that ΔG equals the value of $\Delta G°$ when the molar concentrations of Y and X are equal (log 1 = 0). As expected, ΔG becomes more negative as the ratio of X to Y decreases (the log of a number < 1 is negative).
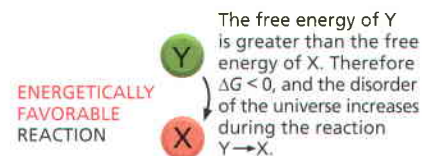
Inspection of the above equation reveals that the ΔG equals the value of $\Delta G°$ when the concentrations of Y and X are equal. But as the favorable reaction $Y \rightarrow X$ proceeds, the concentration of the product X increases and the concentration of the substrate Y decreases. This change in relative concentrations will cause [X]/[Y] to become increasingly large, making the initially favorable ΔG less and less negative. Eventually, when $\Delta G = 0$, a chemical **equilibrium** will be attained; here the concentration effect just balances the push given to the reaction by $\Delta G°$, and the ratio of substrate to product reaches a constant value (**Figure 2–52**).

How far will a reaction proceed before it stops at equilibrium? To address this question, we need to introduce the **equilibrium constant**, K. The value of K is different for different reactions, and it reflects the ratio of product to substrate at equilibrium. For the reaction $Y \rightarrow X$:
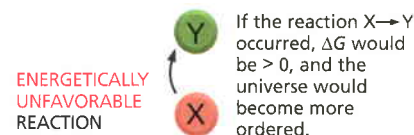
$$K = \frac{[X]}{[Y]}$$

The equation that connects ΔG and the ratio [X]/[Y] allows us to connect $\Delta G°$ directly to K. Since $\Delta G = 0$ at equilibrium, the concentrations of Y and X at this point are such that:

$$\Delta G° = -1.42 \log \frac{[X]}{[Y]} \quad \text{or,} \quad \Delta G° = -1.42 \log K$$

The free energy of Y is greater than the free energy of X. Therefore $\Delta G < 0$, and the disorder of the universe increases during the reaction $Y \rightarrow X$.

ENERGETICALLY FAVORABLE REACTION

this reaction can occur spontaneously

If the reaction $X \rightarrow Y$ occurred, ΔG would be > 0, and the universe would become more ordered.

ENERGETICALLY UNFAVORABLE REACTION

this reaction can occur only if it is coupled to a second, energetically favorable reaction

Figure 2–50 The distinction between energetically favorable and energetically unfavorable reactions.
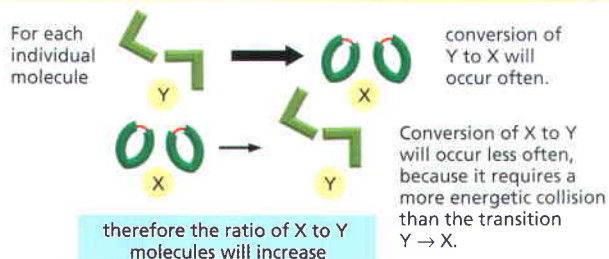

negative ΔG

positive ΔG
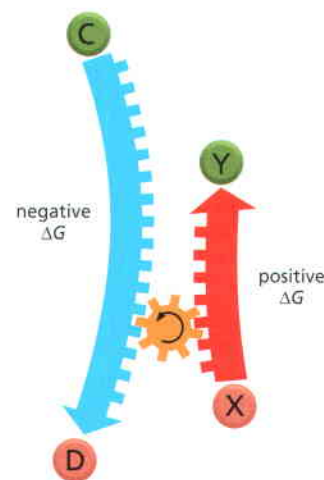
the energetically unfavorable reaction $X \rightarrow Y$ is driven by the energetically favorable reaction $C \rightarrow D$, because the net free-energy change for the pair of coupled reactions is less than zero

**Figure 2–51 How reaction coupling is used to drive energetically unfavorable reactions.**

THE REACTION

The formation of X is energetically favored in this example. In other words, the ΔG of Y → X is negative and the ΔG of X → Y is positive. But because of thermal bombardments, there will always be some X converting to Y and vice versa.

SUPPOSE WE START WITH AN EQUAL NUMBER OF Y AND X MOLECULES

For each individual molecule

conversion of Y to X will occur often.

Conversion of X to Y will occur less often, because it requires a more energetic collision than the transition Y → X.

therefore the ratio of X to Y molecules will increase

EVENTUALLY there will be a large enough excess of X over Y to just compensate for the slow rate of X → Y. Equilibrium will then be attained.
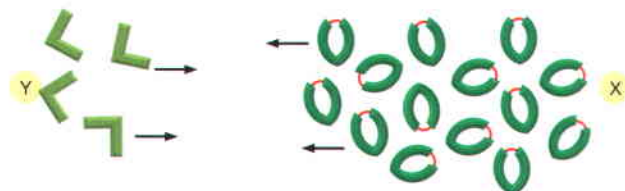
AT EQUILIBRIUM the number of Y molecules being converted to X molecules each second is exactly equal to the number of X molecules being converted to Y molecules each second, so that there is no net change in the ratio of Y to X.

Figure 2–52 Chemical equilibrium. When a reaction reaches equilibrium, the forward and backward fluxes of reacting molecules are equal and opposite.

Table 2–4 Relationship Between the Standard Free-Energy Change, $\Delta G°$, and the Equilibrium Constant

EQUILIBRIUM CONSTANT $\frac{[X]}{[Y]} = K$	FREE ENERGY OF X MINUS FREE ENERGY OF Y kcal/mole (kJ/mole)
10^5	–7.1 (–29.7)
10^4	–5.7 (–23.8)
10^3	–4.3 (–18.0)
10^2	–2.8 (–11.7)
10^1	–1.4 (–5.9)
1	0 (0)
10	1.4 (5.9)
10^{-2}	2.8 (11.7)
10^{-3}	4.3 (18.0)
10^{-4}	5.7 (23.8)
10^{-5}	7.1 (29.7)

Values of the equilibrium constant were calculated for the simple chemical reaction Y ⇌ X using the equation given in the text.

The $\Delta G°$ given here is in kilocalories per mole at 37°C, with kilojoules per mole in parentheses (1 kilocalorie is equal to 4.184 kilojoules). As explained in the text, $\Delta G°$ represents the free-energy difference under standard conditions (where all components are present at a concentration of 1.0 mole/liter).

From this table, we see that if there is a favorable standard free-energy change ($\Delta G°$) of –4.3 kcal/mole (–18.0 kJ/mole) for the transition Y → X, there will be 1000 times more molecules in state X than in state Y at equilibrium ($K = 1000$).

Using the last equation, we can see how the equilibrium ratio of X to Y (expressed as an equilibrium constant, K) depends on the intrinsic character of the molecules, as expressed in the value of $\Delta G°$ (Table 2–4). Note that for every 1.4 kcal/mole (5.9 kJ/mole) difference in free energy at 37°C, the equilibrium constant changes by a factor of 10.

When an enzyme (or any catalyst) lowers the activation energy for the reaction Y → X, it also lowers the activation energy for the reaction X → Y by exactly the same amount (see Figure 2–44). The forward and backward reactions will therefore be accelerated by the same factor by an enzyme, and the equilibrium point for the reaction (and $\Delta G°$) is unchanged (Figure 2–53).

For Sequential Reactions, $\Delta G°$ Values Are Additive

We can predict quantitatively the course of most reactions. A large body of thermodynamic data has been collected that makes it possible to calculate the standard change in free energy, $\Delta G°$, for most of the important metabolic reactions of the cell. The overall free-energy change for a metabolic pathway is then simply the sum of the free-energy changes in each of its component steps. Consider, for example, two sequential reactions

$$X \rightarrow Y \quad \text{and} \quad Y \rightarrow Z$$

whose $\Delta G°$ values are +5 and –13 kcal/mole, respectively. (Recall that a mole is 6 × 10^{23} molecules of a substance.) If these two reactions occur sequentially, the $\Delta G°$ for the coupled reaction will be –8 kcal/mole. Thus, the unfavorable reaction X → Y, which will not occur spontaneously, can be driven by the favorable reaction Y → Z, provided that this second reaction follows the first.

Cells can therefore cause the energetically unfavorable transition, X → Y, to occur if an enzyme catalyzing the X → Y reaction is supplemented by a second enzyme that catalyzes the energetically *favorable* reaction, Y → Z. In effect, the reaction Y → Z will then act as a "siphon" to drive the conversion of all of molecule X to molecule Y, and thence to molecule Z (Figure 2–54). For example,

UNCATALYZED REACTION
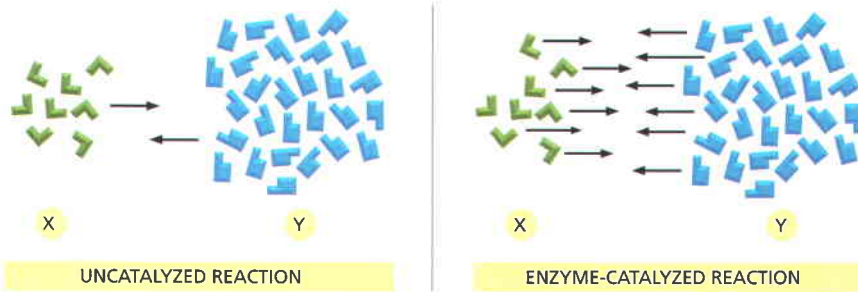
ENZYME-CATALYZED REACTION

Figure 2–53 Enzymes cannot change the equilibrium point for reactions. Enzymes, like all catalysts, speed up the forward and backward rates of a reaction by the same factor. Therefore, for both the catalyzed and the uncatalyzed reactions shown here, the number of molecules undergoing the transition $X \rightarrow Y$ is equal to the number of molecules undergoing the transition $Y \rightarrow X$ when the ratio of Y molecules to X molecules is 3.5 to 1. In other words, the two reactions reach equilibrium at exactly the same point.

several of the reactions in the long pathway that converts sugars into CO_2 and H_2O would be energetically unfavorable if considered on their own. But the pathway nevertheless proceeds because the total $\Delta G°$ for the series of sequential reactions has a large negative value.

But forming a sequential pathway is not adequate for many purposes. Often the desired pathway is simply $X \rightarrow Y$, without further conversion of Y to some other product. Fortunately, there are other more general ways of using enzymes to couple reactions together. How these work is the topic we discuss next.

Activated Carrier Molecules Are Essential for Biosynthesis

The energy released by the oxidation of food molecules must be stored temporarily before it can be channeled into the construction of the many other molecules needed by the cell. In most cases, the energy is stored as chemical bond energy in a small set of activated "carrier molecules," which contain one or more energy-rich covalent bonds. These molecules diffuse rapidly throughout the cell and thereby carry their bond energy from sites of energy generation to the sites where energy is used for biosynthesis and other cell activities (**Figure 2–55**).

The **activated carriers** store energy in an easily exchangeable form, either as a readily transferable chemical group or as high-energy electrons, and they can serve a dual role as a source of both energy and chemical groups in biosynthetic reactions. For historical reasons, these molecules are also sometimes referred to as *coenzymes*. The most important of the activated carrier molecules are ATP and two molecules that are closely related to each other, NADH and NADPH— as we discuss in detail shortly. We shall see that cells use activated carrier molecules like money to pay for reactions that otherwise could not take place.

(A)

equilibrium point for
X→Y reaction alone

(B)

equilibrium point for
Y→Z reaction alone

(C)

equilibrium point for sequential reactions X→Y→Z
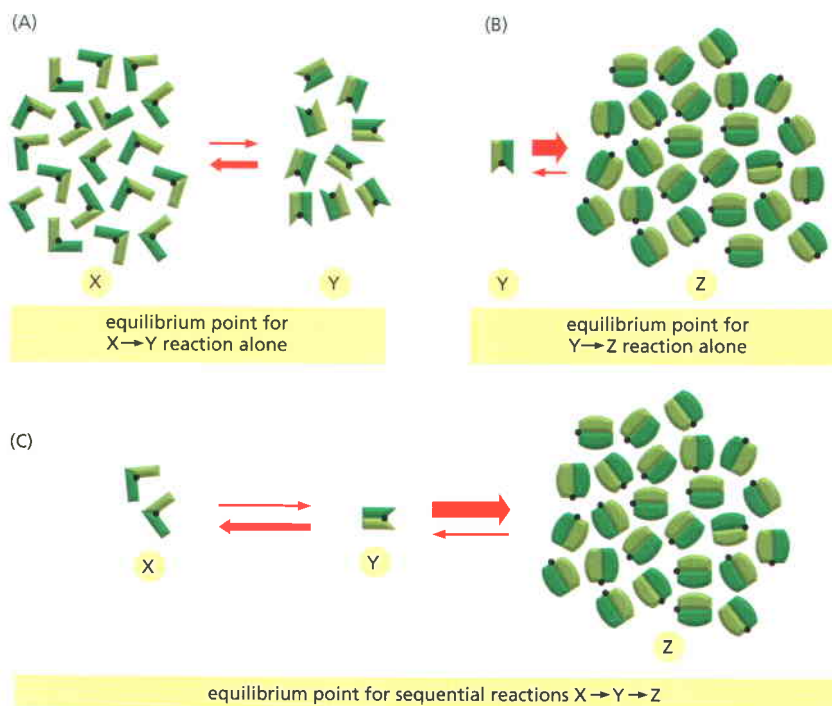
Figure 2–54 How an energetically unfavorable reaction can be driven by a second, following reaction. (A) At equilibrium, there are twice as many X molecules as Y molecules, because X is of lower energy than Y. (B) At equilibrium, there are 25 times more Z molecules than Y molecules, because Z is of much lower energy than Y. (C) If the reactions in (A) and (B) are coupled, nearly all of the X molecules will be converted to Z molecules, as shown.
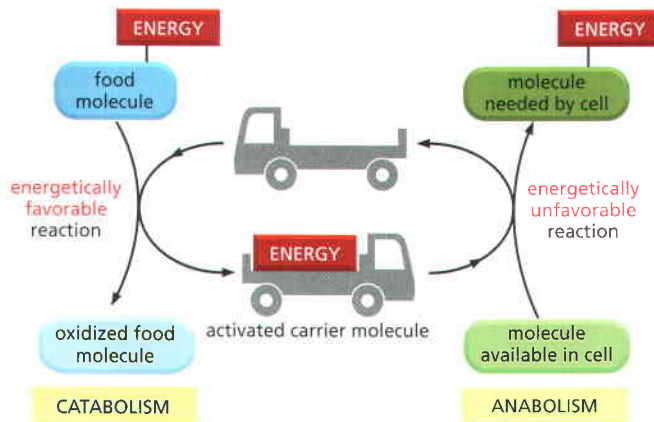
Figure 2–55 **Energy transfer and the role of activated carriers in metabolism.** By serving as energy shuttles, activated carrier molecules perform their function as go-betweens that link the breakdown of food molecules and the release of energy *(catabolism)* to the energy-requiring biosynthesis of small and large organic molecules *(anabolism)*.
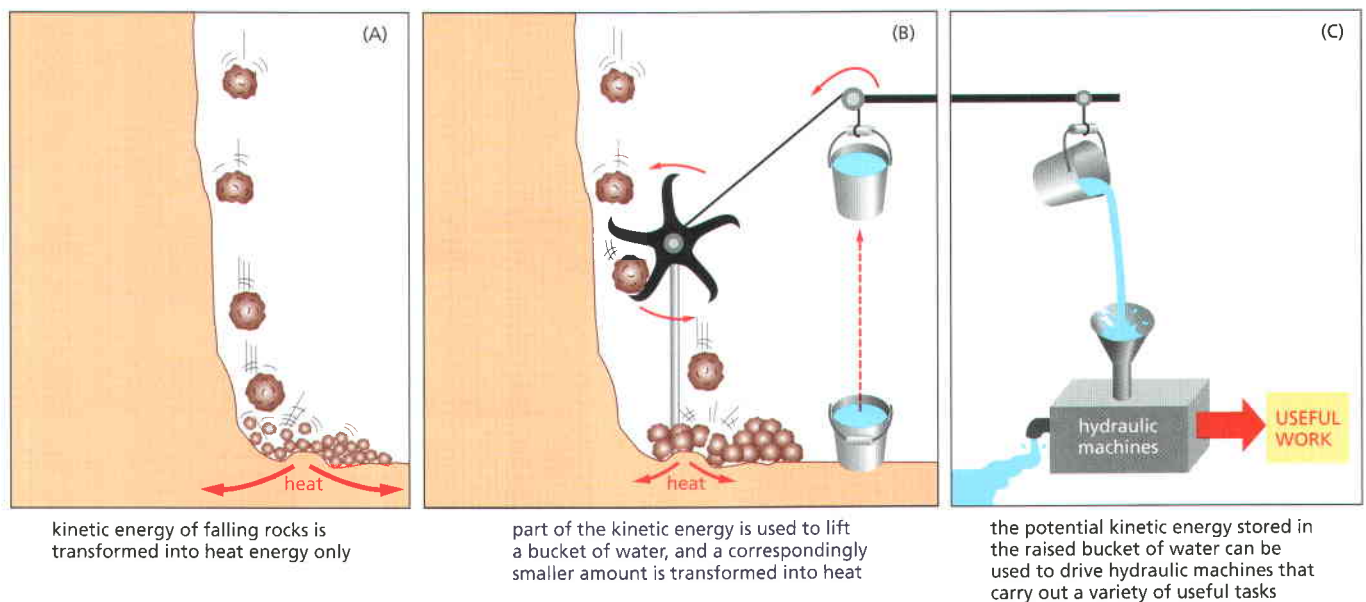
The Formation of an Activated Carrier Is Coupled to an Energetically Favorable Reaction

When a fuel molecule such as glucose is oxidized in a cell, enzyme-catalyzed reactions ensure that a large part of the free energy that is released by oxidation is captured in a chemically useful form, rather than being released as heat. This is achieved by means of a **coupled reaction**, in which an energetically favorable reaction drives an energetically unfavorable one that produces an activated carrier molecule or some other useful energy store. Coupling mechanisms require enzymes and are fundamental to all the energy transactions of the cell.

The nature of a coupled reaction is illustrated by a mechanical analogy in **Figure 2–56**, in which an energetically favorable chemical reaction is represented by rocks falling from a cliff. The energy of falling rocks would normally be entirely wasted in the form of heat generated by friction when the rocks hit the ground (see the falling brick diagram in Figure 2–39). By careful design, however, part of this energy could be used instead to drive a paddle wheel that lifts a bucket of water (Figure 2–56B). Because the rocks can now reach the ground only after moving the paddle wheel, we say that the energetically favorable reaction of rock falling has been directly *coupled* to the energetically unfavorable reaction of lifting the bucket of water. Note that because part of the energy is used to do work in (B), the rocks hit the ground with less velocity than in (A), and correspondingly less energy is dissipated as heat.

Similar processes occur in cells, where enzymes play the role of the paddle wheel in our analogy. By mechanisms that will be discussed later in this chapter, they couple an energetically favorable reaction, such as the oxidation of foodstuffs, to an energetically unfavorable reaction, such as the generation of

Figure 2–56 **A mechanical model illustrating the principle of coupled chemical reactions.** The spontaneous reaction shown in (A) could serve as an analogy for the direct oxidation of glucose to CO_2 and H_2O, which produces heat only. In (B) the same reaction is coupled to a second reaction; this second reaction is analogous to the synthesis of activated carrier molecules. The energy produced in (B) is in a more useful form than in (A) and can be used to drive a variety of otherwise energetically unfavorable reactions (C).

kinetic energy of falling rocks is transformed into heat energy only

part of the kinetic energy is used to lift a bucket of water, and a correspondingly smaller amount is transformed into heat

the potential kinetic energy stored in the raised bucket of water can be used to drive hydraulic machines that carry out a variety of useful tasks
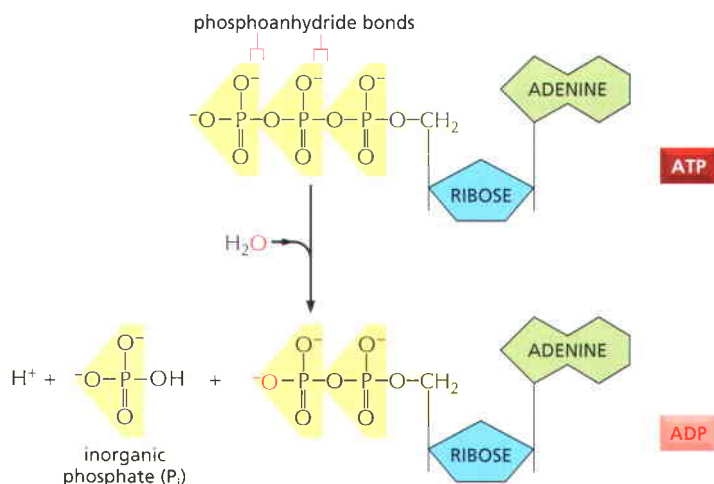
Figure 2–57 The hydrolysis of ATP to ADP and inorganic phosphate. The two outermost phosphates in ATP are held to the rest of the molecule by high-energy phosphoanhydride bonds and are readily transferred. As indicated, water can be added to ATP to form ADP and inorganic phosphate (P_i). This hydrolysis of the terminal phosphate of ATP yields between 11 and 13 kcal/mole of usable energy, depending on the intracellular conditions. The large negative ΔG of this reaction arises from several factors. Release of the terminal phosphate group removes an unfavorable repulsion between adjacent negative charges; in addition, the inorganic phosphate ion (P_i) released is stabilized by resonance and by favorable hydrogen-bond formation with water.

an activated carrier molecule. As a result, the amount of heat released by the oxidation reaction is reduced by exactly the amount of energy that is stored in the energy-rich covalent bonds of the activated carrier molecule. The activated carrier molecule in turn picks up a packet of energy of a size sufficient to power a chemical reaction elsewhere in the cell.

ATP Is the Most Widely Used Activated Carrier Molecule

The most important and versatile of the activated carriers in cells is **ATP** (adenosine triphosphate). Just as the energy stored in the raised bucket of water in Figure 2–56B can drive a wide variety of hydraulic machines, ATP is a convenient and versatile store, or currency, of energy used to drive a variety of chemical reactions in cells. ATP is synthesized in an energetically unfavorable phosphorylation reaction in which a phosphate group is added to **ADP** (adenosine diphosphate). When required, ATP gives up its energy packet through its energetically favorable hydrolysis to ADP and inorganic phosphate (**Figure 2–57**). The regenerated ADP is then available to be used for another round of the phosphorylation reaction that forms ATP.

The energetically favorable reaction of ATP hydrolysis is coupled to many otherwise unfavorable reactions through which other molecules are synthesized. We shall encounter several of these reactions later in this chapter. Many of them involve the transfer of the terminal phosphate in ATP to another molecule, as illustrated by the phosphorylation reaction in **Figure 2–58**.
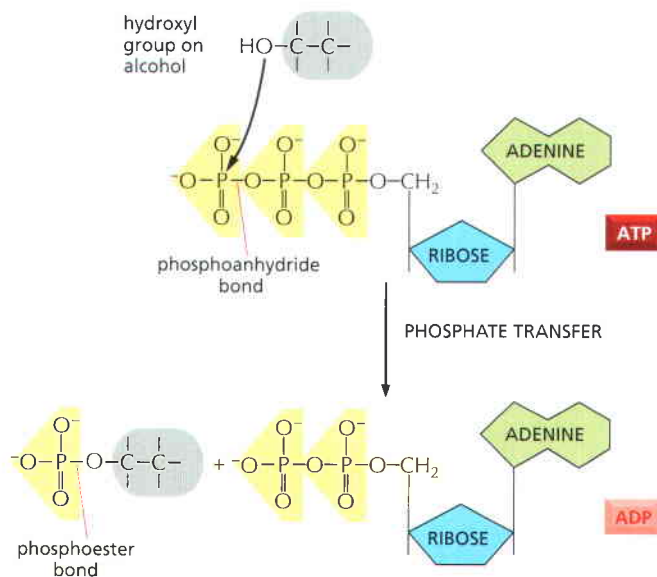
Figure 2–58 An example of a phosphate transfer reaction. Because an energy-rich phosphoanhydride bond in ATP is converted to a phosphoester bond, this reaction is energetically favorable, having a large negative ΔG. Reactions of this type are involved in the synthesis of phospholipids and in the initial steps of reactions that catabolize sugars.
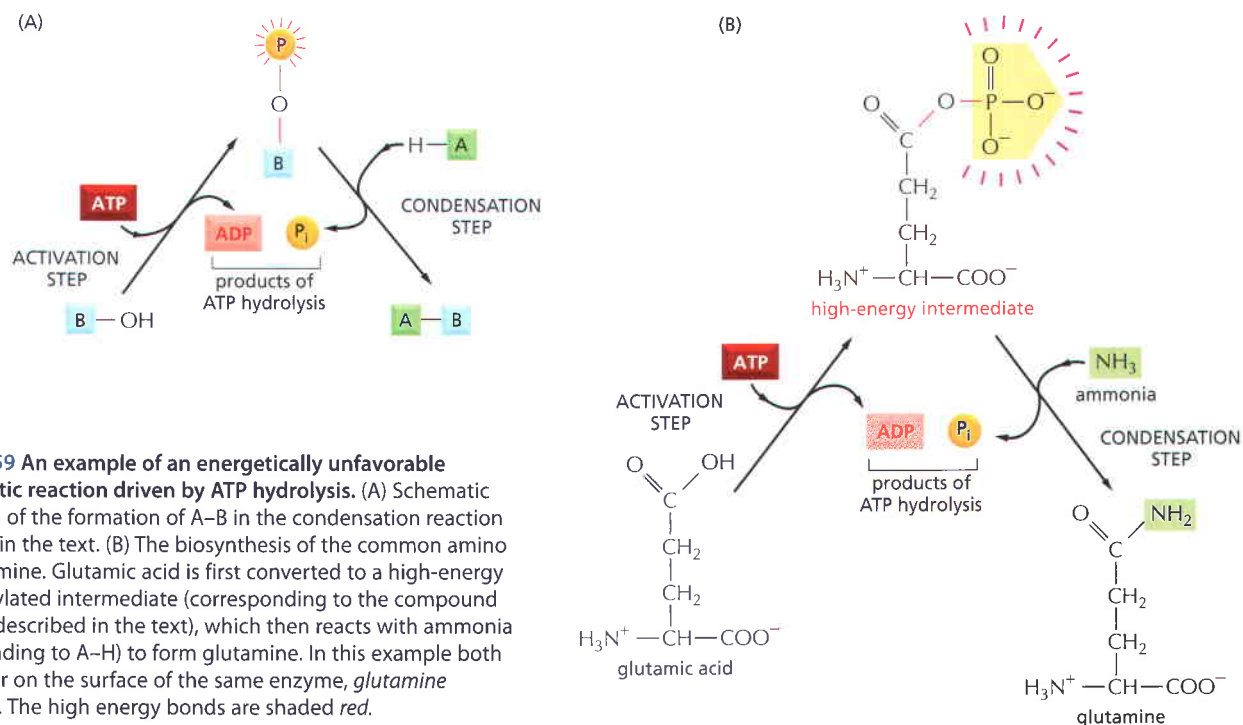
Figure 2–59 An example of an energetically unfavorable biosynthetic reaction driven by ATP hydrolysis. (A) Schematic illustration of the formation of A–B in the condensation reaction described in the text. (B) The biosynthesis of the common amino acid glutamine. Glutamic acid is first converted to a high-energy phosphorylated intermediate (corresponding to the compound B–O–PO$_3$ described in the text), which then reacts with ammonia (corresponding to A–H) to form glutamine. In this example both steps occur on the surface of the same enzyme, *glutamine synthetase*. The high energy bonds are shaded *red*.

ATP is the most abundant activated carrier in cells. As one example, it supplies energy for many of the pumps that transport substances into and out of the cell (discussed in Chapter 11). It also powers the molecular motors that enable muscle cells to contract and nerve cells to transport materials from one end of their long axons to another (discussed in Chapter 16).

Energy Stored in ATP Is Often Harnessed to Join Two Molecules Together

We have previously discussed one way in which an energetically favorable reaction can be coupled to an energetically unfavorable reaction, X → Y, so as to enable it to occur. In that scheme a second enzyme catalyzes the energetically favorable reaction Y → Z, pulling all of the X to Y in the process (see Figure 2–54). But when the required product is Y and not Z, this mechanism is not useful.

A typical biosynthetic reaction is one in which two molecules, A and B, are joined together to produce A–B in the energetically unfavorable condensation reaction

$$A–H + B–OH → A–B + H_2O$$
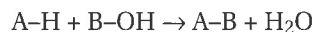
There is an indirect pathway that allows A–H and B–OH to form A–B, in which a coupling to ATP hydrolysis makes the reaction go. Here energy from ATP hydrolysis is first used to convert B–OH to a higher-energy intermediate compound, which then reacts directly with A–H to give A–B. The simplest possible mechanism involves the transfer of a phosphate from ATP to B–OH to make B–OPO$_3$, in which case the reaction pathway contains only two steps:

1.   B–OH + ATP → B–O–PO$_3$ + ADP
2.   A–H + B–O–PO$_3$ → A–B + P$_i$

Net result: B–OH + ATP + A–H → A–B + ADP + P$_i$

The condensation reaction, which by itself is energetically unfavorable, is forced to occur by being directly coupled to ATP hydrolysis in an enzyme-catalyzed reaction pathway (**Figure 2–59A**).

A biosynthetic reaction of exactly this type synthesizes the amino acid glutamine (Figure 2–59B). We will see shortly that similar (but more complex) mechanisms are also used to produce nearly all of the large molecules of the cell.

NADH and NADPH Are Important Electron Carriers

Other important activated carrier molecules participate in oxidation–reduction reactions and are commonly part of coupled reactions in cells. These activated carriers are specialized to carry high-energy electrons and hydrogen atoms. The most important of these electron carriers are **NAD⁺** (nicotinamide adenine dinucleotide) and the closely related molecule **NADP⁺** (nicotinamide adenine dinucleotide phosphate). Later, we examine some of the reactions in which they participate. NAD⁺ and NADP⁺ each pick up a "packet of energy" corresponding to two high-energy electrons plus a proton (H⁺)—being converted to **NADH** (*reduced* nicotinamide adenine dinucleotide) and **NADPH** (*reduced* nicotinamide adenine dinucleotide phosphate), respectively. These molecules can therefore also be regarded as carriers of hydride ions (the H⁺ plus two electrons, or H⁻).

Like ATP, NADPH is an activated carrier that participates in many important biosynthetic reactions that would otherwise be energetically unfavorable. The NADPH is produced according to the general scheme shown in **Figure 2–60A**. During a special set of energy-yielding catabolic reactions, a hydrogen atom plus two electrons are removed from the substrate molecule and added to the nicotinamide ring of NADP⁺ to form NADPH, with a proton (H⁺) being released into solution. This is a typical oxidation–reduction reaction; the substrate is oxidized and NADP⁺ is reduced. The structures of NADP⁺ and NADPH are shown in Figure 2–60B.

NADPH readily gives up the hydride ion it carries in a subsequent oxidation–reduction reaction, because the nicotinamide ring can achieve a more stable arrangement of electrons without it. In this subsequent reaction, which regenerates NADP⁺, it is the NADPH that is oxidized and the substrate that is reduced. The NADPH is an effective donor of its hydride ion to other molecules

(A)

oxidation of molecule 1

reduction of molecule 2

(B)

NADP⁺ oxidized form

NADPH reduced form

nicotinamide ring

RIBOSE

ADENINE

RIBOSE

RIBOSE

ADENINE

RIBOSE

this phosphate group is missing in NAD⁺ and NADH
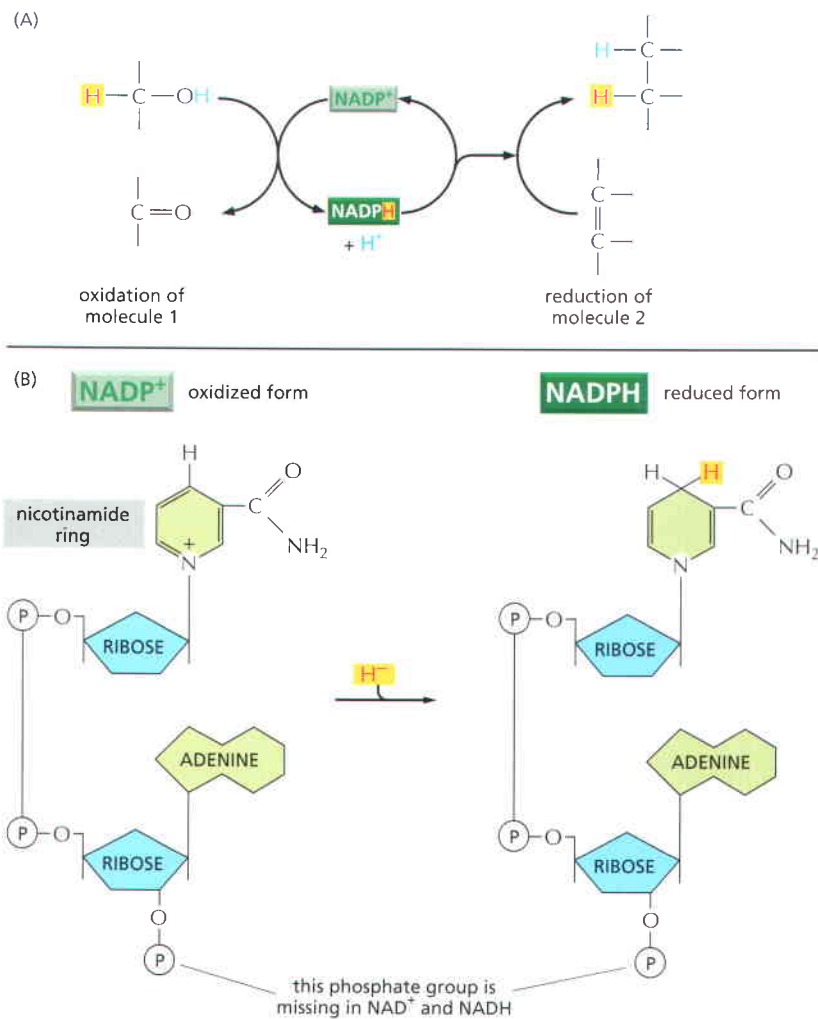
Figure 2–60 NADPH, an important carrier of electrons. (A) NADPH is produced in reactions of the general type shown on the left, in which two hydrogen atoms are removed from a substrate. The oxidized form of the carrier molecule, NADP⁺, receives one hydrogen atom plus an electron (a hydride ion); the proton (H⁺) from the other H atom is released into solution. Because NADPH holds its hydride ion in a high-energy linkage, the added hydride ion can easily be transferred to other molecules, as shown on the right. (B) The structures of NADP⁺ and NADPH. The part of the NADP⁺ molecule known as the nicotinamide ring accepts two electrons together with a proton (the equivalent of a hydride ion, H⁻), forming NADPH. The molecules NAD⁺ and NADH are identical in structure to NADP⁺ and NADPH, respectively, except that the indicated phosphate group is absent from both.

for the same reason that ATP readily transfers a phosphate: in both cases the transfer is accompanied by a large negative free-energy change. One example of the use of NADPH in biosynthesis is shown in **Figure 2–61**.

The extra phosphate group on NADPH has no effect on the electron-transfer properties of NADPH compared with NADH, being far away from the region involved in electron transfer (see Figure 2–60B). It does, however, give a molecule of NADPH a slightly different shape from that of NADH, making it possible for NADPH and NADH to bind as substrates to completely different sets of enzymes. Thus the two types of carriers are used to transfer electrons (or hydride ions) between two different sets of molecules.

Why should there be this division of labor? The answer lies in the need to regulate two sets of electron-transfer reactions independently. NADPH operates chiefly with enzymes that catalyze anabolic reactions, supplying the high-energy electrons needed to synthesize energy-rich biological molecules. NADH, by contrast, has a special role as an intermediate in the catabolic system of reactions that generate ATP through the oxidation of food molecules, as we will discuss shortly. The genesis of NADH from NAD$^+$ and that of NADPH from NADP$^+$ occur by different pathways and are independently regulated, so that the cell can adjust the supply of electrons for these two contrasting purposes. Inside the cell the ratio of NAD$^+$ to NADH is kept high, whereas the ratio of NADP$^+$ to NADPH is kept low. This provides plenty of NAD$^+$ to act as an oxidizing agent and plenty of NADPH to act as a reducing agent—as required for their special roles in catabolism and anabolism, respectively.

There Are Many Other Activated Carrier Molecules in Cells

Other activated carriers also pick up and carry a chemical group in an easily transferred, high-energy linkage. For example, coenzyme A carries an acetyl group in a readily transferable linkage, and in this activated form is known as **acetyl CoA** (acetyl coenzyme A). Acetyl CoA (**Figure 2–62**) is used to add two carbon units in the biosynthesis of larger molecules.

In acetyl CoA as in other carrier molecules, the transferable group makes up only a small part of the molecule. The rest consists of a large organic portion that
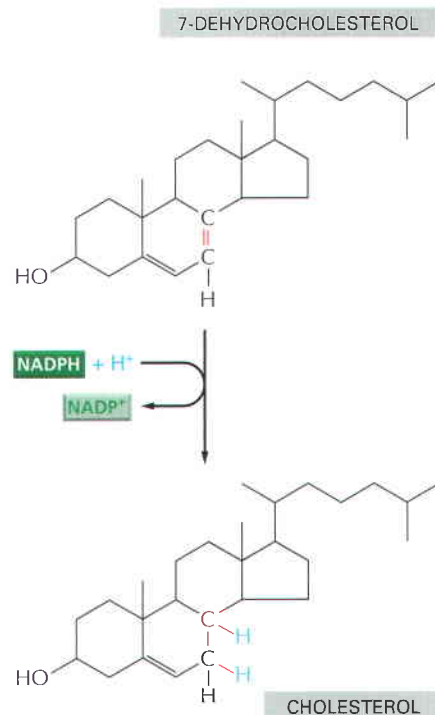
Figure 2–61 The final stage in one of the biosynthetic routes leading to cholesterol. As in many other biosynthetic reactions, the reduction of the C=C bond is achieved by the transfer of a hydride ion from the carrier molecule NADPH, plus a proton (H$^+$) from the solution.
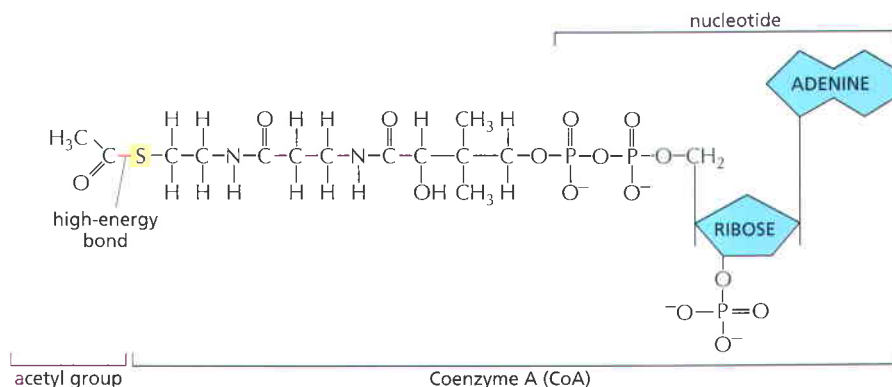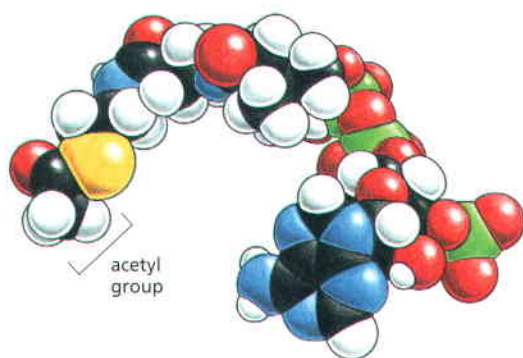
Figure 2–62 The structure of the important activated carrier molecule acetyl CoA. A space-filling model is shown above the structure. The sulfur atom (yellow) forms a thioester bond to acetate. Because this is a high-energy linkage, releasing a large amount of free energy when it is hydrolyzed, the acetate molecule can be readily transferred to other molecules.

Table 2–5 Some Activated Carrier Molecules Widely Used in Metabolism

ACTIVATED CARRIER	GROUP CARRIED IN HIGH-ENERGY LINKAGE
ATP	phosphate
NADH, NADPH, FADH$_2$	electrons and hydrogens
Acetyl CoA	acetyl group
Carboxylated biotin	carboxyl group
S-Adenosylmethionine	methyl group
Uridine diphosphate glucose	glucose

serves as a convenient "handle," facilitating the recognition of the carrier molecule by specific enzymes. As with acetyl CoA, this handle portion very often contains a nucleotide (usually adenosine), a curious fact that may be a relic from an early stage of evolution. It is currently thought that the main catalysts for early life-forms—before DNA or proteins—were RNA molecules (or their close relatives), as described in Chapter 6. It is tempting to speculate that many of the carrier molecules that we find today originated in this earlier RNA world, where their nucleotide portions could have been useful for binding them to RNA enzymes.

Figures 2–58 and 2–61 have presented examples of the type of transfer reactions powered by the activated carrier molecules ATP (transfer of phosphate) and NADPH (transfer of electrons and hydrogen). The reactions of other activated carrier molecules involve the transfer of a methyl, carboxyl, or glucose group for the purpose of biosynthesis (**Table 2–5**). These activated carriers are generated in reactions that are coupled to ATP hydrolysis, as in the example in **Figure 2–63**. Therefore, the energy that enables their groups to be used for biosynthesis ultimately comes from the catabolic reactions that generate ATP. Similar processes occur in the synthesis of the very large molecules of the cell—the nucleic acids, proteins, and polysaccharides—that we discuss next.

The Synthesis of Biological Polymers Is Driven by ATP Hydrolysis

As discussed previously, the macromolecules of the cell constitute most of its dry mass—that is, of the mass not due to water (see Figure 2–29). These

Figure 2–63 A carboxyl group transfer reaction using an activated carrier molecule. Carboxylated biotin is used by the enzyme *pyruvate carboxylase* to transfer a carboxyl group in the production of oxaloacetate, a molecule needed for the citric acid cycle. The acceptor molecule for this group transfer reaction is pyruvate. Other enzymes use biotin to transfer carboxyl groups to other acceptor molecules. Note that synthesis of carboxylated biotin requires energy that is derived from ATP—a general feature of many activated carriers.

CARBOXYL GROUP ACTIVATION

carboxylated biotin

high-energy bond

ADP

ADENINE

RIBOSE

ATP

ADENINE

RIBOSE

ENZYME

P$_i$

pyruvate

biotin

bicarbonate

ENZYME

pyruvate carboxylase

oxaloacetate

CARBOXYL GROUP TRANSFER

Figure 2–64 Condensation and hydrolysis as opposite reactions. The macromolecules of the cell are polymers that are formed from subunits (or monomers) by a condensation reaction and are broken down by hydrolysis. The condensation reactions are all energetically unfavorable.

molecules are made from subunits (or monomers) that are linked together in a *condensation* reaction, in which the constituents of a water molecule (OH plus H) are removed from the two reactants. Consequently, the reverse reaction— the breakdown of all three types of polymers—occurs by the enzyme-catalyzed addition of water (*hydrolysis*). This hydrolysis reaction is energetically favorable, whereas the biosynthetic reactions require an energy input (**Figure 2–64**).

The nucleic acids (DNA and RNA), proteins, and polysaccharides are all polymers that are produced by the repeated addition of a monomer onto one end of a growing chain. The synthesis reactions for these three types of macromolecules are outlined in **Figure 2–65**. As indicated, the condensation step in each case depends on energy from nucleoside triphosphate hydrolysis. And yet, except for the nucleic acids, there are no phosphate groups left in the final product molecules. How are the reactions that release the energy of ATP hydrolysis coupled to polymer synthesis?
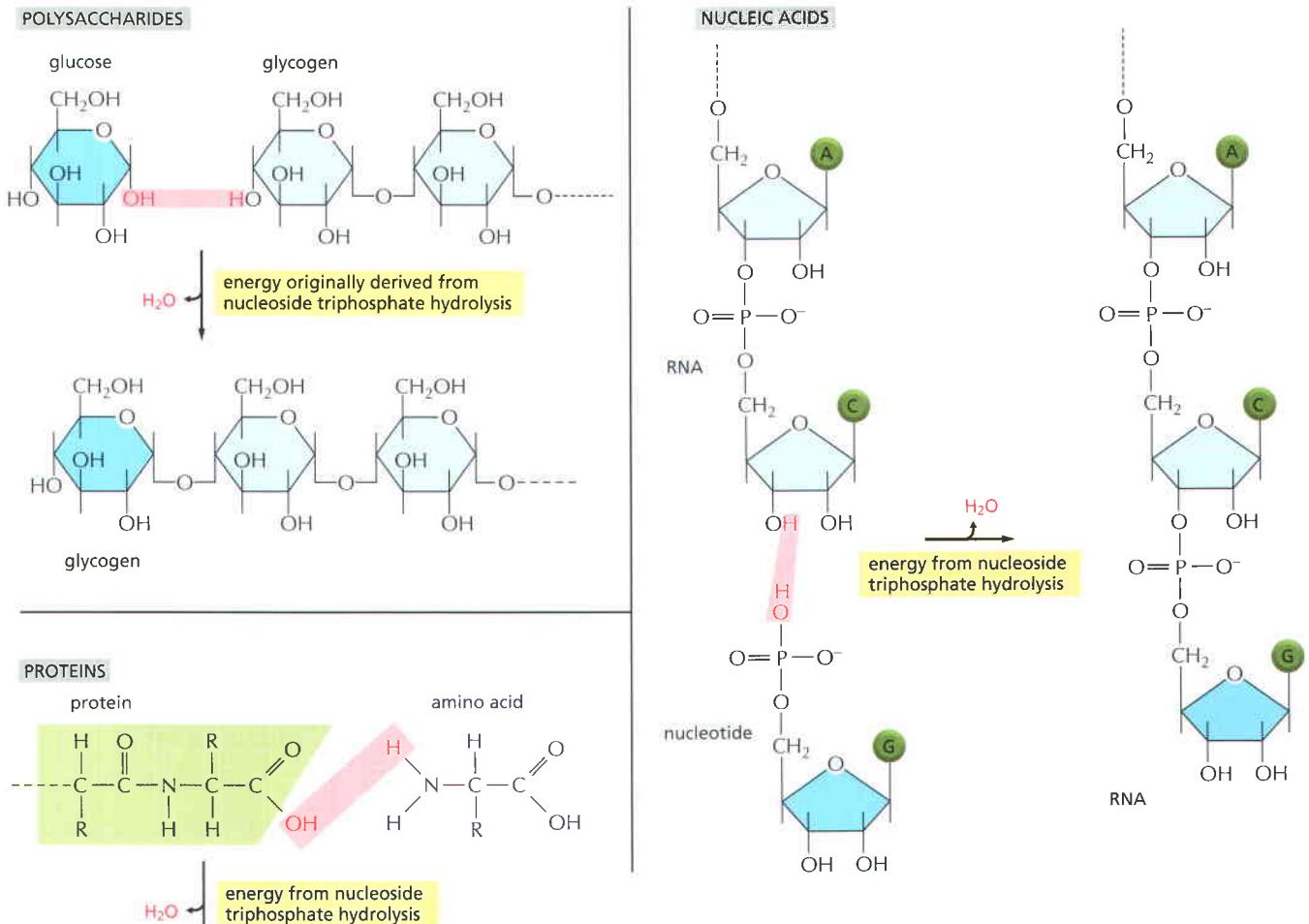
Figure 2–65 The synthesis of polysaccharides, proteins, and nucleic acids. Synthesis of each kind of biological polymer involves the loss of water in a condensation reaction. Not shown is the consumption of high-energy nucleoside triphosphates that is required to activate each monomer before its addition. In contrast, the reverse reaction—the breakdown of all three types of polymers—occurs by the simple addition of water (hydrolysis).

For each type of macromolecule, an enzyme-catalyzed pathway exists which resembles that discussed previously for the synthesis of the amino acid glutamine (see Figure 2–59). The principle is exactly the same, in that the OH group that will be removed in the condensation reaction is first activated by becoming involved in a high-energy linkage to a second molecule. However, the actual mechanisms used to link ATP hydrolysis to the synthesis of proteins and polysaccharides are more complex than that used for glutamine synthesis, since a series of high-energy intermediates is required to generate the final high-energy bond that is broken during the condensation step (discussed in Chapter 6 for protein synthesis).

Each activated carrier has limits in its ability to drive a biosynthetic reaction. The ΔG for the hydrolysis of ATP to ADP and inorganic phosphate (P_i) depends on the concentrations of all of the reactants, but under the usual conditions in a cell it is between –11 and –13 kcal/mole (between –46 and –54 kJ/mole). In principle, this hydrolysis reaction could drive an unfavorable reaction with a ΔG of, perhaps, +10 kcal/mole, provided that a suitable reaction path is available. For some biosynthetic reactions, however, even –13 kcal/mole may not be enough. In these cases the path of ATP hydrolysis can be altered so that it initially produces AMP and pyrophosphate (PP_i), which is itself then hydrolyzed in a subsequent step (**Figure 2–66**). The whole process makes available a total free-energy change of about –26 kcal/mole. An important type of biosynthetic reaction that is driven in this way is the synthesis of nucleic acids (polynucleotides) from nucleoside triphosphates, as illustrated on the right side of **Figure 2–67**.

Note that the repetitive condensation reactions that produce macromolecules can be oriented in one of two ways, giving rise to either the head polymerization or the tail polymerization of monomers. In so-called *head polymerization* the reactive bond required for the condensation reaction is carried on the end of the growing polymer, and it must therefore be regenerated each time that a monomer is added. In this case, each monomer brings with it the reactive bond that will be used in adding the *next* monomer in the series. In *tail polymerization* the reactive bond carried by each monomer is instead used immediately for its own addition (**Figure 2–68**).

We shall see in later chapters that both these types of polymerization are used. The synthesis of polynucleotides and some simple polysaccharides occurs by tail polymerization, for example, whereas the synthesis of proteins occurs by a head polymerization process.

(A)

adenosine triphosphate (ATP)

pyrophosphate

adenosine monophosphate (AMP)

phosphate   phosphate

(B)
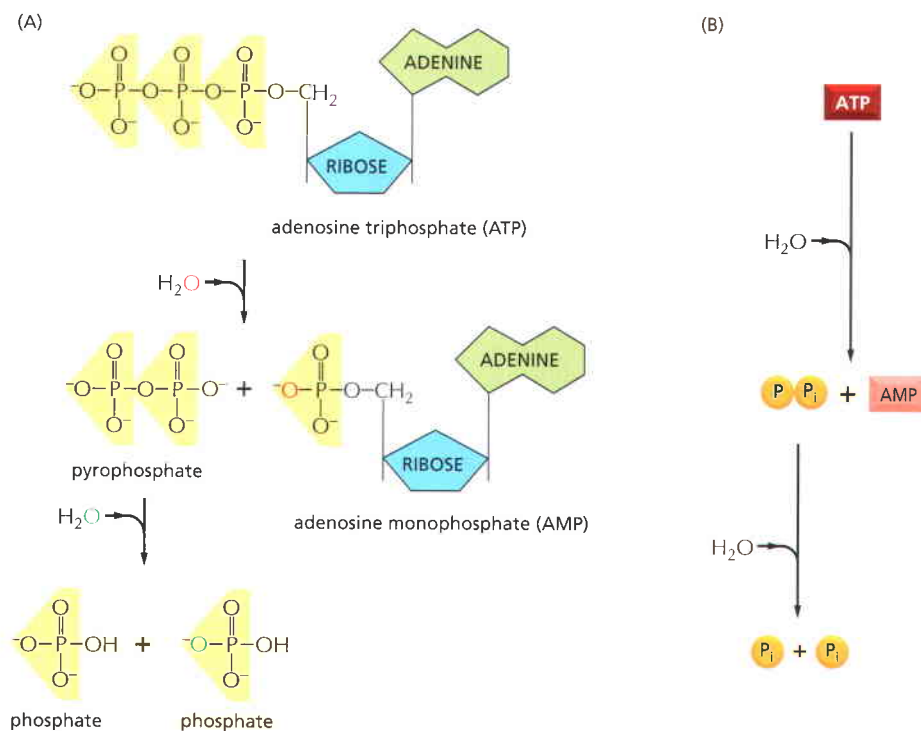
Figure 2–66 An alternative pathway of ATP hydrolysis, in which pyrophosphate is first formed and then hydrolyzed. This route releases about twice as much free energy as the reaction shown earlier in Figure 2–57, and it forms AMP instead of ADP. (A) In the two successive hydrolysis reactions, oxygen atoms from the participating water molecules are retained in the products, as indicated, whereas the hydrogen atoms dissociate to form free hydrogen ions (H+, not shown). (B) Diagram of overall reaction in summary form.
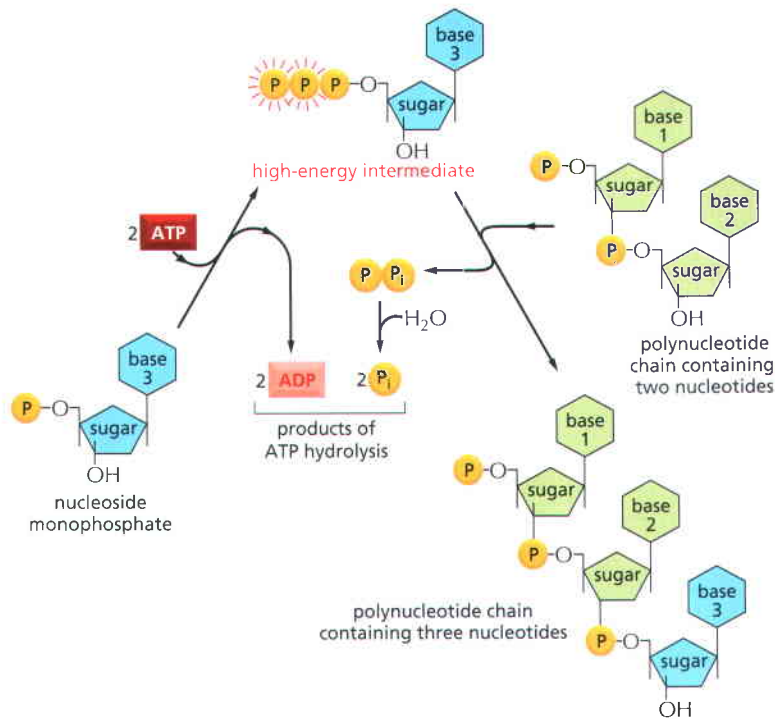
Figure 2–67 Synthesis of a polynucleotide, RNA or DNA, is a multistep process driven by ATP hydrolysis. In the first step, a nucleoside monophosphate is activated by the sequential transfer of the terminal phosphate groups from two ATP molecules. The high-energy intermediate formed—a nucleoside triphosphate—exists free in solution until it reacts with the growing end of an RNA or a DNA chain with release of pyrophosphate. Hydrolysis of the latter to inorganic phosphate is highly favorable and helps to drive the overall reaction in the direction of polynucleotide synthesis. For details, see Chapter 5.

Summary

Living cells are highly ordered and need to create order within themselves to survive and grow. This is thermodynamically possible only because of a continual input of energy, part of which must be released from the cells to their environment as heat. The energy comes ultimately from the electromagnetic radiation of the sun, which drives the formation of organic molecules in photosynthetic organisms such as green plants. Animals obtain their energy by eating these organic molecules and oxidizing them in a series of enzyme-catalyzed reactions that are coupled to the formation of ATP—a common currency of energy in all cells.

To make possible the continual generation of order in cells, the energetically favorable hydrolysis of ATP is coupled to energetically unfavorable reactions. In the biosynthesis of macromolecules, this is accomplished by the transfer of phosphate groups to form reactive phosphorylated intermediates. Because the energetically unfavorable reaction now becomes energetically favorable, ATP hydrolysis is said to drive the reaction. Polymeric molecules such as proteins, nucleic acids, and polysaccharides are assembled from small activated precursor molecules by repetitive condensation reactions that are driven in this way. Other reactive molecules, called either active carriers or coenzymes, transfer other chemical groups in the course of biosynthesis: NADPH transfers hydrogen as a proton plus two electrons (a hydride ion), for example, whereas acetyl CoA transfers an acetyl group.
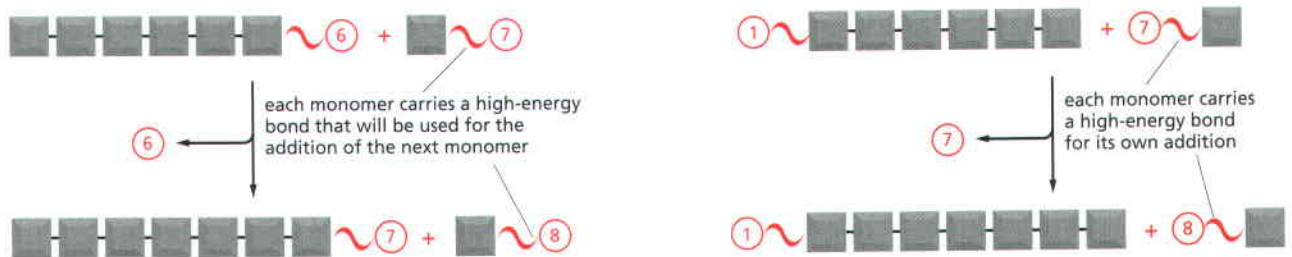
Figure 2–68 The orientation of the active intermediates in the repetitive condensation reactions that form biological polymers. The head growth of polymers is compared with its alternative, tail growth. As indicated, these two mechanisms are used to produce different types of biological macromolecules.

HOW CELLS OBTAIN ENERGY FROM FOOD

The constant supply of energy that cells need to generate and maintain the biological order that keeps them alive comes from the chemical bond energy in food molecules, which thereby serve as fuel for cells.

The proteins, lipids, and polysaccharides that make up most of the food we eat must be broken down into smaller molecules before our cells can use them—either as a source of energy or as building blocks for other molecules. Enzymatic digestion breaks down the large polymeric molecules in food into their monomer subunits—proteins into amino acids, polysaccharides into sugars, and fats into fatty acids and glycerol. After digestion, the small organic molecules derived from food enter the cytosol of cells, where their gradual oxidation begins.

Sugars are particularly important fuel molecules, and they are oxidized in small controlled steps to carbon dioxide (CO_2) and water (**Figure 2–69**). In this section we trace the major steps in the breakdown, or catabolism, of sugars and show how they produce ATP, NADH, and other activated carrier molecules in animal cells. A very similar pathway also operates in plants, fungi, and many bacteria. As we shall see, the oxidation of fatty acids is equally important for cells. Other molecules, such as proteins, can also serve as energy sources when they are funneled through appropriate enzymatic pathways.

Glycolysis Is a Central ATP-Producing Pathway

The major process for oxidizing sugars is the sequence of reactions known as **glycolysis**—from the Greek *glukus*, "sweet," and *lusis*, "rupture." Glycolysis produces ATP without the involvement of molecular oxygen (O_2 gas). It occurs in the cytosol of most cells, including many anaerobic microorganisms (those that can live without using molecular oxygen). Glycolysis probably evolved early in the history of life, before photosynthetic organisms introduced oxygen into the atmosphere. During glycolysis, a glucose molecule with six carbon atoms is converted into two molecules of *pyruvate*, each of which contains three carbon atoms. For each glucose molecule, two molecules of ATP are hydrolyzed to provide energy to drive the early steps, but four molecules of ATP are produced in the later steps. At the end of glycolysis, there is consequently a net gain of two molecules of ATP for each glucose molecule broken down.

The glycolytic pathway is outlined in **Figure 2–70** and shown in more detail in Panel 2–8 (pp. 120–121). Glycolysis involves a sequence of 10 separate reactions, each producing a different sugar intermediate and each catalyzed by a

(A) stepwise oxidation of sugar in cells

(B) direct burning of sugar

small activation energies overcome at body temperature owing to the presence of enzymes

large activation energy overcome by the heat from a fire

activated carrier molecules store energy

all free energy is released as heat; none is stored
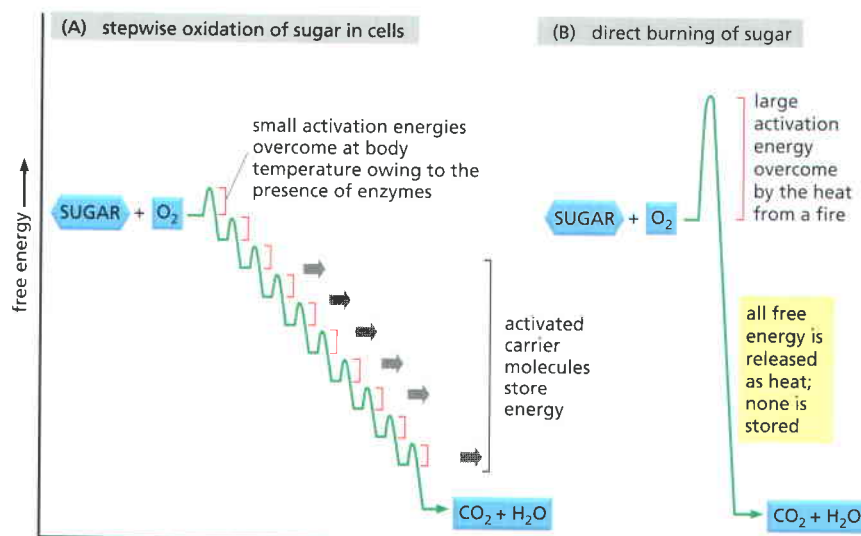
Figure 2–69 Schematic representation of the controlled stepwise oxidation of sugar in a cell, compared with ordinary burning. (A) In the cell, enzymes catalyze oxidation via a series of small steps in which free energy is transferred in conveniently sized packets to carrier molecules—most often ATP and NADH. At each step, an enzyme controls the reaction by reducing the activation energy barrier that has to be surmounted before the specific reaction can occur. The total free energy released is exactly the same in (A) and (B). But if the sugar were instead oxidized to CO_2 and H_2O in a single step, as in (B), it would release an amount of energy much larger than could be captured for useful purposes.
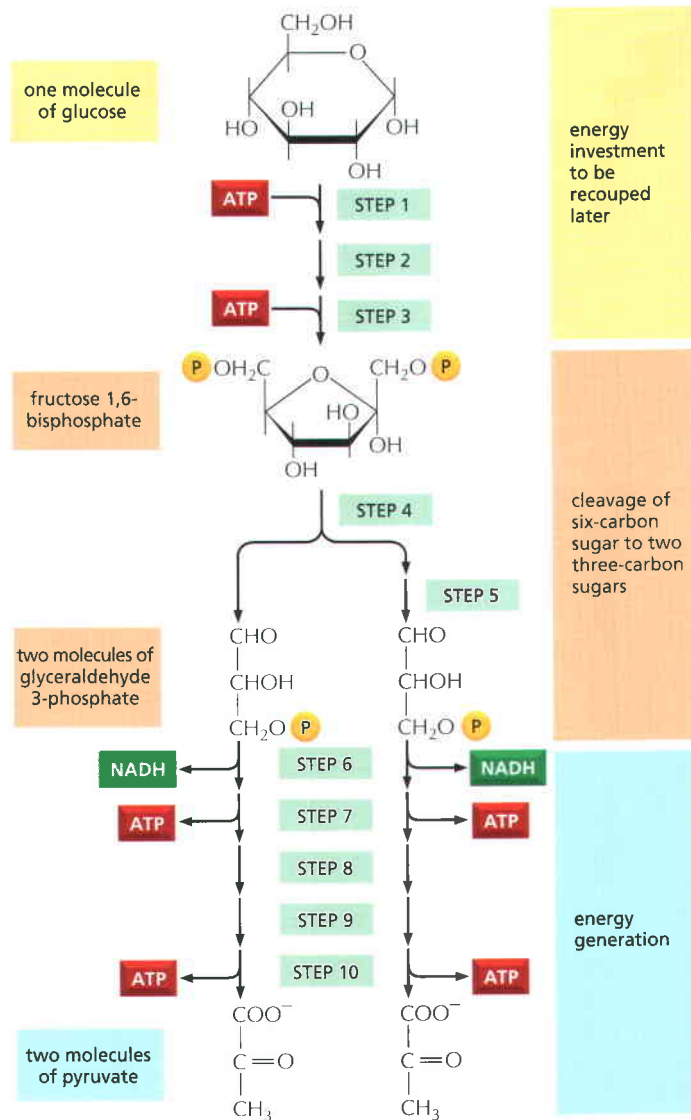
Figure 2–70 An outline of glycolysis. <GGGC> Each of the 10 steps shown is catalyzed by a different enzyme. Note that step 4 cleaves a six-carbon sugar into two three-carbon sugars, so that the number of molecules at every stage after this doubles. As indicated, step 6 begins the energy generation phase of glycolysis. Because two molecules of ATP are hydrolyzed in the early, energy investment phase, glycolysis results in the net synthesis of 2 ATP and 2 NADH molecules per molecule of glucose (see also Panel 2–8).

different enzyme. Like most enzymes, these have names ending in *ase*—such as isomer*ase* and dehydrogen*ase*—to indicate the type of reaction they catalyze.

Although no molecular oxygen is used in glycolysis, oxidation occurs, in that electrons are removed by NAD^+ (producing NADH) from some of the carbons derived from the glucose molecule. The stepwise nature of the process releases the energy of oxidation in small packets, so that much of it can be stored in activated carrier molecules rather than all of it being released as heat (see Figure 2–69). Thus, some of the energy released by oxidation drives the direct synthesis of ATP molecules from ADP and P_i, and some remains with the electrons in the high-energy electron carrier NADH.

Two molecules of NADH are formed per molecule of glucose in the course of glycolysis. In aerobic organisms (those that require molecular oxygen to live), these NADH molecules donate their electrons to the electron-transport chain described in Chapter 14, and the NAD^+ formed from the NADH is used again for glycolysis (see step 6 in Panel 2–8, pp. 120–121).

Fermentations Produce ATP in the Absence of Oxygen

For most animal and plant cells, glycolysis is only a prelude to the final stage of the breakdown of food molecules. In these cells, the pyruvate formed by glycolysis is

rapidly transported into the mitochondria, where it is converted into CO_2 plus acetyl CoA, which is then completely oxidized to CO_2 and H_2O.

In contrast, for many anaerobic organisms—which do not utilize molecular oxygen and can grow and divide without it—glycolysis is the principal source of the cell's ATP. This is also true for certain animal tissues, such as skeletal muscle, that can continue to function when molecular oxygen is limiting. In these anaerobic conditions, the pyruvate and the NADH electrons stay in the cytosol. The pyruvate is converted into products excreted from the cell—for example, into ethanol and CO_2 in the yeasts used in brewing and breadmaking, or into lactate in muscle. In this process, the NADH gives up its electrons and is converted back into NAD⁺. This regeneration of NAD⁺ is required to maintain the reactions of glycolysis (**Figure 2–71**).

Anaerobic energy-yielding pathways like these are called **fermentations**. Studies of the commercially important fermentations carried out by yeasts inspired much of early biochemistry. Work in the nineteenth century led in 1896 to the then startling recognition that these processes could be studied outside living organisms, in cell extracts. This revolutionary discovery eventually made it possible to dissect out and study each of the individual reactions in the fermentation process. The piecing together of the complete glycolytic pathway in the 1930s was a major triumph of biochemistry, and it was quickly followed by the recognition of the central role of ATP in cell processes. Thus, most of the fundamental concepts discussed in this chapter have been understood for many years.
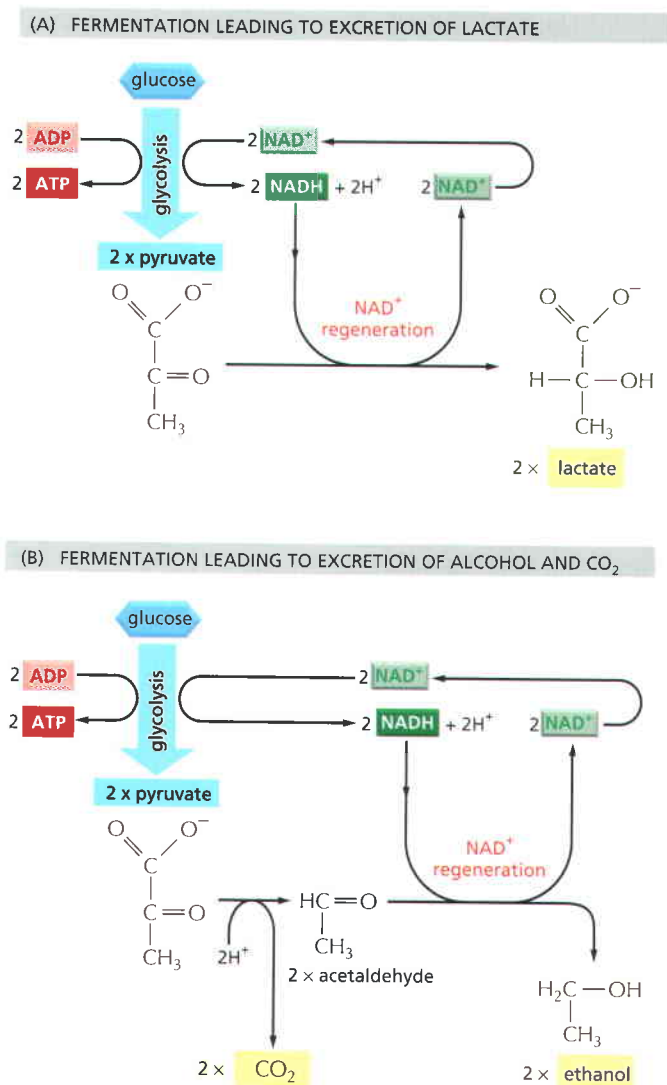
Figure 2–71 **Two pathways for the anaerobic breakdown of pyruvate.** (A) When there is inadequate oxygen, for example, in a muscle cell undergoing vigorous contraction, the pyruvate produced by glycolysis is converted to lactate as shown. This reaction regenerates the NAD⁺ consumed in step 6 of glycolysis, but the whole pathway yields much less energy overall than complete oxidation. (B) In some organisms that can grow anaerobically, such as yeasts, pyruvate is converted via acetaldehyde into carbon dioxide and ethanol. Again, this pathway regenerates NAD⁺ from NADH, as required to enable glycolysis to continue. Both (A) and (B) are examples of *fermentations*.

Glycolysis Illustrates How Enzymes Couple Oxidation to Energy Storage

Returning to the paddle-wheel analogy that we used to introduce coupled reactions (see Figure 2–56), we can now equate enzymes with the paddle wheel. Enzymes act to harvest useful energy from the oxidation of organic molecules by coupling an energetically unfavorable reaction with a favorable one. To demonstrate this coupling, we examine a step in glycolysis to see exactly how such coupled reactions occur.

Two central reactions in glycolysis (steps 6 and 7) convert the three-carbon sugar intermediate glyceraldehyde 3-phosphate (an aldehyde) into 3-phospho-glycerate (a carboxylic acid; see Panel 2–8, pp. 120–121). This entails the oxidation of an aldehyde group to a carboxylic acid group in a reaction that occurs in two steps. The overall reaction releases enough free energy to convert a molecule of ADP to ATP and to transfer two electrons from the aldehyde to NAD^+ to form NADH, while still releasing enough heat to the environment to make the overall reaction energetically favorable ($\Delta G°$ for the overall reaction is –3.0 kcal/mole).

Figure 2–72 outlines the means by which this remarkable feat of energy harvesting is accomplished. The indicated chemical reactions are precisely guided by two enzymes to which the sugar intermediates are tightly bound. In fact, as detailed in Figure 2–72, the first enzyme (glyceraldehyde 3-phosphate dehydrogenase) forms a short-lived covalent bond to the aldehyde through a reactive –SH group on the enzyme, and catalyzes its oxidation by NAD^+ in this attached state. The reactive enzyme–substrate bond is then displaced by an inorganic phosphate ion to produce a high-energy phosphate intermediate, which is released from the enzyme. This intermediate binds to the second enzyme (phosphoglycerate kinase), which catalyzes the energetically favorable transfer of the high-energy phosphate just created to ADP, forming ATP and completing the process of oxidizing an aldehyde to a carboxylic acid.

We have shown this particular oxidation process in some detail because it provides a clear example of enzyme-mediated energy storage through coupled reactions (**Figure 2–73**). Steps 6 and 7 are the only reactions in glycolysis that create a high-energy phosphate linkage directly from inorganic phosphate. As such, they account for the net yield of two ATP molecules and two NADH molecules per molecule of glucose (see Panel 2–8, pp. 120–121).

As we have just seen, ATP can be formed readily from ADP when a reaction intermediate is formed with a phosphate bond of higher-energy than the phosphate bond in ATP. Phosphate bonds can be ordered in energy by comparing the standard free-energy change $(\Delta G°)$ for the breakage of each bond by hydrolysis. **Figure 2–74** compares the high-energy phosphoanhydride bonds in ATP with the energy of some other phosphate bonds, several of which are generated during glycolysis.

Organisms Store Food Molecules in Special Reservoirs

All organisms need to maintain a high ATP/ADP ratio to maintain biological order in their cells. Yet animals have only periodic access to food, and plants need to survive overnight without sunlight, when they are unable to produce sugar from photosynthesis. For this reason, both plants and animals convert sugars and fats to special forms for storage (**Figure 2–75**).

To compensate for long periods of fasting, animals store fatty acids as fat droplets composed of water-insoluble triacylglycerols, largely in the cytoplasm of specialized fat cells, called adipocytes. For shorter-term storage, sugar is stored as glucose subunits in the large branched polysaccharide **glycogen**, which is present as small granules in the cytoplasm of many cells, including liver and muscle. The synthesis and degradation of glycogen are rapidly regulated according to need. When cells need more ATP than they can generate from the food molecules taken in from the bloodstream, they break down glycogen in a reaction that produces glucose 1-phosphate, which is rapidly converted to glucose 6-phosphate for glycolysis.

(A)

glyceraldehyde 3-phosphate
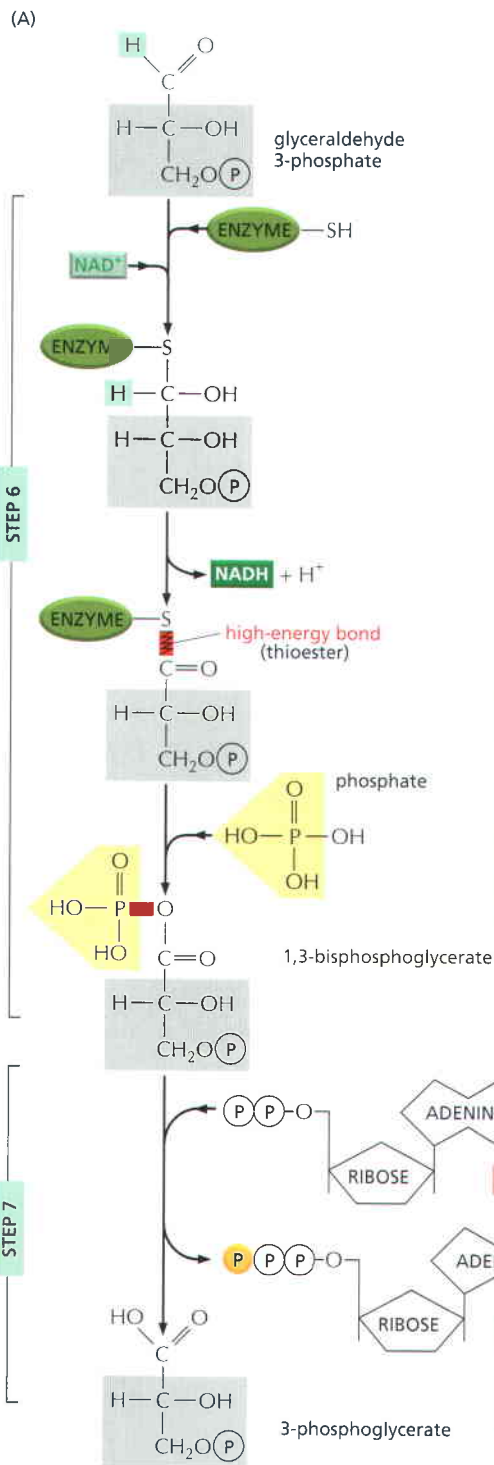
A covalent bond is formed between glyceraldehyde 3-phosphate (the substrate) and the –SH group of a cysteine side chain of the enzyme glyceraldehyde 3-phosphate dehydrogenase, which also binds noncovalently to NAD⁺.

STEP 6

Oxidation of glyceraldehyde 3-phosphate occurs, as two electrons plus a proton (a hydride ion, see Figure 2–60) are transferred from glyceraldehyde 3-phosphate to the bound NAD⁺, forming NADH. Part of the energy released by the oxidation of the aldehyde is thus stored in NADH, and part goes into converting the bond between the enzyme and its substrate glyceraldehyde 3-phosphate into a high-energy thioester bond.

high-energy bond (thioester)

phosphate

A molecule of inorganic phosphate displaces the high-energy bond to the enzyme to create 1,3-bis-phosphoglycerate, which contains a high-energy acyl-anhydride bond.

1,3-bisphosphoglycerate

STEP 7

ADENINE
RIBOSE
ADP

ADENINE
RIBOSE
ATP

3-phosphoglycerate

The high-energy bond to phosphate is transferred to ADP to form ATP.

Figure 2–72 **Energy storage in steps 6 and 7 of glycolysis.** In these steps the oxidation of an aldehyde to a carboxylic acid is coupled to the formation of ATP and NADH. (A) Step 6 begins with the formation of a covalent bond between the substrate (glyceraldehyde 3-phosphate) and an –SH group exposed on the surface of the enzyme (glyceraldehyde 3-phosphate dehydrogenase). The enzyme then catalyzes transfer of hydrogen (as a hydride ion––a proton plus two electrons) from the bound glyceraldehyde 3-phosphate to a molecule of NAD⁺. Part of the energy released in this oxidation is used to form a molecule of NADH and part is used to convert the original linkage between the enzyme and its substrate to a high-energy thioester bond (shown in *red*). A molecule of inorganic phosphate then displaces this high-energy bond on the enzyme, creating a high-energy sugar-phosphate bond instead *(red)*. At this point the enzyme has not only stored energy in NADH, but also coupled the energetically favorable oxidation of an aldehyde to the energetically unfavorable formation of a high-energy phosphate bond. The second reaction has been driven by the first, thereby acting like the "paddle-wheel" coupler in Figure 2–56.

In reaction step 7, the high-energy sugar-phosphate intermediate just made, 1,3-bisphosphoglycerate, binds to a second enzyme, phosphoglycerate kinase. The reactive phosphate is transferred to ADP, forming a molecule of ATP and leaving a free carboxylic acid group on the oxidized sugar.

(B) Summary of the overall chemical change produced by reactions 6 and 7.

(B)

SUMMARY OF STEPS 6 AND 7

NADH

ATP

aldehyde   carboxylic acid
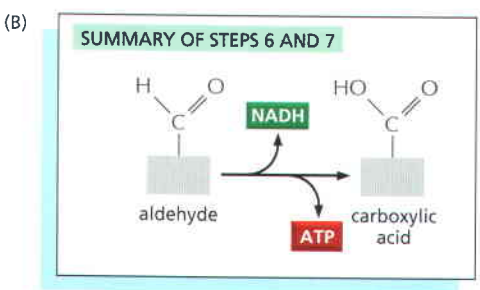
Much of the energy of oxidation has been stored in the activated carriers ATP and NADH.
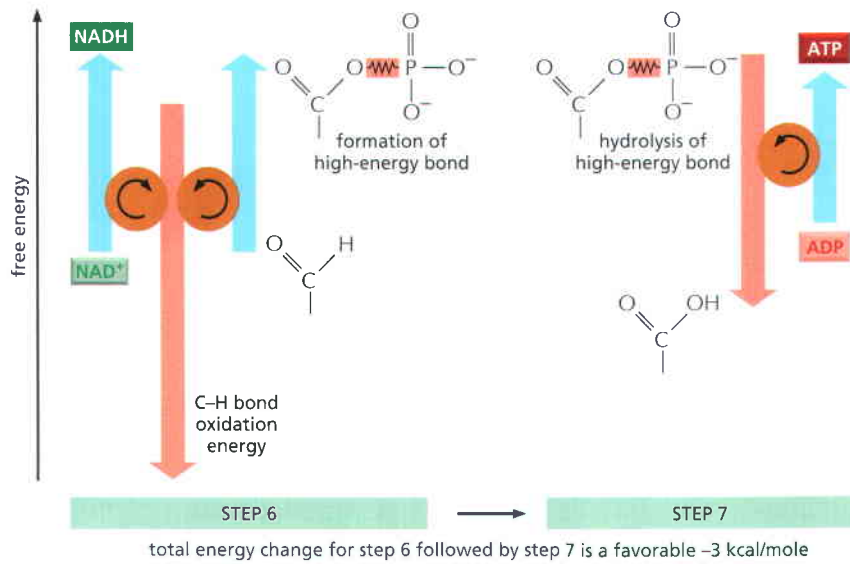
Figure 2–73 **Schematic view of the coupled reactions that form NADH and ATP in steps 6 and 7 of glycolysis.** The C–H bond oxidation energy drives the formation of both NADH and a high-energy phosphate bond. The breakage of the high-energy bond then drives ATP formation.

C–H bond oxidation energy

STEP 6 → STEP 7

total energy change for step 6 followed by step 7 is a favorable –3 kcal/mole

type of phosphate bond		specific examples showing the standard free-energy change (ΔG°) for hydrolysis of phosphate bond	
enol phosphate bond		phosphoenolpyruvate (see Panel 2–8, pp. 120–121)	−14.8 (−61.9)
anhydride bond to carbon		for example, 1,3-bisphosphoglycerate (see Panel 2–8)	−11.7 (−49.0)
phosphate bond in creatine phosphate		creatine phosphate (activated carrier that stores energy in muscle)	−10.3 (−43.0)
anhydride bond to phosphate (phospho-anhydride bond)		for example, ATP when hydrolyzed to ADP	−7.3 (−30.6)
phosphoester bond		for example, glucose 6-phosphate (see Panel 2–8)	−3.3 (−17.5)

ΔG° FOR HYDROLYSIS

Figure 2–74 **Phosphate bonds have different energies.** Examples of different types of phosphate bonds with their sites of hydrolysis are shown in the molecules depicted on the left. Those starting with a *gray* carbon atom show only part of a molecule. Examples of molecules containing such bonds are given on the right, with the free-energy change for hydrolysis in kilocalories (kilojoules in parentheses). The transfer of a phosphate group from one molecule to another is energetically favorable if the standard free-energy change (ΔG°) for hydrolysis of the phosphate bond of the first molecule is more negative than that for hydrolysis of the phosphate bond in the second. Thus, a phosphate group is readily transferred from 1,3-bisphosphoglycerate to ADP to form ATP. The hydrolysis reaction can be viewed as the transfer of the phosphate group to water.

(A)

branch point   glucose subunits

α 1,4-glycosidic
bond in backbone

α 1,6-glycosidic bond
at branch point

(B)

glycogen
granules in
the cytoplasm
of a liver cell

1 μm
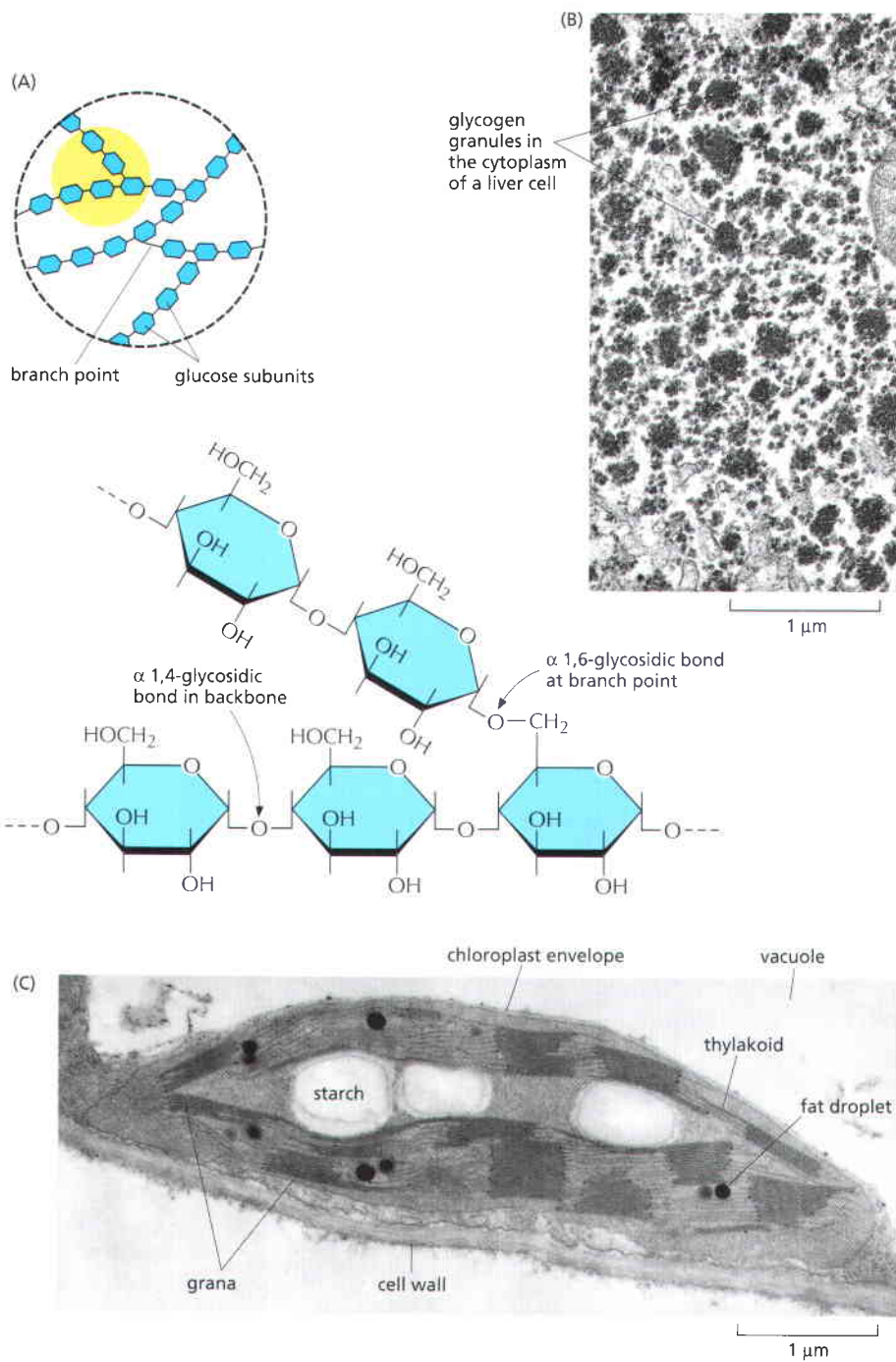
Figure 2–75 The storage of sugars and fats in animal and plant cells. (A) The structures of starch and glycogen, the storage form of sugars in plants and animals, respectively. Both are storage polymers of the sugar glucose and differ only in the frequency of branch points (the region in *yellow* is shown enlarged below). There are many more branches in glycogen than in starch. (B) An electron micrograph shows glycogen granules in the cytoplasm of a liver cell. (C) A thin section of a single chloroplast from a plant cell, showing the starch granules and lipid (fat droplets) that have accumulated as a result of the biosyntheses occurring there. (D) Fat droplets (stained *red)* beginning to accumulate in developing fat cells of an animal. (B, courtesy of Robert Fletterick and Daniel S. Friend; C, courtesy of K. Plaskitt; D, courtesy of Ronald M. Evans and Peter Totonoz.)

(C)

chloroplast envelope   vacuole

thylakoid

starch

fat droplet

grana   cell wall

1 μm

(D)

50 μm
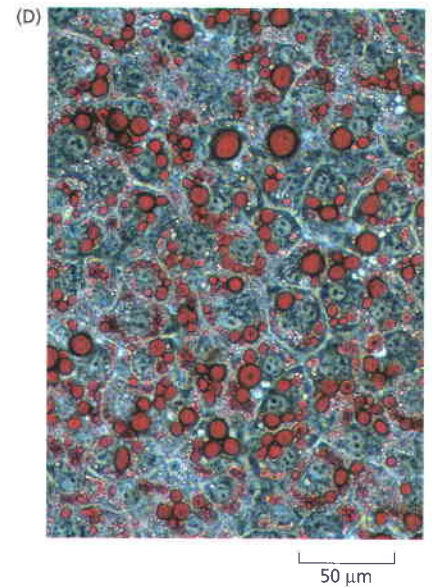
Quantitatively, **fat** is far more important than glycogen as an energy store for animals, presumably because it provides for more efficient storage. The oxidation of a gram of fat releases about twice as much energy as the oxidation of a gram of glycogen. Moreover, glycogen differs from fat in binding a great deal of water, producing a sixfold difference in the actual mass of glycogen required to store the same amount of energy as fat. An average adult human stores enough glycogen for only about a day of normal activities but enough fat to last for nearly a month. If our main fuel reservoir had to be carried as glycogen instead of fat, body weight would increase by an average of about 60 pounds.

Although plants produce NADPH and ATP by photosynthesis, this important process occurs in a specialized organelle, called a chloroplast, which is isolated from the rest of the plant cell by a membrane that is impermeable to both types of activated carrier molecules. Moreover, the plant contains many other cells—such as those in the roots—that lack chloroplasts and therefore cannot produce their own sugars. Therefore, for most of its ATP production, the plant relies on an
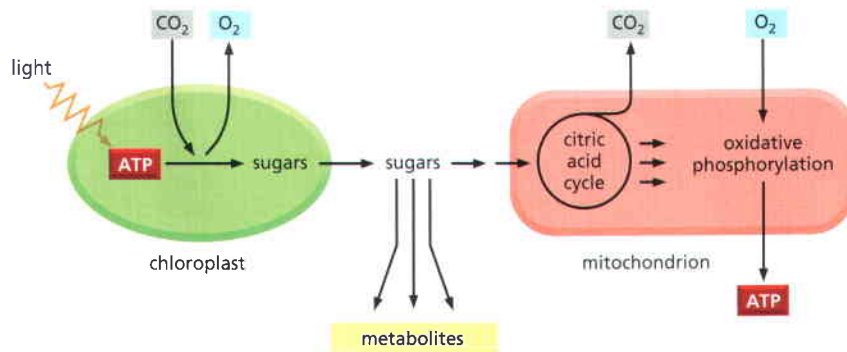
Figure 2–76 **How the ATP needed for most plant cell metabolism is made.** In plants, the chloroplasts and mitochondria collaborate to supply cells with metabolites and ATP. (For details, see Chapter 14.)

export of sugars from its chloroplasts to the mitochondria that are located in all cells of the plant. Most of the ATP needed by the plant is synthesized in these mitochondria and exported from them to the rest of the plant cell, using exactly the same pathways for the oxidative breakdown of sugars as in nonphotosynthetic organisms (**Figure 2–76**).

During periods of excess photosynthetic capacity during the day, chloroplasts convert some of the sugars that they make into fats and into **starch**, a polymer of glucose analogous to the glycogen of animals. The fats in plants are triacylglycerols, just like the fats in animals, and differ only in the types of fatty acids that predominate. Fat and starch are both stored in the chloroplast as reservoirs to be mobilized as an energy source during periods of darkness (see Figure 2–75C).

The embryos inside plant seeds must live on stored sources of energy for a prolonged period, until they germinate to produce leaves that can harvest the energy in sunlight. For this reason plant seeds often contain especially large amounts of fats and starch—which makes them a major food source for animals, including ourselves (**Figure 2–77**).

Most Animal Cells Derive Their Energy from Fatty Acids Between Meals

After a meal, most of the energy that an animal needs is derived from sugars derived from food. Excess sugars, if any, are used to replenish depleted glycogen stores, or to synthesize fats as a food store. But soon the fat stored in adipose tissue is called into play, and by the morning after an overnight fast, fatty acid oxidation generates most of the ATP we need.

Low glucose levels in the blood trigger the breakdown of fats for energy production. As illustrated in **Figure 2–78**, the triacylglycerols stored in fat droplets in adipocytes are hydrolyzed to produce fatty acids and glycerol, and the fatty acids released are transferred to cells in the body through the bloodstream. While animals readily convert sugars to fats, they cannot convert fatty acids to sugars. Instead, the fatty acids are oxidized directly.

Figure 2–77 **Some plant seeds that serve as important foods for humans.** Corn, nuts, and peas all contain rich stores of starch and fat that provide the young plant embryo in the seed with energy and building blocks for biosynthesis. (Courtesy of the John Innes Foundation.)
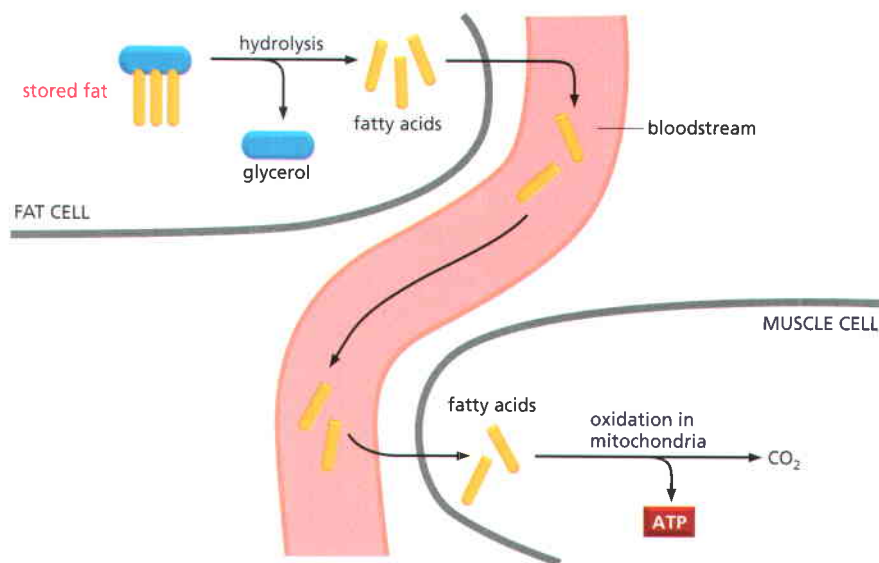
Figure 2–78 How stored fats are mobilized for energy production in animals. Low glucose levels in the blood trigger the hydrolysis of the triacylglycerol molecules in fat droplets to free fatty acids and glycerol, as illustrated. These fatty acids enter the bloodstream, where they bind to the abundant blood protein, serum albumin. Special fatty acid transporters in the plasma membrane of cells that oxidize fatty acids, such as muscle cells, then pass these fatty acids into the cytosol, from which they are moved into mitochondria for energy production (see Figure 2–80).

Sugars and Fats Are Both Degraded to Acetyl CoA in Mitochondria

In aerobic metabolism, the pyruvate that was produced by glycolysis from sugars in the cytosol is transported into the *mitochondria* of eucaryotic cells. There, it is rapidly decarboxylated by a giant complex of three enzymes, called the *pyruvate dehydrogenase complex*. The products of pyruvate decarboxylation are a molecule of CO_2 (a waste product), a molecule of NADH, and acetyl CoA (**Figure 2–79**).

The fatty acids imported from the bloodstream are moved into mitochondria, where all of their oxidation takes place (**Figure 2–80**). Each molecule of fatty acid (as the activated molecule *fatty acyl CoA*) is broken down completely by a cycle of reactions that trims two carbons at a time from its carboxyl end, generating one molecule of acetyl CoA for each turn of the cycle. A molecule of NADH and a molecule of $FADH_2$ are also produced in this process (**Figure 2–81**).

Sugars and fats are the major energy sources for most non-photosynthetic organisms, including humans. However, most of the useful energy that can be
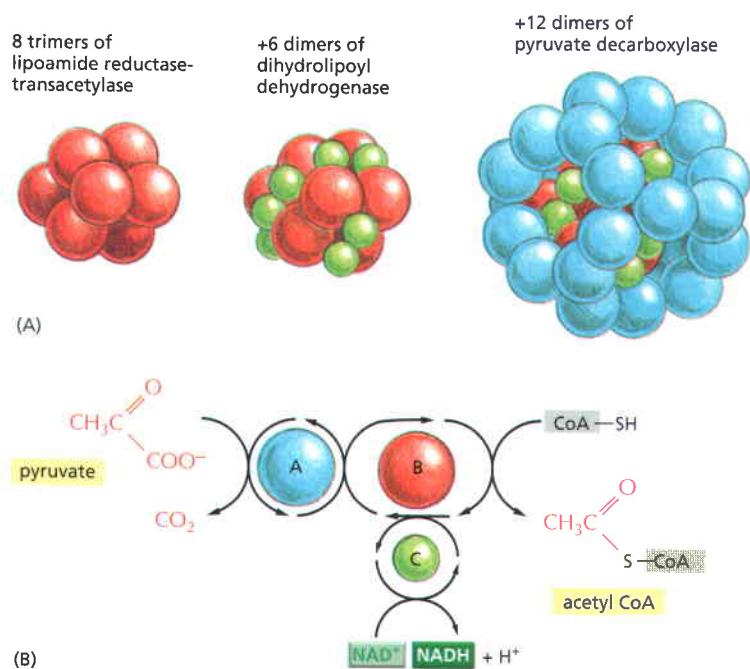
Figure 2–79 The oxidation of pyruvate to acetyl CoA and CO_2. (A) The structure of the pyruvate dehydrogenase complex, which contains 60 polypeptide chains. This is an example of a large multienzyme complex in which reaction intermediates are passed directly from one enzyme to another. In eucaryotic cells it is located in the mitochondrion. (B) The reactions carried out by the pyruvate dehydrogenase complex. The complex converts pyruvate to acetyl CoA in the mitochondrial matrix; NADH is also produced in this reaction. A, B, and C are the three enzymes *pyruvate decarboxylase, lipoamide reductase-transacetylase,* and *dihydrolipoyl dehydrogenase,* respectively. These enzymes are illustrated in (A); their activities are linked as shown.
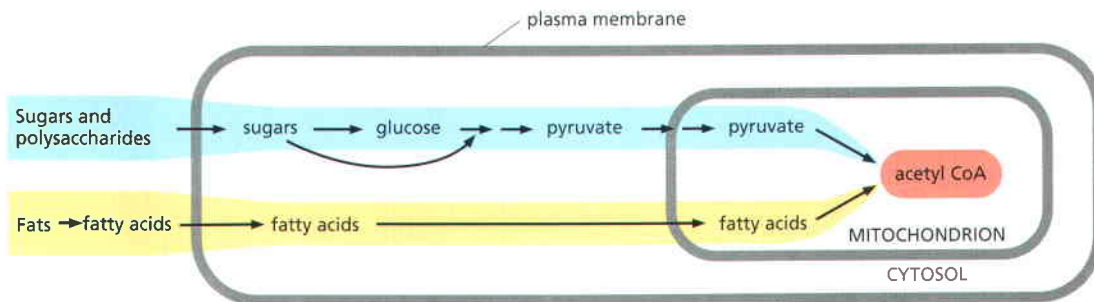
Figure 2–80 **Pathways for the production of acetyl CoA from sugars and fats.** The mitochondrion in eucaryotic cells is the place where acetyl CoA is produced from both types of major food molecules. It is therefore the place where most of the cell's oxidation reactions occur and where most of its ATP is made. The structure and function of mitochondria are discussed in detail in Chapter 14.

extracted from the oxidation of both types of foodstuffs remains stored in the acetyl CoA molecules that are produced by the two types of reactions just described. The citric acid cycle of reactions, in which the acetyl group in acetyl CoA is oxidized to CO_2 and H_2O, is therefore central to the energy metabolism of aerobic organisms. In eucaryotes these reactions all take place in mitochondria. We should therefore not be surprised to discover that the mitochondrion is the place where most of the ATP is produced in animal cells. In contrast, aerobic bacteria carry out all of their reactions in a single compartment, the cytosol, and it is here that the citric acid cycle takes place in these cells.

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

In the nineteenth century, biologists noticed that in the absence of air (anaerobic conditions) cells produce lactic acid (for example, in muscle) or ethanol (for example, in yeast), while in its presence (aerobic conditions) they consume O_2 and produce CO_2 and H_2O. Efforts to define the pathways of aerobic metabolism

Figure 2–81 **The oxidation of fatty acids to acetyl CoA.** (A) Electron micrograph of a lipid droplet in the cytoplasm *(top)*, and the structure of fats *(bottom)*. Fats are triacylglycerols. The glycerol portion, to which three fatty acids are linked through ester bonds, is shown here in *blue*. Fats are insoluble in water and form large lipid droplets in the specialized fat cells (called adipocytes) in which they are stored. (B) The fatty acid oxidation cycle. The cycle is catalyzed by a series of four enzymes in the mitochondrion. Each turn of the cycle shortens the fatty acid chain by two carbons (shown in *red*) and generates one molecule of acetyl CoA and one molecule each of NADH and $FADH_2$. The structure of $FADH_2$ is presented in Figure 2–83B. (A, courtesy of Daniel S. Friend.)

eventually focused on the oxidation of pyruvate and led in 1937 to the discovery of the **citric acid cycle**, also known as the *tricarboxylic acid cycle* or the *Krebs cycle*. The citric acid cycle accounts for about two-thirds of the total oxidation of carbon compounds in most cells, and its major end products are CO_2 and high-energy electrons in the form of NADH. The CO_2 is released as a waste product, while the high-energy electrons from NADH are passed to a membrane-bound electron-transport chain (discussed in Chapter 14), eventually combining with O_2 to produce H_2O. Although the citric acid cycle itself does not use O_2, it requires O_2 in order to proceed because there is no other efficient way for the NADH to get rid of its electrons and thus regenerate the NAD^+ that is needed to keep the cycle going.

The citric acid cycle takes place inside mitochondria in eucaryotic cells. It results in the complete oxidation of the carbon atoms of the acetyl groups in acetyl CoA, converting them into CO_2. But the acetyl group is not oxidized directly. Instead, this group is transferred from acetyl CoA to a larger, four-carbon molecule, *oxaloacetate*, to form the six-carbon tricarboxylic acid, *citric acid*, for which the subsequent cycle of reactions is named. The citric acid molecule is then gradually oxidized, allowing the energy of this oxidation to be harnessed to produce energy-rich activated carrier molecules. The chain of eight reactions forms a cycle because at the end the oxaloacetate is regenerated and enters a new turn of the cycle, as shown in outline in **Figure 2–82**.

We have thus far discussed only one of the three types of activated carrier molecules that are produced by the citric acid cycle, the NAD^+–NADH pair (see Figure 2–60). In addition to three molecules of NADH, each turn of the cycle also produces one molecule of **FADH$_2$** (reduced flavin adenine dinucleotide) from FAD and one molecule of the ribonucleotide **GTP** (guanosine triphosphate) from GDP. The structures of these two activated carrier molecules are illustrated in **Figure 2–83**. GTP is a close relative of ATP, and the transfer of its terminal phosphate group to ADP produces one ATP molecule in each cycle. Like NADH, FADH$_2$ is a carrier of high-energy electrons and hydrogen. As we discuss shortly, the energy that is stored in the readily transferred high-energy electrons of NADH and FADH$_2$ will be utilized subsequently for ATP production through the process of *oxidative phosphorylation*, the only step in the oxidative catabolism of foodstuffs that directly requires gaseous oxygen (O_2) from the atmosphere.

Panel 2–9 (pp. 122–123) presents the complete citric acid cycle. Water, rather than molecular oxygen, supplies the extra oxygen atoms required to make CO_2 from the acetyl groups entering the citric acid cycle. As illustrated in the panel,

NET RESULT: ONE TURN OF THE CYCLE PRODUCES THREE NADH, ONE GTP, AND ONE FADH$_2$, AND RELEASES TWO MOLECULES OF CO_2
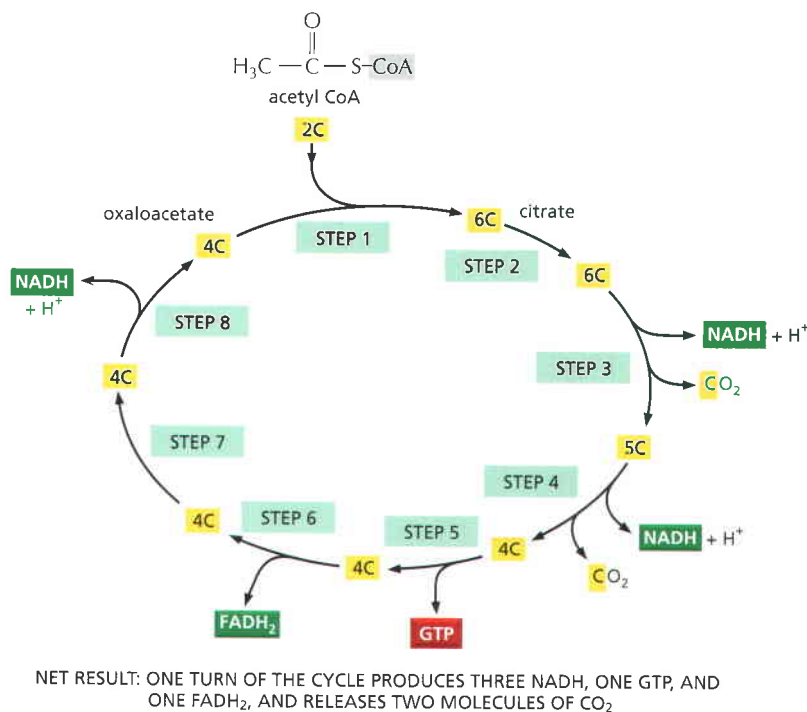
Figure 2–82 Simple overview of the citric acid cycle. <TAGT> The reaction of acetyl CoA with oxaloacetate starts the cycle by producing citrate (citric acid). In each turn of the cycle, two molecules of CO_2 are produced as waste products, plus three molecules of NADH, one molecule of GTP, and one molecule of FADH$_2$. The number of carbon atoms in each intermediate is shown in a *yellow box.* For details, see Panel 2–9 (pp. 122–123).
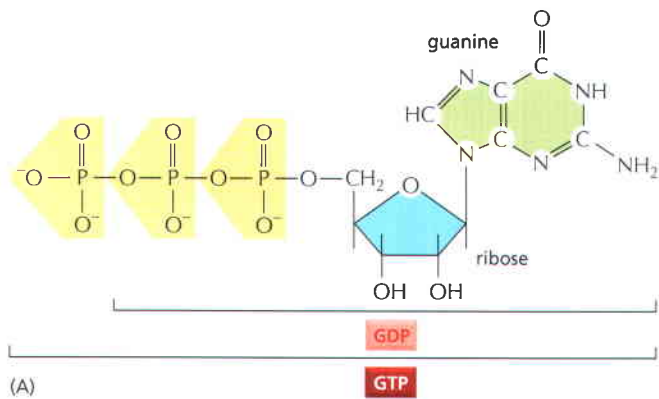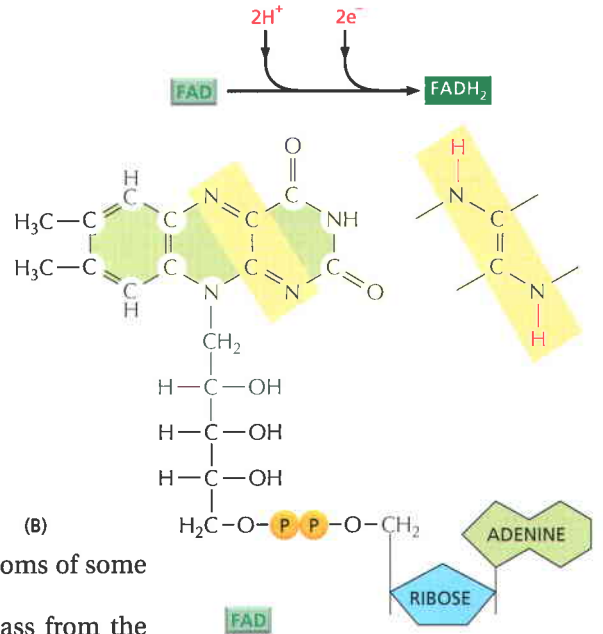
three molecules of water are split in each cycle, and the oxygen atoms of some of them are ultimately used to make CO_2.

In addition to pyruvate and fatty acids, some amino acids pass from the cytosol into mitochondria, where they are also converted into acetyl CoA or one of the other intermediates of the citric acid cycle. Thus, in the eucaryotic cell, the mitochondrion is the center toward which all energy-yielding processes lead, whether they begin with sugars, fats, or proteins.

Both the citric acid cycle and glycolysis also function as starting points for important biosynthetic reactions by producing vital carbon-containing intermediates, such as *oxaloacetate* and *α-ketoglutarate*. Some of these substances produced by catabolism are transferred back from the mitochondrion to the cytosol, where they serve in anabolic reactions as precursors for the synthesis of many essential molecules, such as amino acids (**Figure 2–84**).

Figure 2–83 The structures of GTP and FADH$_2$. (A) GTP and GDP are close relatives of ATP and ADP, respectively. (B) FADH$_2$ is a carrier of hydrogens and high-energy electrons, like NADH and NADPH. It is shown here in its oxidized form (FAD) with the hydrogen-carrying atoms highlighted in *yellow*.
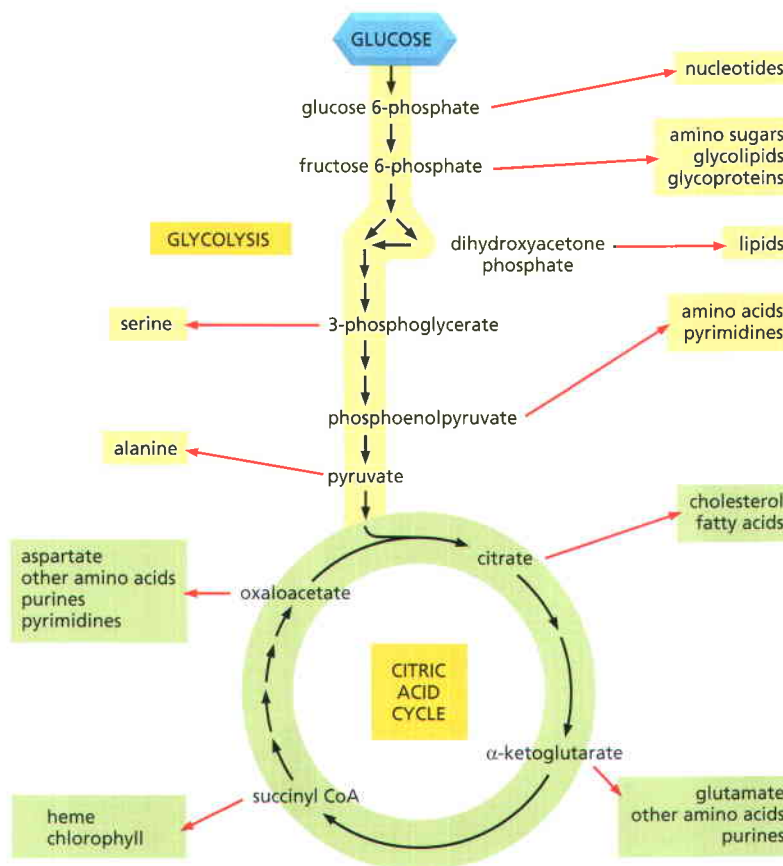
Figure 2–84 Glycolysis and the citric acid cycle provide the precursors needed to synthesize many important biological molecules. The amino acids, nucleotides, lipids, sugars, and other molecules—shown here as products—in turn serve as the precursors for the many macromolecules of the cell. Each *black arrow* in this diagram denotes a single enzyme-catalyzed reaction; the *red arrows* generally represent pathways with many steps that are required to produce the indicated products.

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

Most chemical energy is released in the last step in the degradation of a food molecule. In this final process the electron carriers NADH and $FADH_2$ transfer the electrons that they have gained when oxidizing other molecules to the **electron-transport chain**, which is embedded in the inner membrane of the mitochondrion (see Figure 14–10). As the electrons pass along this long chain of specialized electron acceptor and donor molecules, they fall to successively lower energy states. The energy that the electrons release in this process pumps H^+ ions (protons) across the membrane—from the inner mitochondrial compartment to the outside—generating a gradient of H^+ ions (**Figure 2–85**). This gradient serves as a source of energy, being tapped like a battery to drive a variety of energy-requiring reactions. The most prominent of these reactions is the generation of ATP by the phosphorylation of ADP.

At the end of this series of electron transfers, the electrons are passed to molecules of oxygen gas (O_2) that have diffused into the mitochondrion, which simultaneously combine with protons (H^+) from the surrounding solution to produce water molecules. The electrons have now reached their lowest energy level, and therefore all the available energy has been extracted from the oxidized food molecule. This process, termed **oxidative phosphorylation** (**Figure 2–86**), also occurs in the plasma membrane of bacteria. As one of the most remarkable achievements of cell evolution, it is a central topic of Chapter 14.

In total, the complete oxidation of a molecule of glucose to H_2O and CO_2 is used by the cell to produce about 30 molecules of ATP. In contrast, only 2 molecules of ATP are produced per molecule of glucose by glycolysis alone.

Amino Acids and Nucleotides Are Part of the Nitrogen Cycle

So far we have concentrated mainly on carbohydrate metabolism and have not yet considered the metabolism of nitrogen or sulfur. These two elements are important constituents of biological macromolecules. Nitrogen and sulfur atoms pass from compound to compound and between organisms and their environment in a series of reversible cycles.

Although molecular nitrogen is abundant in the Earth's atmosphere, nitrogen is chemically unreactive as a gas. Only a few living species are able to incorporate it into organic molecules, a process called **nitrogen fixation**. Nitrogen fixation occurs in certain microorganisms and by some geophysical processes, such as lightning discharge. It is essential to the biosphere as a whole, for without it life could not exist on this planet. Only a small fraction of the nitrogenous compounds in today's organisms, however, is due to fresh products of nitrogen fixation from the atmosphere. Most organic nitrogen has been in circulation for
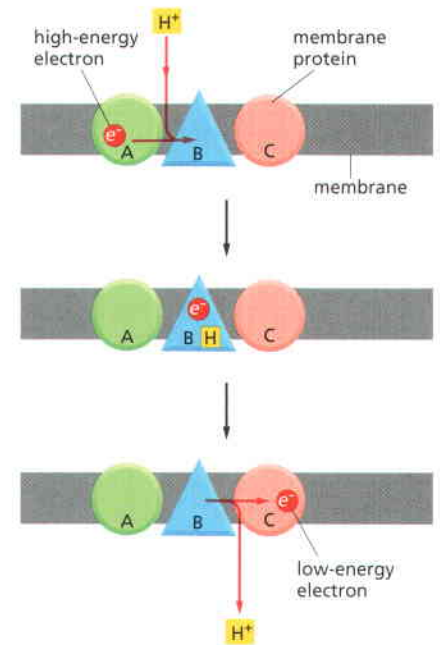
Figure 2–85 The generation of an H^+ gradient across a membrane by electron-transport reactions. A high-energy electron (derived, for example, from the oxidation of a metabolite) is passed sequentially by carriers A, B, and C to a lower energy state. In this diagram carrier B is arranged in the membrane in such a way that it takes up H^+ from one side and releases it to the other as the electron passes. The result is an H^+ gradient. As discussed in Chapter 14, this gradient is an important form of energy that is harnessed by other membrane proteins to drive the formation of ATP.
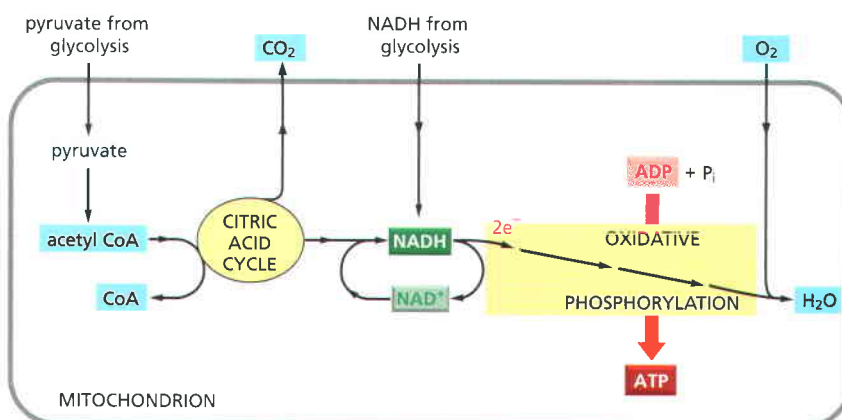
Figure 2–86 The final stages of oxidation of food molecules. Molecules of NADH and $FADH_2$ ($FADH_2$ is not shown) are produced by the citric acid cycle. These activated carriers donate high-energy electrons that are eventually used to reduce oxygen gas to water. A major portion of the energy released during the transfer of these electrons along an electron-transfer chain in the mitochondrial inner membrane (or in the plasma membrane of bacteria) is harnessed to drive the synthesis of ATP—hence the name oxidative phosphorylation (discussed in Chapter 14).

some time, passing from one living organism to another. Thus present-day nitrogen-fixing reactions can be said to perform a "topping-up" function for the total nitrogen supply.

Vertebrates receive virtually all of their nitrogen from their dietary intake of proteins and nucleic acids. In the body these macromolecules are broken down to amino acids and the components of nucleotides, and the nitrogen they contain is used to produce new proteins and nucleic acids—or utilized to make other molecules. About half of the 20 amino acids found in proteins are essential amino acids for vertebrates (**Figure 2–87**), which means that they cannot be synthesized from other ingredients of the diet. The others can be so synthesized, using a variety of raw materials, including intermediates of the citric acid cycle as described previously. The essential amino acids are made by plants and other organisms, usually by long and energetically expensive pathways that have been lost in the course of vertebrate evolution. RoshanKetab 021-66950639

The nucleotides needed to make RNA and DNA can be synthesized using specialized biosynthetic pathways. All of the nitrogens in the purine and pyrimidine bases (as well as some of the carbons) are derived from the plentiful amino acids glutamine, aspartic acid, and glycine, whereas the ribose and deoxyribose sugars are derived from glucose. There are no "essential nucleotides" that must be provided in the diet.

Amino acids not used in biosynthesis can be oxidized to generate metabolic energy. Most of their carbon and hydrogen atoms eventually form CO_2 or H_2O, whereas their nitrogen atoms are shuttled through various forms and eventually appear as urea, which is excreted. Each amino acid is processed differently, and a whole constellation of enzymatic reactions exists for their catabolism.

Sulfur is abundant on Earth in its most oxidized form, sulfate (SO_4^{2-}). To convert it to forms useful for life, sulfate must be reduced to sulfide (S^{2-}), the oxidation state of sulfur required for the synthesis of essential biological molecules. These molecules include the amino acids methionine and cysteine, coenzyme A (see Figure 2–62), and the iron-sulfur centers essential for electron transport (see Figure 14–23). The process begins in bacteria, fungi, and plants, where a special group of enzymes use ATP and reducing power to create a sulfate assimilation pathway. Humans and other animals cannot reduce sulfate and must therefore acquire the sulfur they need for their metabolism in the food that they eat.

Metabolism Is Organized and Regulated

One gets a sense of the intricacy of a cell as a chemical machine from the relation of glycolysis and the citric acid cycle to the other metabolic pathways sketched out in **Figure 2–88**. This type of chart, which was used earlier in this chapter to introduce metabolism, represents only some of the enzymatic pathways in a cell. It is obvious that our discussion of cell metabolism has dealt with only a tiny fraction of cellular chemistry.

All these reactions occur in a cell that is less than 0.1 mm in diameter, and each requires a different enzyme. As is clear from Figure 2–88, the same molecule can often be part of many different pathways. Pyruvate, for example, is a substrate for half a dozen or more different enzymes, each of which modifies it chemically in a different way. One enzyme converts pyruvate to acetyl CoA, another to oxaloacetate; a third enzyme changes pyruvate to the amino acid alanine, a fourth to lactate, and so on. All of these different pathways compete for the same pyruvate molecule, and similar competitions for thousands of other small molecules go on at the same time.

The situation is further complicated in a multicellular organism. Different cell types will in general require somewhat different sets of enzymes. And different tissues make distinct contributions to the chemistry of the organism as a whole. In addition to differences in specialized products such as hormones or antibodies, there are significant differences in the "common" metabolic pathways among various types of cells in the same organism.

Although virtually all cells contain the enzymes of glycolysis, the citric acid cycle, lipid synthesis and breakdown, and amino acid metabolism, the levels of

THE ESSENTIAL AMINO ACIDS

THREONINE

METHIONINE

LYSINE

VALINE

LEUCINE

ISOLEUCINE

HISTIDINE

PHENYLALANINE

TRYPTOPHAN

Figure 2–87 The nine essential amino acids. These cannot be synthesized by human cells and so must be supplied in the diet.
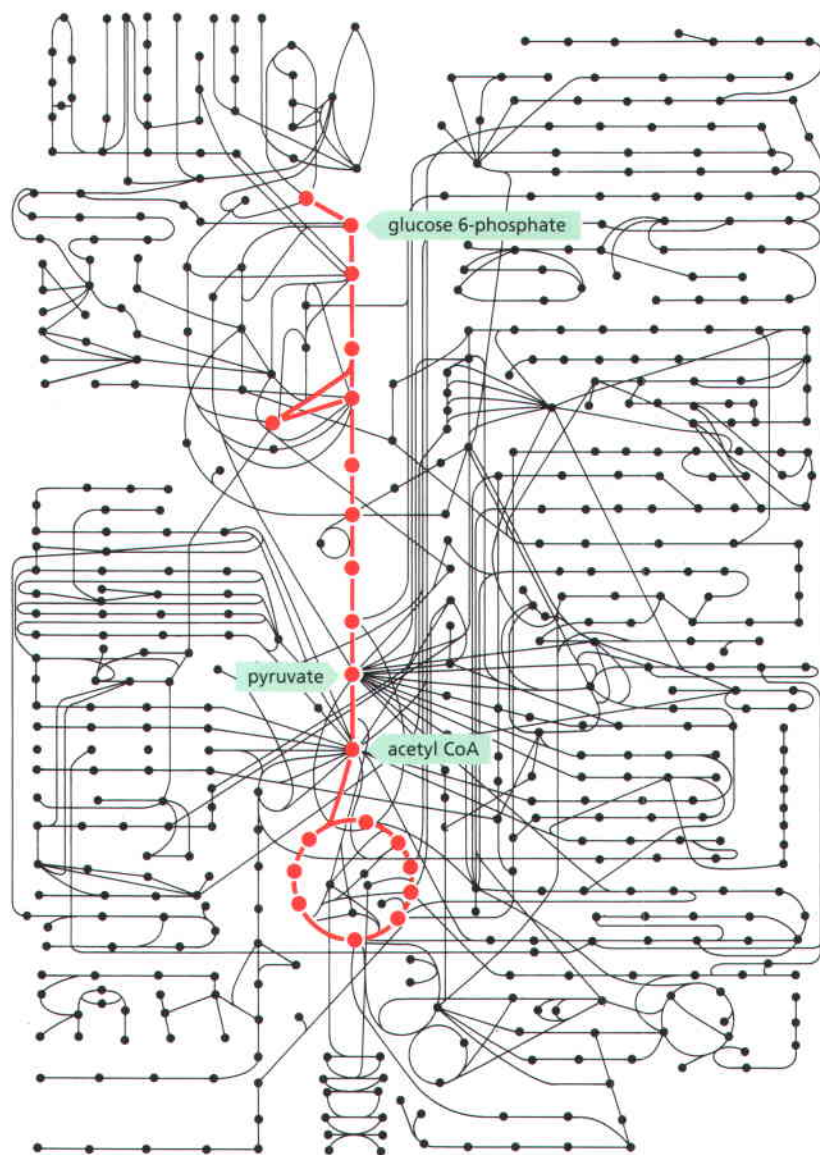
Figure 2–88 Glycolysis and the citric acid cycle are at the center of metabolism. Some 500 metabolic reactions of a typical cell are shown schematically with the reactions of glycolysis and the citric acid cycle in *red*. Other reactions either lead into these two central pathways—delivering small molecules to be catabolized with production of energy—or they lead outward and thereby supply carbon compounds for the purpose of biosynthesis.

these processes required in different tissues are not the same. For example, nerve cells, which are probably the most fastidious cells in the body, maintain almost no reserves of glycogen or fatty acids and rely almost entirely on a constant supply of glucose from the bloodstream. In contrast, liver cells supply glucose to actively contracting muscle cells and recycle the lactic acid produced by muscle cells back into glucose. All types of cells have their distinctive metabolic traits, and they cooperate extensively in the normal state, as well as in response to stress and starvation. One might think that the whole system would need to be so finely balanced that any minor upset, such as a temporary change in dietary intake, would be disastrous.

In fact, the metabolic balance of a cell is amazingly stable. Whenever the balance is perturbed, the cell reacts so as to restore the initial state. The cell can adapt and continue to function during starvation or disease. Mutations of many kinds can damage or even eliminate particular reaction pathways, and yet—provided that certain minimum requirements are met—the cell survives. It does so because an elaborate network of *control mechanisms* regulates and coordinates the rates of all of its reactions. These controls rest, ultimately, on the remarkable abilities of proteins to change their shape and their chemistry in response to changes in their immediate environment. The principles that underlie how large molecules such as proteins are built and the chemistry behind their regulation will be our next concern.

Summary

Glucose and other food molecules are broken down by controlled stepwise oxidation to provide chemical energy in the form of ATP and NADH. There are three main sets of reactions that act in series—the products of each being the starting material for the next: glycolysis (which occurs in the cytosol), the citric acid cycle (in the mitochondrial matrix), and oxidative phosphorylation (on the inner mitochondrial membrane). The intermediate products of glycolysis and the citric acid cycle are used both as sources of metabolic energy and to produce many of the small molecules used as the raw materials for biosynthesis. Cells store sugar molecules as glycogen in animals and starch in plants; both plants and animals also use fats extensively as a food store. These storage materials in turn serve as a major source of food for humans, along with the proteins that comprise the majority of the dry mass of most of the cells in the foods we eat.

PROBLEMS

Which statements are true? Explain why or why not.

2–1   Of the original radioactivity in a sample, only about 1/1000 will remain after 10 half-lives.

2–2   A 10^{-8} M solution of HCl has a pH of 8.

2–3   Most of the interactions between macromolecules could be mediated just as well by covalent bonds as by non-covalent bonds.

2–4   Animals and plants use oxidation to extract energy from food molecules.

2–5   If an oxidation occurs in a reaction, it must be accompanied by a reduction.

2–6   Linking the energetically unfavorable reaction A → B to a second, favorable reaction B → C will shift the equilibrium constant for the first reaction.

2–7   The criterion for whether a reaction proceeds spontaneously is ΔG not $\Delta G°$, because ΔG takes into account the concentrations of the substrates and products.

2–8   Because glycolysis is only a prelude to the oxidation of glucose in mitochondria, which yields 15-fold more ATP, glycolysis is not really important for human cells.

2–9   The oxygen consumed during the oxidation of glucose in animal cells is returned as CO_2 to the atmosphere.

Discuss the following problems.

2–10   The organic chemistry of living cells is said to be special for two reasons: it occurs in an aqueous environment and it accomplishes some very complex reactions. But do you suppose it is really all that much different from the organic chemistry carried out in the top laboratories in the world? Why or why not?

2–11   The molecular weight of ethanol (CH_3CH_2OH) is 46 and its density is 0.789 g/cm^3.
A.   What is the molarity of ethanol in beer that is 5% ethanol by volume? [Alcohol content of beer varies from about 4% (lite beer) to 8% (stout beer).]
B.   The legal limit for a driver's blood alcohol content varies, but 80 mg of ethanol per 100 mL of blood (usually

Table Q2–1 Radioactive isotopes and some of their properties (Problem 2–12).

RADIOACTIVE ISOTOPE	EMISSION	HALF-LIFE	MAXIMUM SPECIFIC ACTIVITY (Ci/mmol)
^{14}C	β particle	5730 years	0.062
^{3}H	β particle	12.3 years	29
^{35}S	β particle	87.4 days	1490
^{32}P	β particle	14.3 days	9120

referred to as a blood alcohol level of 0.08) is typical. What is the molarity of ethanol in a person at this legal limit?
C.   How many 12-oz (355-mL) bottles of 5% beer could a 70-kg person drink and remain under the legal limit? A 70-kg person contains about 40 liters of water. Ignore the metabolism of ethanol, and assume that the water content of the person remains constant.
D.   Ethanol is metabolized at a constant rate of about 120 mg per hour per kg body weight, regardless of its concentration. If a 70-kg person were at twice the legal limit (160 mg/100 mL), how long would it take for their blood alcohol level to fall below the legal limit?

2–12   Specific activity refers to the amount of radioactivity per unit amount of substance, usually in biology expressed on a molar basis, for example, as Ci/mmol. [One curie (Ci) corresponds to 2.22×10^{12} disintegrations per minute (dpm).] As apparent in **Table Q2–1**, which lists properties of four isotopes commonly used in biology, there is an inverse relationship between maximum specific activity and half-life. Do you suppose this is just a coincidence or is there an underlying reason? Explain your answer.

2–13   By a convenient coincidence the ion product of water, $K_w = [H^+][OH^-]$, is a nice round number: 1.0×10^{-14} M^2.
A.   Why is a solution at pH 7.0 said to be neutral?
B.   What is the H^+ concentration and pH of a 1 mM solution of NaOH?
C.   If the pH of a solution is 5.0, what is the concentration of OH^- ions?

2–14   Suggest a rank order for the pK values (from lowest to highest) for the carboxyl group on the aspartate side chain

in the following environments in a protein. Explain your ranking.

1. An aspartate side chain on the surface of a protein with no other ionizable groups nearby.
2. An aspartate side chain buried in a hydrophobic pocket on the surface of a protein.
3. An aspartate side chain in a hydrophobic pocket adjacent to a glutamate side chain.
4. An aspartate side chain in a hydrophobic pocket adjacent to a lysine side chain.

2–15   A histidine side chain is known to play an important role in the catalytic mechanism of an enzyme; however, it is not clear whether histidine is required in its protonated (charged) or unprotonated (uncharged) state. To answer this question you measure enzyme activity over a range of pH, with the results shown in **Figure Q2–1**. Which form of histidine is required for enzyme activity?
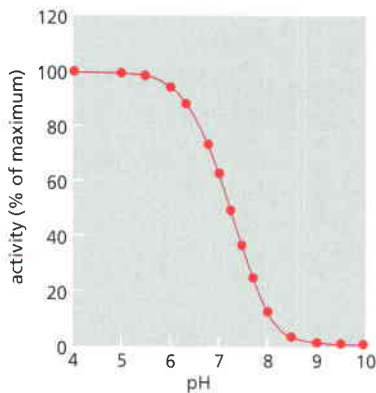
Figure Q2–1 Enzyme activity as a function of pH (Problem 2–15).

2–16   During an all-out sprint, muscles metabolize glucose anaerobically, producing a high concentration of lactic acid, which lowers the pH of the blood and of the cytosol and contributes to the fatigue sprinters experience well before their fuel reserves are exhausted. The main blood buffer against pH changes is the bicarbonate/CO_2 system.

$$CO_2 \underset{\text{(gas)}}{\rightleftharpoons} \underset{\text{(dissolved)}}{CO_2} \overset{pK_1 =}{\underset{2.3}{\rightleftharpoons}} H_2CO_3 \overset{pK_2 =}{\underset{3.8}{\rightleftharpoons}} H^+ + HCO_3^- \overset{pK_3 =}{\underset{10.3}{\rightleftharpoons}} H^+ + CO_3^{2-}$$
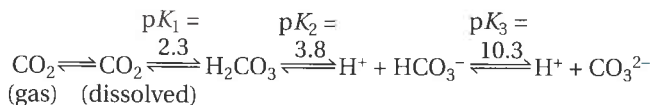
To improve their performance, would you advise sprinters to hold their breath or to breathe rapidly for a minute immediately before the race? Explain your answer.

2–17   The three molecules in **Figure Q2–2** contain the seven most common reactive groups in biology. Most molecules in the cell are built from these functional groups. Indicate and name the functional groups in these molecules.

2–18   "Diffusion" sounds slow—and over everyday distances it is—but on the scale of a cell it is very fast. The average instantaneous velocity of a particle in solution, that is, the velocity between collisions, is

$$v = (kT/m)^{1/2}$$

where $k = 1.38 \times 10^{-16}$ g cm^2/K sec^2, T = temperature in K (37°C is 310 K), m = mass in g/molecule.
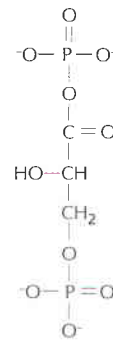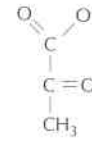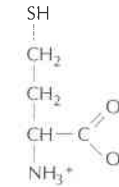
Figure Q2–2 Three molecules that illustrate the seven most common functional groups in biology (Problem 2–17). 1,3-Bisphosphoglycerate and pyruvate are intermediates in glycolysis and cysteine is an amino acid.

Calculate the instantaneous velocity of a water molecule (molecular mass = 18 daltons), a glucose molecule (molecular mass = 180 daltons), and a myoglobin molecule (molecular mass = 15,000 daltons) at 37°C. Just for fun, convert these numbers into kilometers/hour. Before you do any calculations, try to guess whether the molecules are moving at a slow crawl (<1 km/hr), an easy walk (5 km/hr), or a record-setting sprint (40 km/hr).

2–19   Polymerization of tubulin subunits into microtubules occurs with an increase in the orderliness of the subunits (**Figure Q2–3**). Yet tubulin polymerization occurs with an increase in entropy (decrease in order). How can that be?
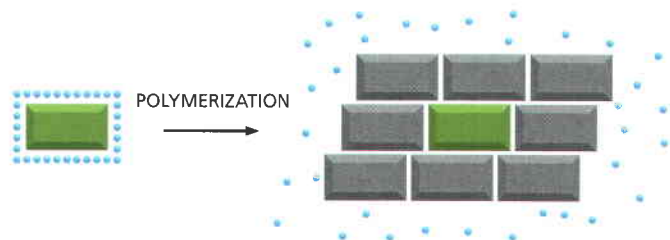
Figure Q2–3 Polymerization of tubulin subunits into a microtubule (Problem 2–19). The fates of one subunit (*shaded*) and its associated water molecules (*small spheres*) are shown.

2–20   A 70-kg adult human (154 lb) could meet his or her entire energy needs for one day by eating 3 moles of glucose (540 g). (We don't recommend this.) Each molecule of glucose generates 30 ATP when it is oxidized to CO_2. The concentration of ATP is maintained in cells at about 2 mM, and a 70-kg adult has about 25 liters of intracellular fluid. Given that the ATP concentration remains constant in cells, calculate how many times per day, on average, each ATP molecule in the body is hydrolyzed and resynthesized.

2–21   Assuming that there are 5×10^{13} cells in the human body and that ATP is turning over at a rate of 10^9 ATP per minute in each cell, how many watts is the human body consuming? (A watt is a joule per second, and there are 4.18 joules/calorie.) Assume that hydrolysis of ATP yields 12 kcal/mole.

2–22   Does a Snickers™ candy bar (65 g, 325 kcal) provide enough energy to climb from Zermatt (elevation 1660 m) to the top of the Matterhorn (4478 m, **Figure Q2–4**), or might

Figure Q2–4 The Matterhorn (Problem 2–22). (Courtesy of Zermatt Tourism.)
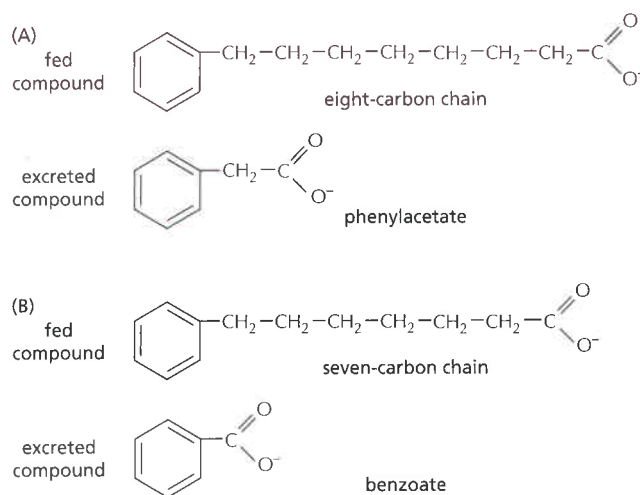

Figure Q2–5 The original labeling experiment to analyze fatty acid oxidation (Problem 2–26). (A) Fed and excreted derivatives of an even-number fatty acid chain. (B) Fed and excreted derivatives of an odd-number fatty acid chain.

you need to stop at Hörnli Hut (3260 m) to eat another one? Imagine that you and your gear have a mass of 75 kg, and that all of your work is done against gravity (that is, you are just climbing straight up). Remember from your introductory physics course that

work (J) = mass (kg) × g (m/sec²) × height gained (m)

where g is acceleration due to gravity (9.8 m/sec²). One joule is 1 kg m²/sec² and there are 4.18 kJ per kcal.

What assumptions made here will greatly underestimate how much candy you need?

2–23 At first glance, fermentation of pyruvate to lactate appears to be an optional add-on reaction to glycolysis. After all, could cells growing in the absence of oxygen not simply discard pyruvate as a waste product? In the absence of fermentation, which products derived from glycolysis would accumulate in cells under anaerobic conditions? Could the metabolism of glucose via the glycolytic pathway continue in the absence of oxygen in cells that cannot carry out fermentation? Why or why not?

2–24 In the absence of oxygen, cells consume glucose at a high, steady rate. When oxygen is added, glucose consumption drops precipitously and is then maintained at the lower rate. Why is glucose consumed at a high rate in the absence of oxygen and at a low rate in its presence?

2–25 The liver provides glucose to the rest of the body between meals. It does so by breaking down glycogen, forming glucose 6-phosphate in the penultimate step. Glucose 6-phosphate is converted to glucose by splitting off the phosphate ($\Delta G° = -3.3$ kcal/mole). Why do you suppose the liver removes the phosphate by hydrolysis, rather than reversing the reaction by which glucose 6-phosphate (G6P) is formed from glucose (glucose + ATP → G6P + ADP, $\Delta G° = -4.0$ kcal/mole)? By reversing this reaction the liver could generate both glucose and ATP.

2–26 In 1904 Franz Knoop performed what was probably the first successful labeling experiment to study metabolic pathways. He fed many different fatty acids labeled with a terminal benzene ring to dogs and analyzed their urine for excreted benzene derivatives. Whenever the fatty acid had an even number of carbon atoms, phenylacetate was excreted (Figure Q2–5A). Whenever the fatty acid had an odd number of carbon atoms, benzoate was excreted (Figure Q2–5B).

From these experiments Knoop deduced that oxidation of fatty acids to CO_2 and H_2O involved the removal of two-carbon fragments from the carboxylic acid end of the chain.

Can you explain the reasoning that led him to conclude that two-carbon fragments, as opposed to any other number, were removed, and that degradation was from the carboxylic acid end, as opposed to the other end?

2–27 Pathways for synthesis of amino acids in microorganisms were worked out in part by cross-feeding experiments among mutant organisms that were defective for individual steps in the pathway. Results of cross-feeding experiments for three mutants defective in the tryptophan pathway—TrpB⁻, TrpD⁻, and TrpE⁻—are shown in **Figure Q2–6**. The mutants were streaked on a Petri dish and allowed to grow briefly in the presence of a very small amount of tryptophan, producing three pale streaks. As shown, heavier growth was observed at points where some streaks were close to other streaks. These spots of heavier growth indicate that one mutant can cross-feed (supply an intermediate) to the other one.

From the pattern of cross-feeding shown in Figure Q2–6, deduce the order of the steps controlled by the products of the TrpB, TrpD, and TrpE genes. Explain your reasoning.

CROSS-FEEDING RESULT
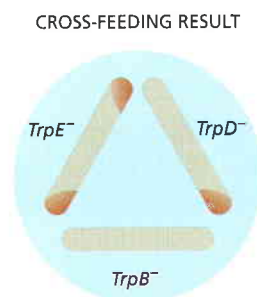
Figure Q2–6 Defining the pathway for tryptophan synthesis using cross-feeding experiments (Problem 2–27). Results of a cross-feeding experiment among mutants defective for steps in the tryptophan biosynthetic pathway. Dark areas on the Petri dish show regions of cell growth.

CARBON SKELETONS

Carbon has a unique role in the cell because of its ability to form strong covalent bonds with other carbon atoms. Thus carbon atoms can join to form chains.
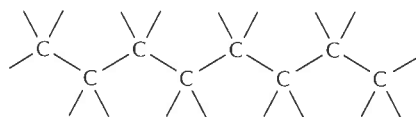
or branched trees

or rings

also written as

also written as
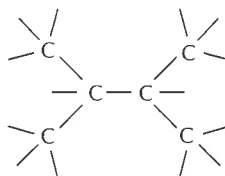
also written as
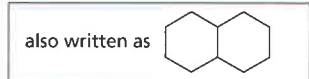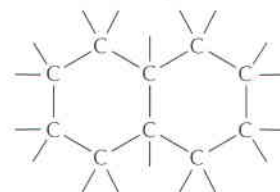
COVALENT BONDS

A covalent bond forms when two atoms come very close together and share one or more of their electrons. In a single bond one electron from each of the two atoms is shared; in a double bond a total of four electrons are shared.

Each atom forms a fixed number of covalent bonds in a defined spatial arrangement. For example, carbon forms four single bonds arranged tetrahedrally, whereas nitrogen forms three single bonds and oxygen forms two single bonds arranged as shown below.
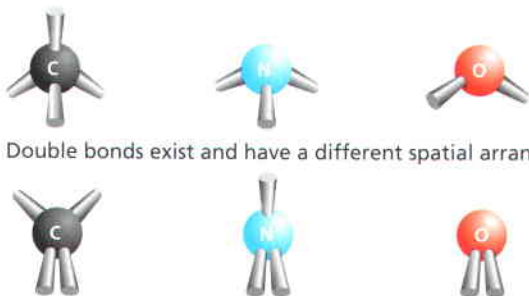
Double bonds exist and have a different spatial arrangement.
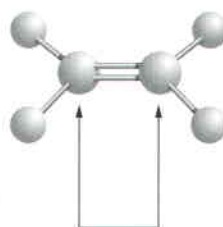
Atoms joined by two or more covalent bonds cannot rotate freely around the bond axis. This restriction is a major influence on the three-dimensional shape of many macromolecules.

HYDROCARBONS

Carbon and hydrogen combine together to make stable compounds (or chemical groups) called hydrocarbons. These are nonpolar, do not form hydrogen bonds, and are generally insoluble in water.
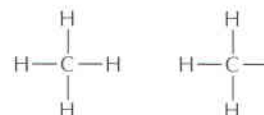
methane

methyl group

ALTERNATING DOUBLE BONDS

The carbon chain can include double bonds. If these are on alternate carbon atoms, the bonding electrons move within the molecule, stabilizing the structure by a phenomenon called resonance.

the truth is somewhere between these two structures
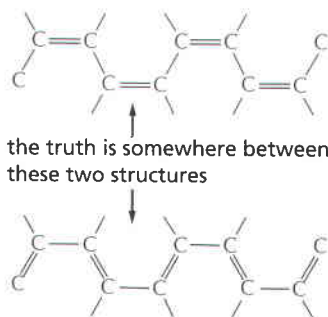
Alternating double bonds in a ring can generate a very stable structure.
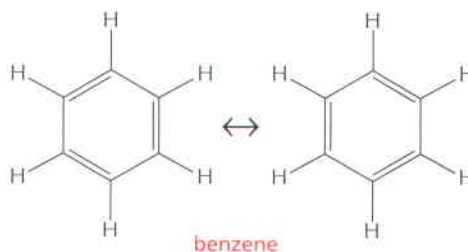
benzene

often written as
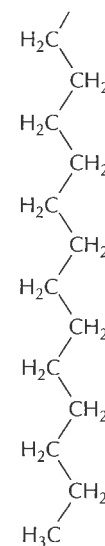
part of the hydrocarbon "tail" of a fatty acid molecule

C–O CHEMICAL GROUPS

Many biological compounds contain a carbon bonded to an oxygen. For example,

alcohol
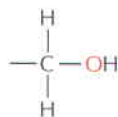
The –OH is called a hydroxyl group.

aldehyde
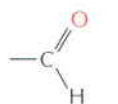
ketone

The C=O is called a carbonyl group.
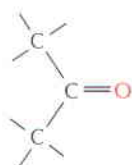
carboxylic acid
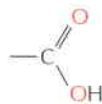
The –COOH is called a carboxyl group. In water this loses an H$^+$ ion to become –COO$^-$.

esters

Esters are formed by a condensation reaction between acid and an alcohol.

acid   alcohol   ester
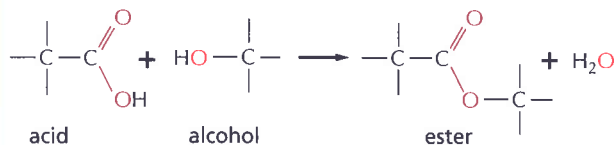
C–N CHEMICAL GROUPS

Amines and amides are two important examples of compounds containing a carbon linked to a nitrogen.

Amines in water combine with an H$^+$ ion to become positively charged.
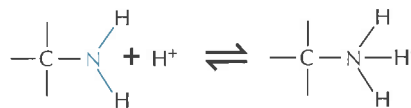
Amides are formed by combining an acid and an amine. Unlike amines, amides are uncharged in water. An example is the peptide bond that joins amino acids in a protein.

acid   amine   amide
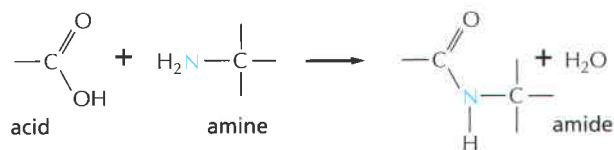
Nitrogen also occurs in several ring compounds, including important constituents of nucleic acids: purines and pyrimidines.

cytosine (a pyrimidine)
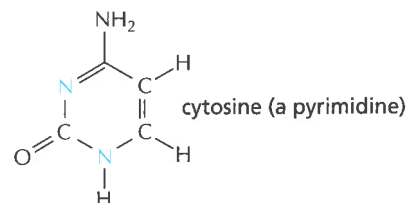
SULFHYDRYL GROUP

The $-\overset{|}{\underset{|}{C}}-SH$ is called a sulfhydryl group. In the amino acid cysteine the sulfhydryl group may exist in the reduced form, $-\overset{|}{\underset{|}{C}}-SH$ or more rarely in an oxidized, cross-bridging form, $-\overset{|}{\underset{|}{C}}-S-S-\overset{|}{\underset{|}{C}}-$

PHOSPHATES

Inorganic phosphate is a stable ion formed from phosphoric acid, H_3PO_4. It is often written as P_i.

Phosphate esters can form between a phosphate and a free hydroxyl group. Phosphate groups are often attached to proteins in this way.
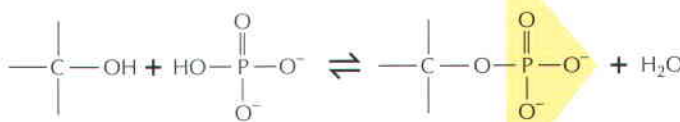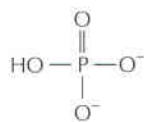
also written as
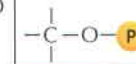
The combination of a phosphate and a carboxyl group, or two or more phosphate groups, gives an acid anhydride.
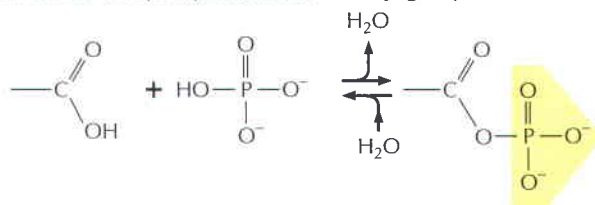
high-energy acyl phosphate bond (carboxylic–phosphoric acid anhydride) found in some metabolites

also written as
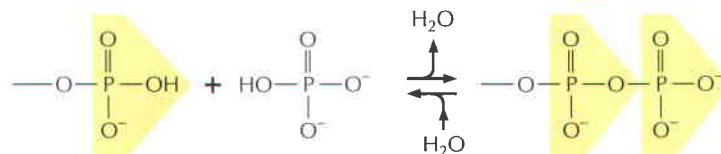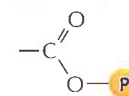
phosphoanhydride—a high-energy bond found in molecules such as ATP

also written as

WATER

Two atoms, connected by a covalent bond, may exert different attractions for the electrons of the bond. In such cases the bond is polar, with one end slightly negatively charged (δ^-) and the other slightly positively charged (δ^+).

electropositive region

electronegative region
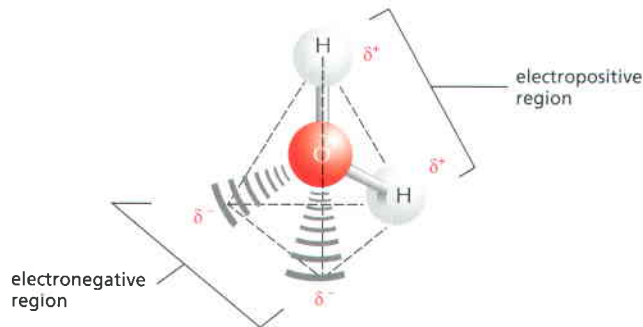
Although a water molecule has an overall neutral charge (having the same number of electrons and protons), the electrons are asymmetrically distributed, which makes the molecule polar. The oxygen nucleus draws electrons away from the hydrogen nuclei, leaving these nuclei with a small net positive charge. The excess of electron density on the oxygen atom creates weakly negative regions at the other two corners of an imaginary tetrahedron.

WATER STRUCTURE

Molecules of water join together transiently in a hydrogen-bonded lattice. Even at 37°C, 15% of the water molecules are joined to four others in a short-lived assembly known as a "flickering cluster."
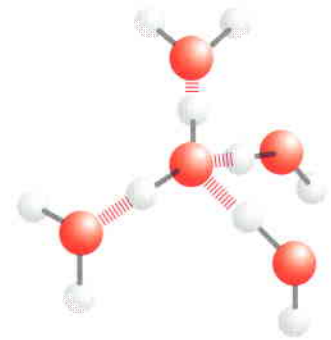
The cohesive nature of water is responsible for many of its unusual properties, such as high surface tension, specific heat, and heat of vaporization.

HYDROGEN BONDS

Because they are polarized, two adjacent H_2O molecules can form a linkage known as a hydrogen bond. Hydrogen bonds have only about 1/20 the strength of a covalent bond.

Hydrogen bonds are strongest when the three atoms lie in a straight line.

hydrogen bond

bond lengths

hydrogen bond 0.27 nm

0.10 nm covalent bond
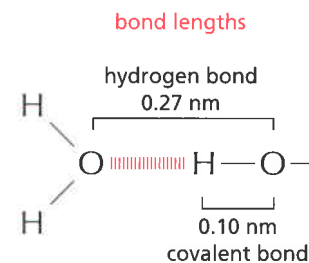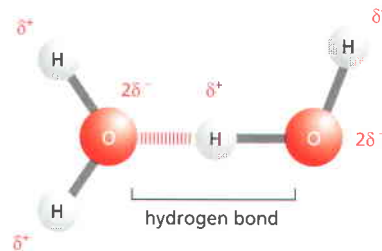
HYDROPHILIC MOLECULES

Substances that dissolve readily in water are termed hydrophilic. They are composed of ions or polar molecules that attract water molecules through electrical charge effects. Water molecules surround each ion or polar molecule on the surface of a solid substance and carry it into solution.
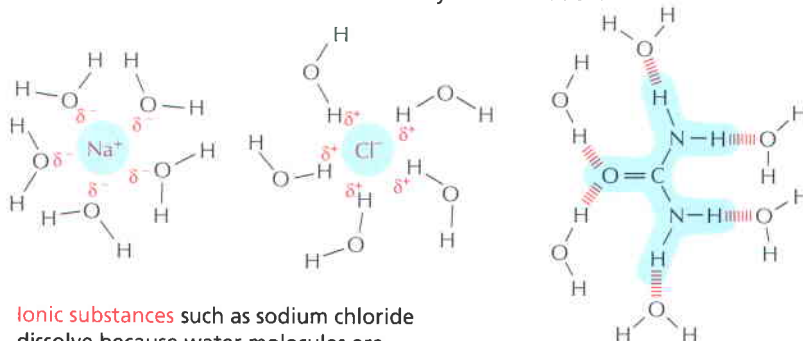
Ionic substances such as sodium chloride dissolve because water molecules are attracted to the positive (Na^+) or negative (Cl^-) charge of each ion.

Polar substances such as urea dissolve because their molecules form hydrogen bonds with the surrounding water molecules.

HYDROPHOBIC MOLECULES

Molecules that contain a preponderance of nonpolar bonds are usually insoluble in water and are termed hydrophobic. This is true, especially, of hydrocarbons, which contain many C–H bonds. Water molecules are not attracted to such molecules and so have little tendency to surround them and carry them into solution.
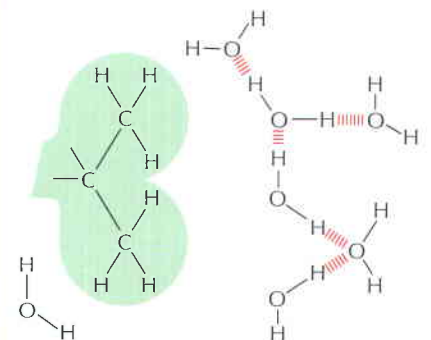
WATER AS A SOLVENT

Many substances, such as household sugar, dissolve in water. That is, their molecules separate from each other, each becoming surrounded by water molecules.

water molecule
sugar dissolves
sugar crystal
sugar molecule
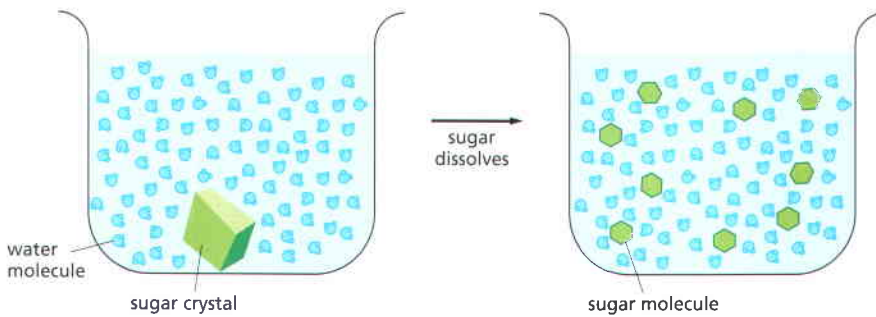
When a substance dissolves in a liquid, the mixture is termed a solution. The dissolved substance (in this case sugar) is the solute, and the liquid that does the dissolving (in this case water) is the solvent. Water is an excellent solvent for many substances because of its polar bonds.

ACIDS

Substances that release hydrogen ions into solution are called acids.

HCl $\longrightarrow$ H$^+$ + Cl$^-$
hydrochloric acid   hydrogen ion   chloride ion
(strong acid)
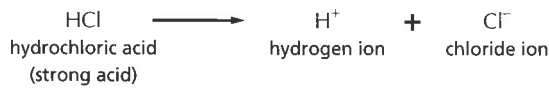
Many of the acids important in the cell are only partially dissociated, and they are therefore weak acids—for example, the carboxyl group (–COOH), which dissociates to give a hydrogen ion in solution

(weak acid)

Note that this is a reversible reaction.

HYDROGEN ION EXCHANGE

Positively charged hydrogen ions (H$^+$) can spontaneously move from one water molecule to another, thereby creating two ionic species.

hydronium ion   hydroxyl ion
(water acting as   (water acting as
a weak base)   a weak acid)

often written as: $H_2O \rightleftharpoons H^+ + OH^-$
hydrogen   hydroxyl
ion   ion
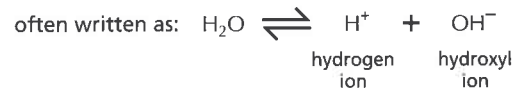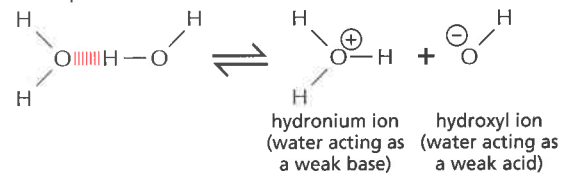
Since the process is rapidly reversible, hydrogen ions are continually shuttling between water molecules. Pure water contains a steady-state concentration of hydrogen ions and hydroxyl ions (both 10^{-7} M).

pH

The acidity of a solution is defined by the concentration of H$^+$ ions it possesses. For convenience we use the pH scale, where

$pH = -\log_{10}[H^+]$

For pure water

$[H^+] = 10^{-7}$ moles/liter

H$^+$ conc. moles/liter	pH
10^{-1}	1
10^{-2}	2
10^{-3}	3
10^{-4}	4
10^{-5}	5
10^{-6}	6
10^{-7}	7
10^{-8}	8
10^{-9}	9
10^{-10}	10
10^{-11}	11
10^{-12}	12
10^{-13}	13
10^{-14}	14

ACIDIC
ALKALINE
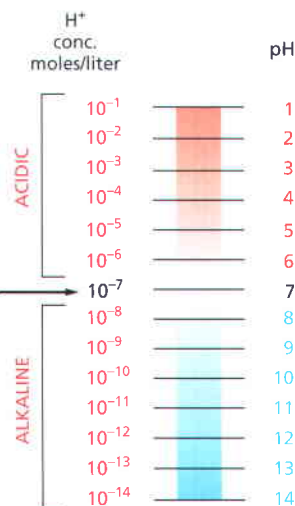
BASES

Substances that reduce the number of hydrogen ions in solution are called bases. Some bases, such as ammonia, combine directly with hydrogen ions.

NH$_3$ + H$^+$ $\longrightarrow$ NH$_4^+$
ammonia   hydrogen ion   ammonium ion

Other bases, such as sodium hydroxide, reduce the number of H$^+$ ions indirectly, by making OH$^-$ ions that then combine directly with H$^+$ ions to make H$_2$O.

NaOH $\longrightarrow$ Na$^+$ + OH$^-$
sodium hydroxide   sodium   hydroxyl
(strong base)   ion   ion

Many bases found in cells are partially dissociated and are termed weak bases. This is true of compounds that contain an amino group (–NH$_2$), which has a weak tendency to reversibly accept an H$^+$ ion from water, increasing the quantity of free OH$^-$ ions.

–NH$_2$ + H$^+$ $\rightleftharpoons$ –NH$_3^+$

WEAK CHEMICAL BONDS

Organic molecules can interact with other molecules through three types of short-range attractive forces known as *noncovalent bonds:* van der Waals attractions, electrostatic attractions, and hydrogen bonds. The repulsion of hydrophobic groups from water is also important for ordering biological macromolecules.
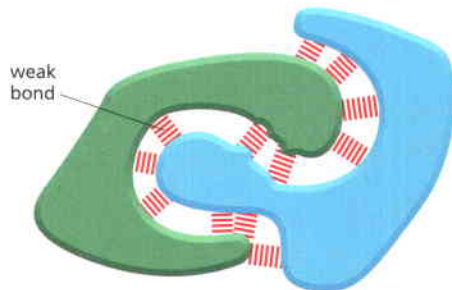
Weak chemical bonds have less than 1/20 the strength of a strong covalent bond. They are strong enough to provide tight binding only when many of them are formed simultaneously.

HYDROGEN BONDS

As already described for water (see Panel 2–2), hydrogen bonds form when a hydrogen atom is "sandwiched" between two electron-attracting atoms (usually oxygen or nitrogen).

Hydrogen bonds are strongest when the three atoms are in a straight line:

Examples in macromolecules:

Amino acids in polypeptide chains hydrogen-bonded together.
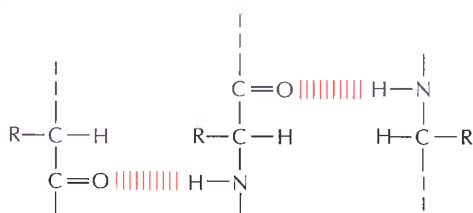
Two bases, G and C, hydrogen-bonded in DNA or RNA.
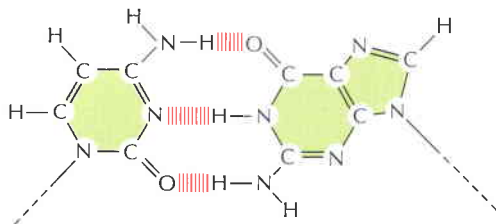
VAN DER WAALS ATTRACTIONS

If two atoms are too close together they repel each other very strongly. For this reason, an atom can often be treated as a sphere with a fixed radius. The characteristic "size" for each atom is specified by a unique van der Waals radius. The contact distance between any two noncovalently bonded atoms is the sum of their van der Waals radii.

| 0.12 nm radius | 0.2 nm radius | 0.15 nm radius | 0.14 nm radius |
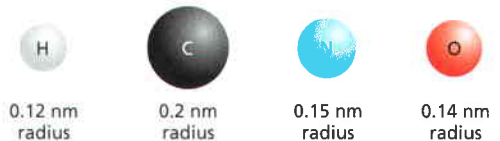
At very short distances any two atoms show a weak bonding interaction due to their fluctuating electrical charges. The two atoms will be attracted to each other in this way until the distance between their nuclei is approximately equal to the sum of their van der Waals radii. Although they are individually very weak, van der Waals attractions can become important when two macromolecular surfaces fit very close together, because many atoms are involved.

Note that when two atoms form a covalent bond, the centers of the two atoms (the two atomic nuclei) are much closer together than the sum of the two van der Waals radii. Thus,

| 0.4 nm two non-bonded carbon atoms | 0.15 nm single-bonded carbons | 0.13 nm double-bonded carbons |
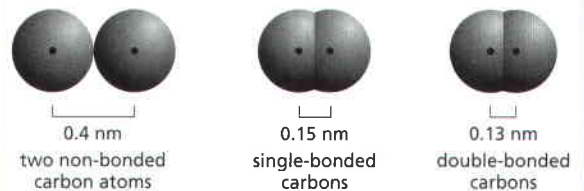
HYDROGEN BONDS IN WATER

Any molecules that can form hydrogen bonds to each other can alternatively form hydrogen bonds to water molecules. Because of this competition with water molecules, the hydrogen bonds formed between two molecules dissolved in water are relatively weak.
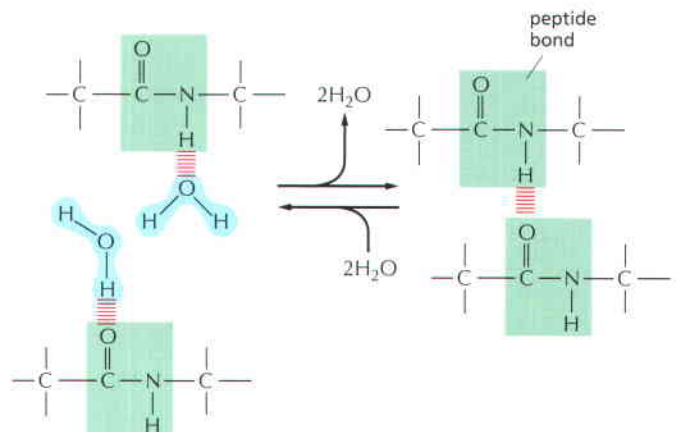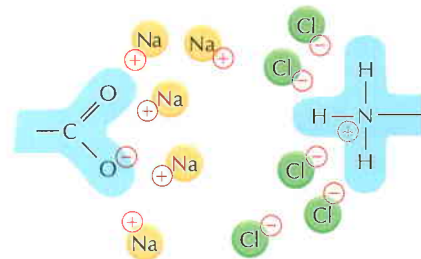
HYDROPHOBIC FORCES
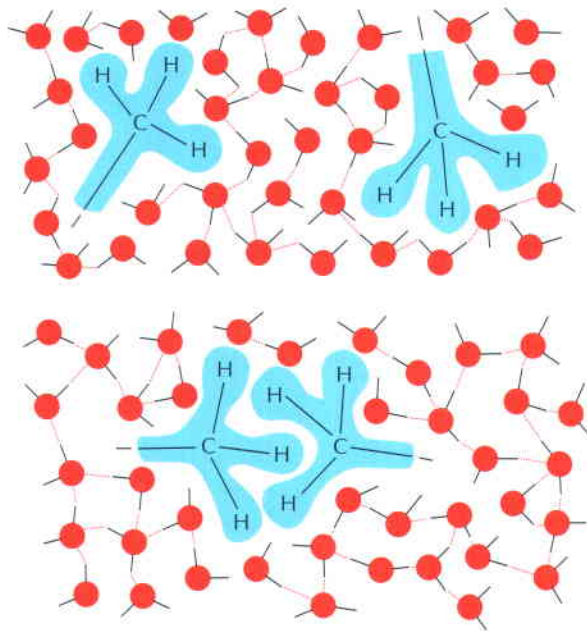
Water forces hydrophobic groups together, because doing so minimizes their disruptive effects on the hydrogen-bonded water network. Hydrophobic groups held together in this way are sometimes said to be held together by "hydrophobic bonds," even though the apparent attraction is actually caused by a repulsion from the water.
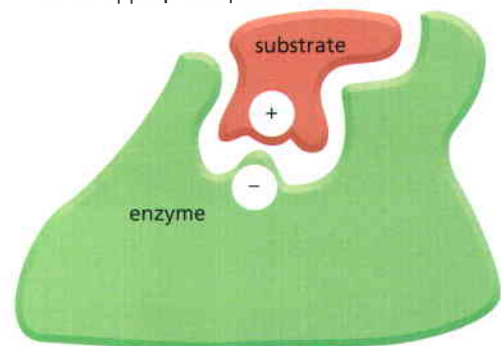
ELECTROSTATIC ATTRACTIONS IN AQUEOUS SOLUTIONS

Charged groups are shielded by their interactions with water molecules. Electrostatic attractions are therefore quite weak in water.
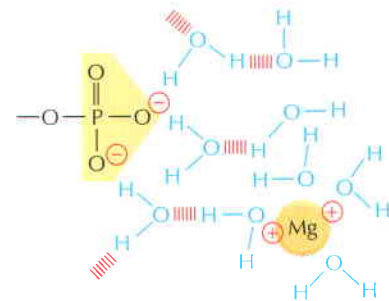
Similarly, ions in solution can cluster around charged groups and further weaken these attractions.

ELECTROSTATIC ATTRACTIONS

Attractive forces occur both between fully charged groups (ionic bond) and between the partially charged groups on polar molecules.

The force of attraction between the two charges, δ^+ and δ^-, falls off rapidly as the distance between the charges increases.

In the absence of water, electrostatic forces are very strong. They are responsible for the strength of such minerals as marble and agate, and for crystal formation in common table salt, NaCl.

Despite being weakened by water and salt, electrostatic attractions are very important in biological systems. For example, an enzyme that binds a positively charged substrate will often have a negatively charged amino acid side chain at the appropriate place.

Cl^-

Na^+
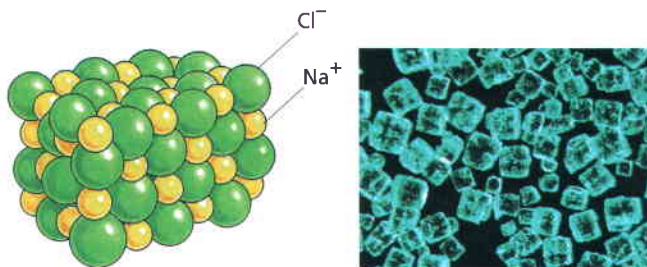
a crystal of salt, NaCl

1 mm

substrate

enzyme

MONOSACCHARIDES

Monosaccharides usually have the general formula $(CH_2O)_n$, where n can be 3, 4, 5, 6, 7, or 8, and have two or more hydroxyl groups. They either contain an aldehyde group ($-C\!\!\begin{smallmatrix}O\\H\end{smallmatrix}$) and are called aldoses or a ketone group ($>\!C\!=\!O$) and are called ketoses.

	3-carbon (TRIOSES)	5-carbon (PENTOSES)	6-carbon (HEXOSES)
ALDOSES	glyceraldehyde	ribose	glucose
KETOSES	dihydroxyacetone	ribulose	fructose
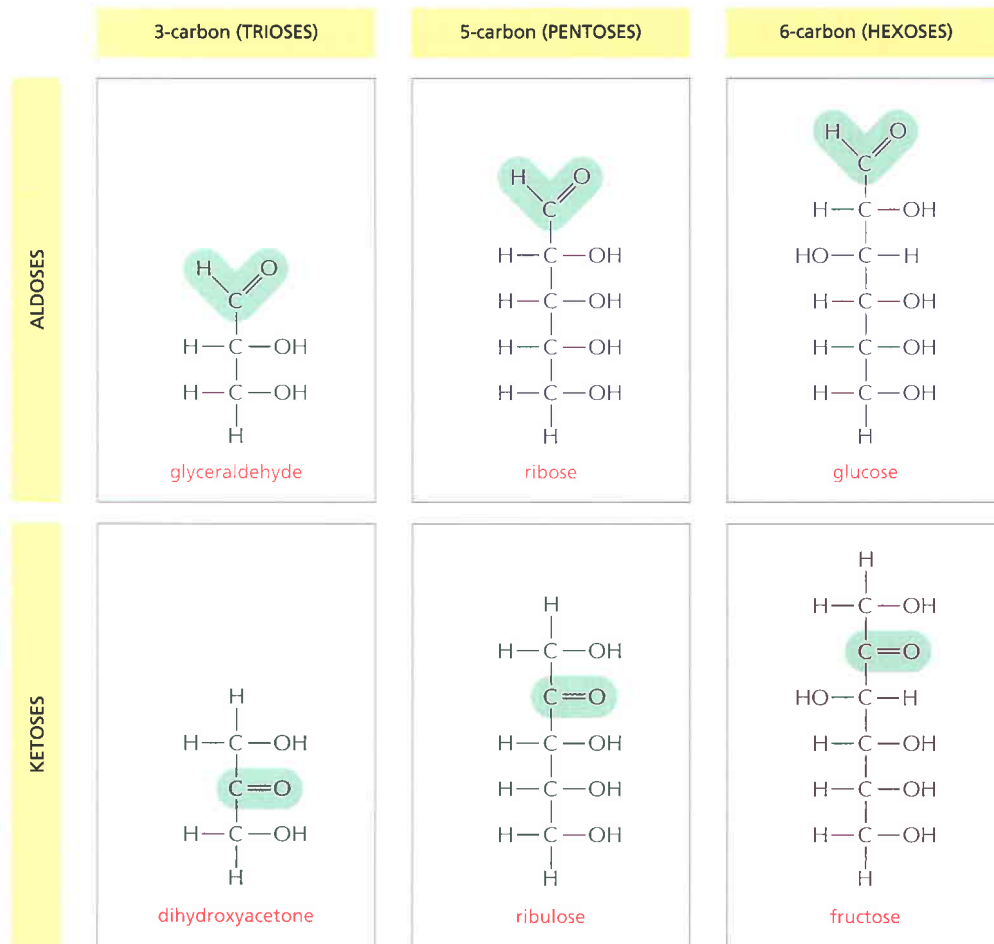
RING FORMATION

In aqueous solution, the aldehyde or ketone group of a sugar molecule tends to react with a hydroxyl group of the same molecule, thereby closing the molecule into a ring.

glucose

ribose

Note that each carbon atom has a number.
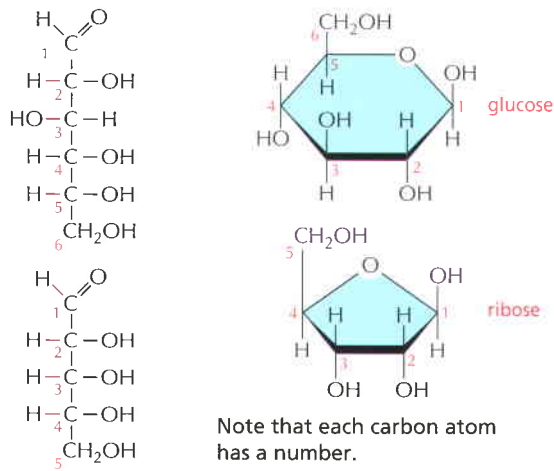
ISOMERS

Many monosaccharides differ only in the spatial arrangement of atoms—that is, they are isomers. For example, glucose, galactose, and mannose have the same formula ($C_6H_{12}O_6$) but differ in the arrangement of groups around one or two carbon atoms.

glucose

galactose

mannose
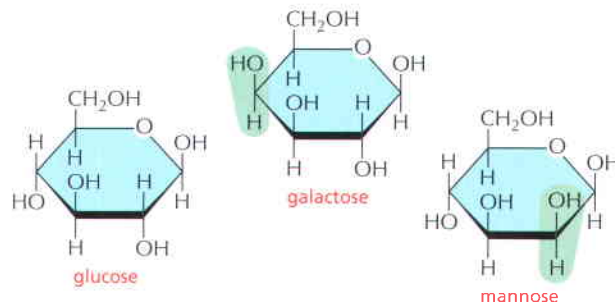
These small differences make only minor changes in the chemical properties of the sugars. But they are recognized by enzymes and other proteins and therefore can have important biological effects.

α AND β LINKS

The hydroxyl group on the carbon that carries the aldehyde or ketone can rapidly change from one position to the other. These two positions are called α and β.

β hydroxyl   α hydroxyl
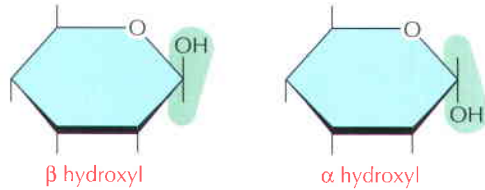
As soon as one sugar is linked to another, the α or β form is frozen.

SUGAR DERIVATIVES

The hydroxyl groups of a simple monosaccharide can be replaced by other groups. For example,

glucosamine

N-acetylglucosamine

glucuronic acid
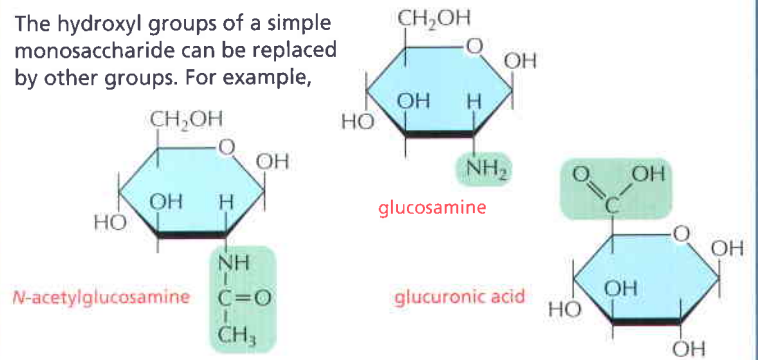
DISACCHARIDES

The carbon that carries the aldehyde or the ketone can react with any hydroxyl group on a second sugar molecule to form a disaccharide. The linkage is called a glycosidic bond.

Three common disaccharides are

maltose (glucose + glucose)
lactose (galactose + glucose)
sucrose (glucose + fructose)

The reaction forming sucrose is shown here.

α glucose   β fructose

H_2O

sucrose
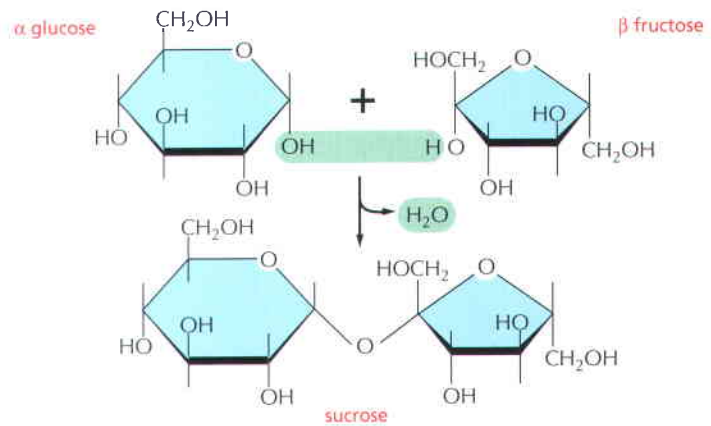
OLIGOSACCHARIDES AND POLYSACCHARIDES

Large linear and branched molecules can be made from simple repeating sugar subunits. Short chains are called oligosaccharides, while long chains are called polysaccharides. Glycogen, for example, is a polysaccharide made entirely of glucose units joined together.

branch points   glycogen
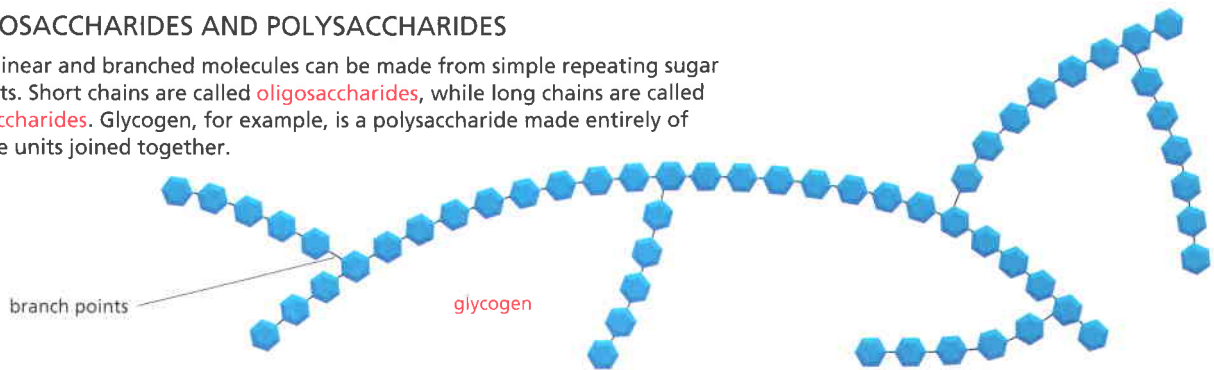
COMPLEX OLIGOSACCHARIDES

In many cases a sugar sequence is nonrepetitive. Many different molecules are possible. Such complex oligosaccharides are usually linked to proteins or to lipids, as is this oligosaccharide, which is part of a cell-surface molecule that defines a particular blood group.
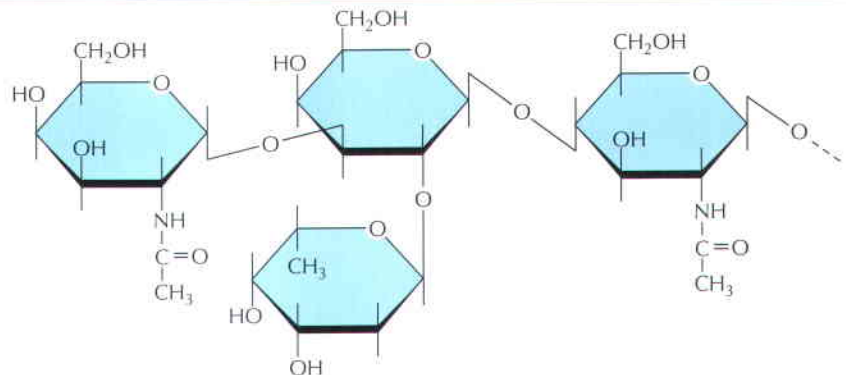
COMMON FATTY ACIDS

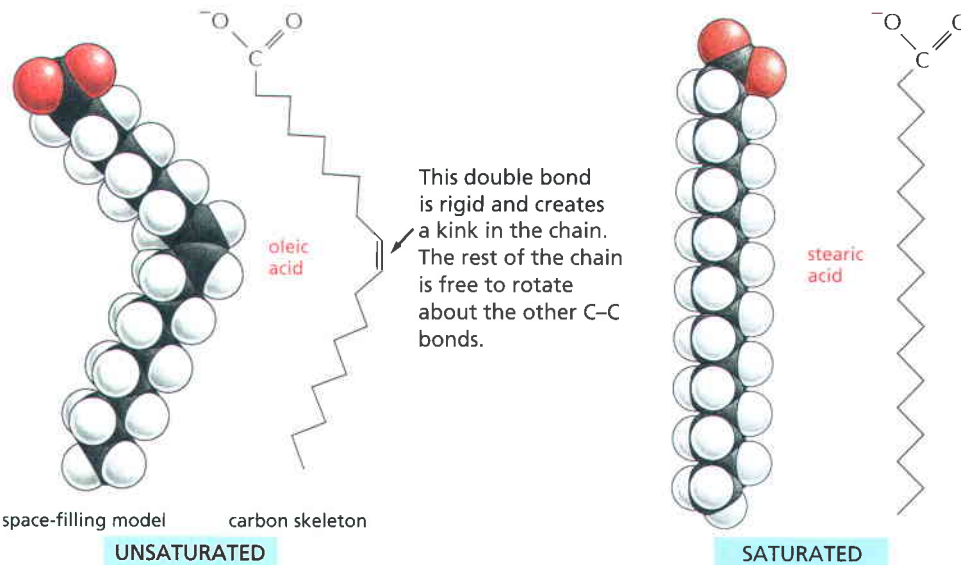These are carboxylic acids with long hydrocarbon tails.

COOH   COOH   COOH
| | |
CH_2 CH_2 CH_2
| | |
CH_2 CH_2 CH_2
| | |
CH_2 CH_2 CH_2
| | |
CH_2 CH_2 CH_2
| | |
CH_2 CH_2 CH_2
| | |
CH_2 CH_2 CH_2
| | |
CH_2 CH_2 CH_2
| | |
CH_2 CH_2 CH
| | ||
CH_2 CH_2 CH
| | |
CH_2 CH_2 CH_2
| | |
CH_2 CH_2 CH_2
| | |
CH_2 CH_2 CH_2
| | |
CH_2 CH_2 CH_2
| | |
CH_2 CH_3 CH_2
| palmitic |
CH_2 acid CH_2
| (C_{16}) |
CH_3 CH_3

stearic   oleic
acid (C_{18}) acid (C_{18})
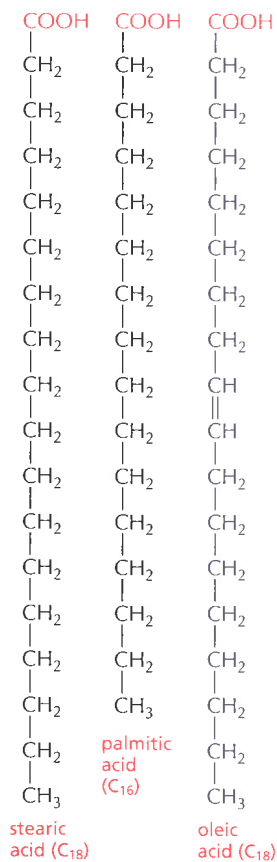
TRIACYLGLYCEROLS

Fatty acids are stored as an energy reserve (fats and oils) through an ester linkage to glycerol to form triacylglycerols, also known as triglycerides.

glycerol
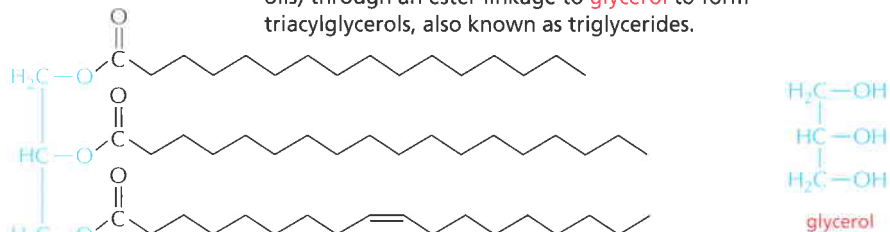
Hundreds of different kinds of fatty acids exist. Some have one or more double bonds in their hydrocarbon tail and are said to be unsaturated. Fatty acids with no double bonds are saturated.

This double bond is rigid and creates a kink in the chain. The rest of the chain is free to rotate about the other C–C bonds.

oleic acid

stearic acid

space-filling model   carbon skeleton

UNSATURATED   **SATURATED**

CARBOXYL GROUP

If free, the carboxyl group of a fatty acid will be ionized.
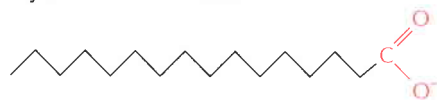
But more usually it is linked to other groups to form either esters
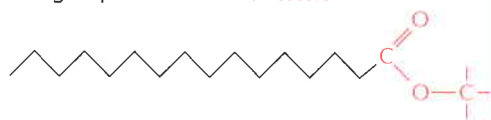
or amides.
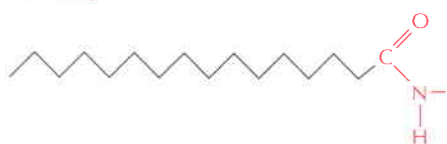
PHOSPHOLIPIDS

Phospholipids are the major constituents of cell membranes.

hydrophilic group

choline

$O=P-O^-$

$CH_2 - CH - CH_2$

hydrophobic fatty acid tails

general structure of a phospholipid

space-filling model of the phospholipid phosphatidylcholine

In phospholipids two of the –OH groups in glycerol are linked to fatty acids, while the third –OH group is linked to phosphoric acid. The phosphate is further linked to one of a variety of small polar groups (alcohols).

LIPID AGGREGATES

Fatty acids have a hydrophilic head and a hydrophobic tail.

micelle

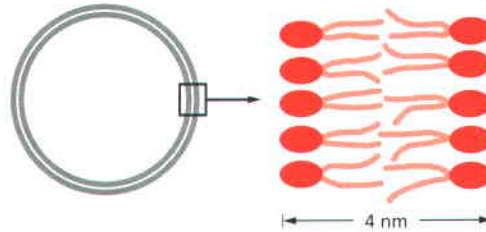In water they can form a surface film or form small micelles.
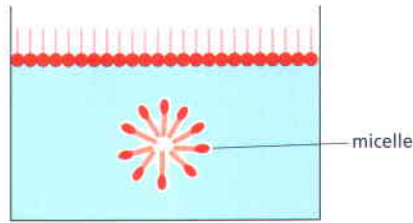
Their derivatives can form larger aggregates held together by hydrophobic forces:

Triglycerides can form large spherical fat droplets in the cell cytoplasm.

Phospholipids and glycolipids form self-sealing lipid bilayers that are the basis for all cell membranes.

200 nm or more

4 nm

OTHER LIPIDS

Lipids are defined as the water-insoluble molecules in cells that are soluble in organic solvents. Two other common types of lipids are steroids and polyisoprenoids. Both are made from isoprene units.

$$CH_3$$
$$C-CH=CH_2$$
$$CH_2$$
isoprene
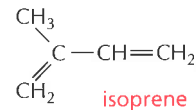
STEROIDS

Steroids have a common multiple-ring structure.

OH

HO
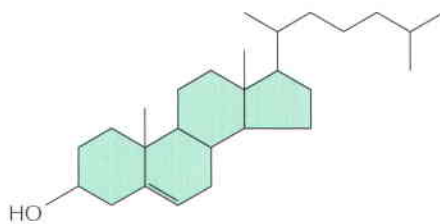
cholesterol—found in many membranes

O
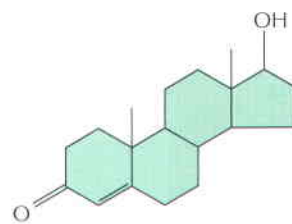
testosterone—male steroid hormone

GLYCOLIPIDS

Like phospholipids, these compounds are composed of a hydrophobic region, containing two long hydrocarbon tails, and a polar region, which, however, contains one or more sugars and no phosphate.

galactose

sugar

$$OH$$
$$H \quad C \quad O$$
$$C=C \quad C \quad CH_2$$
$$H \quad H$$

$$C-NH$$
$$\parallel$$
$$O$$
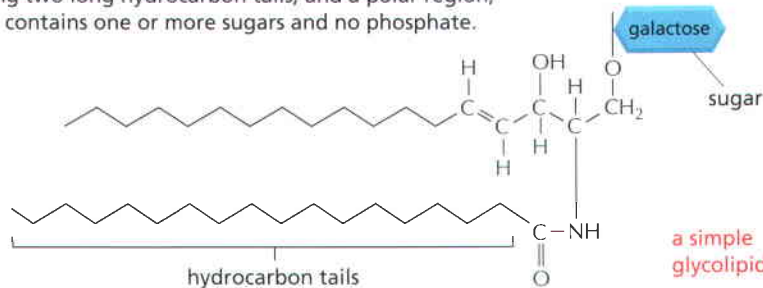
a simple glycolipid

hydrocarbon tails

POLYISOPRENOIDS

long-chain polymers of isoprene

$$O^-$$
$$O=P-O^-$$
$$O$$
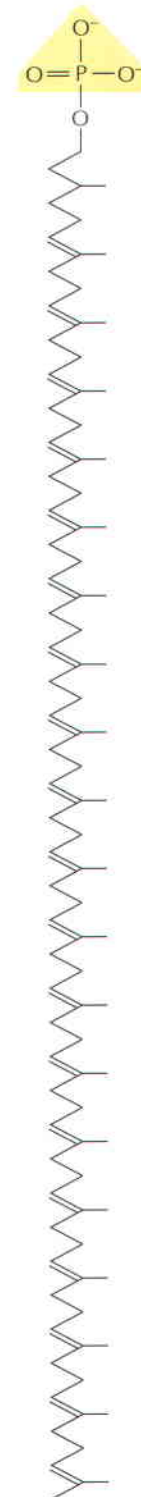
dolichol phosphate—used to carry activated sugars in the membrane-associated synthesis of glycoproteins and some polysaccharides
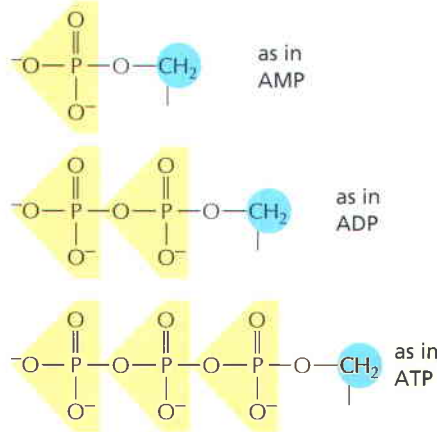
NUCLEOTIDES

A nucleotide consists of a nitrogen-containing base, a five-carbon sugar, and one or more phosphate groups.

BASE
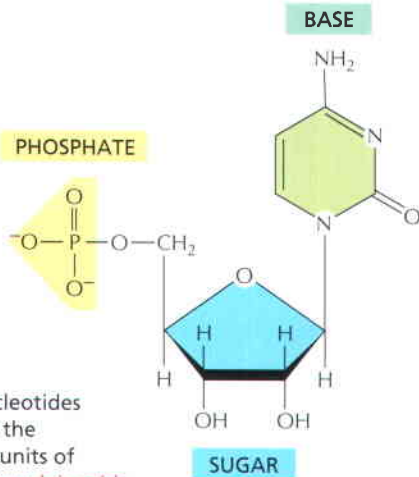
PHOSPHATE

SUGAR

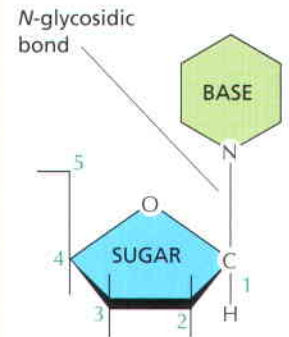Nucleotides are the subunits of the nucleic acids.

PHOSPHATES

The phosphates are normally joined to the C5 hydroxyl of the ribose or deoxyribose sugar (designated 5'). Mono-, di-, and triphosphates are common.
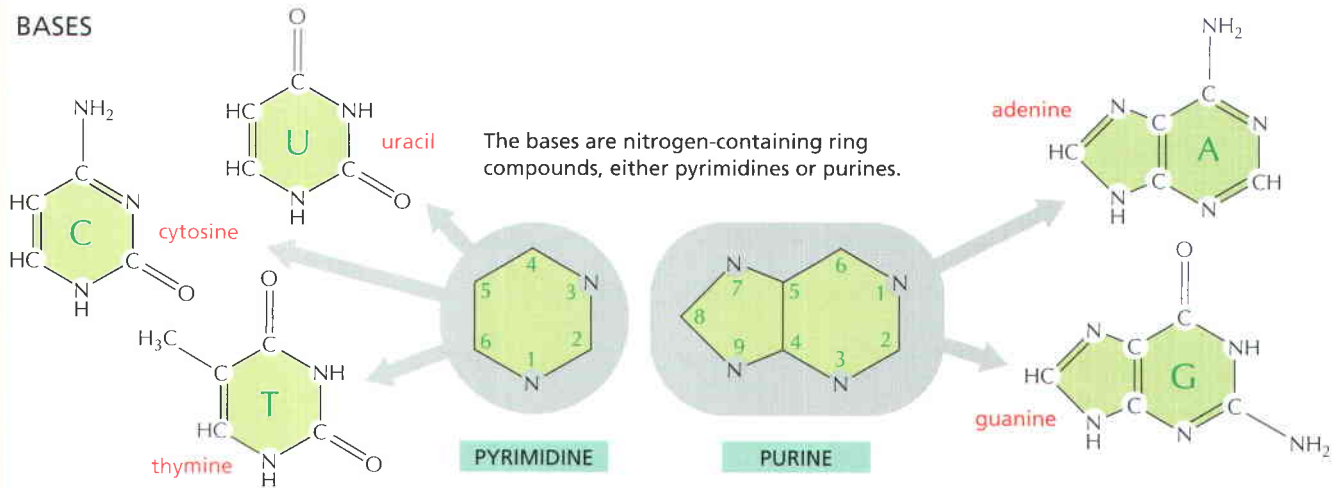
as in AMP

as in ADP

as in ATP

The phosphate makes a nucleotide negatively charged.

BASIC SUGAR LINKAGE

N-glycosidic bond

BASE

SUGAR

The base is linked to the same carbon (C1) used in sugar–sugar bonds.

BASES

The bases are nitrogen-containing ring compounds, either pyrimidines or purines.

cytosine

uracil

thymine

adenine

guanine

PYRIMIDINE

PURINE

SUGARS

PENTOSE
a five-carbon sugar
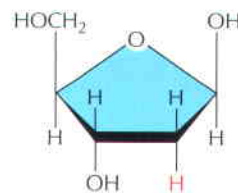
two kinds are used
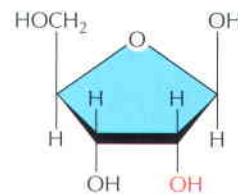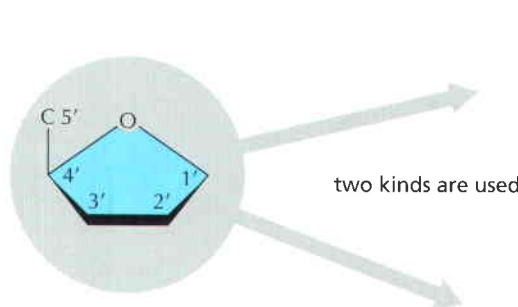
β-D-ribose
used in ribonucleic acid

β-D-2-deoxyribose
used in deoxyribonucleic acid

Each numbered carbon on the sugar of a nucleotide is followed by a prime mark; therefore, one speaks of the "5-prime carbon," etc.

NOMENCLATURE

A nucleoside or nucleotide is named according to its nitrogenous base.

Single letter abbreviations are used variously as shorthand for (1) the base alone, (2) the nucleoside, or (3) the whole nucleotide—the context will usually make clear which of the three entities is meant. When the context is not sufficient, we will add the terms "base", "nucleoside", "nucleotide", or—as in the examples below—use the full 3-letter nucleotide code.

BASE	NUCLEOSIDE	ABBR.
adenine	adenosine	A
guanine	guanosine	G
cytosine	cytidine	C
uracil	uridine	U
thymine	thymidine	T

AMP = adenosine monophosphate
dAMP = deoxyadenosine monophosphate
UDP = uridine diphosphate
ATP = adenosine triphosphate

BASE + SUGAR = NUCLEOSIDE

BASE + SUGAR + PHOSPHATE = NUCLEOTIDE
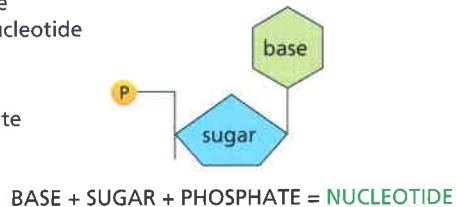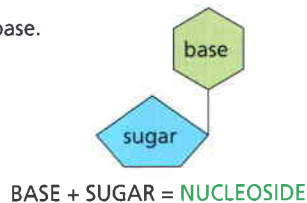
NUCLEIC ACIDS

Nucleotides are joined together by a phosphodiester linkage between 5′ and 3′ carbon atoms to form nucleic acids. The linear sequence of nucleotides in a nucleic acid chain is commonly abbreviated by a one-letter code, A—G—C—T—T—A—C—A, with the 5′ end of the chain at the left.
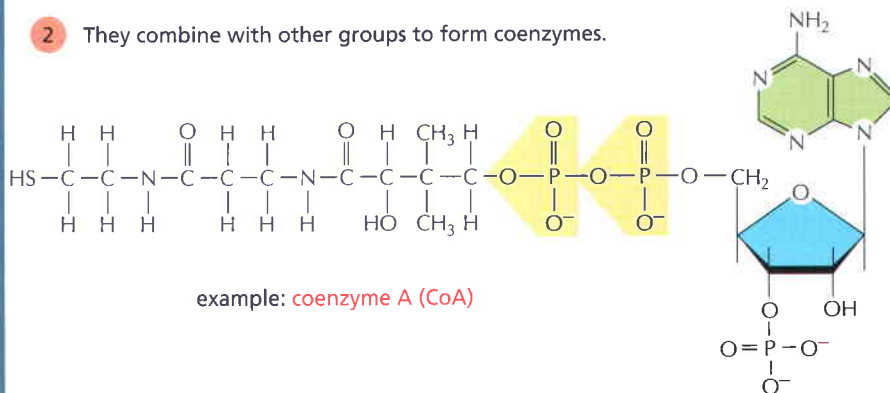
5′ end of chain

phosphodiester linkage

example: DNA

3′ end of chain
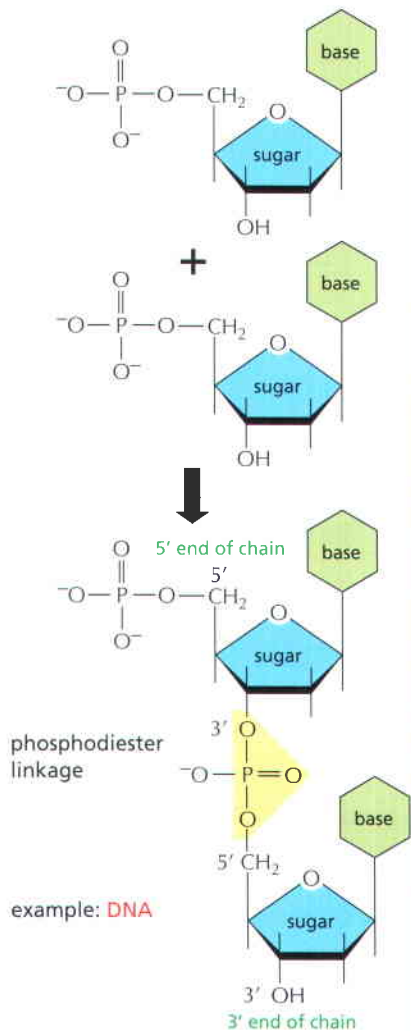
NUCLEOTIDES HAVE MANY OTHER FUNCTIONS

1 They carry chemical energy in their easily hydrolyzed phosphoanhydride bonds.

phosphoanhydride bonds

example: ATP (or ATP)
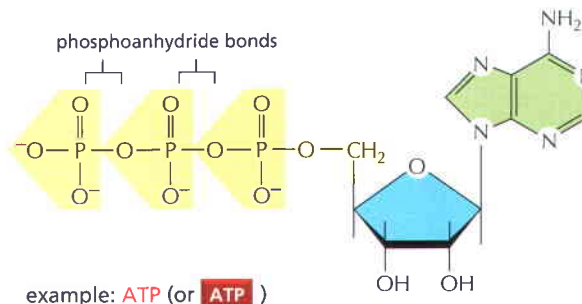
2 They combine with other groups to form coenzymes.

example: coenzyme A (CoA)

3 They are used as specific signaling molecules in the cell.

example: cyclic AMP (cAMP)
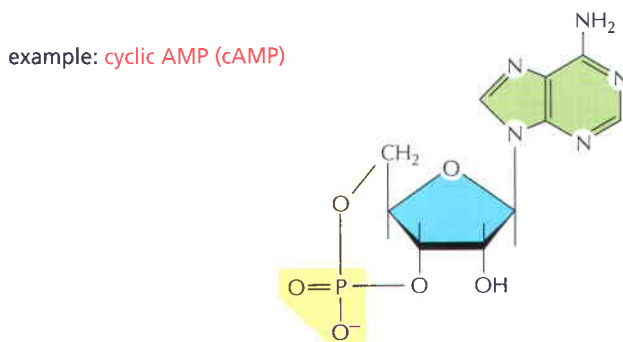
THE IMPORTANCE OF FREE ENERGY FOR CELLS

Life is possible because of the complex network of interacting chemical reactions occurring in every cell. In viewing the metabolic pathways that comprise this network, one might suspect that the cell has had the ability to evolve an enzyme to carry out any reaction that it needs. But this is not so. Although enzymes are powerful catalysts, they can speed up only those reactions that are thermodynamically possible; other reactions proceed in cells only because they are *coupled* to very favorable reactions that drive them. The question of whether a reaction can occur spontaneously, or instead needs to be coupled to another reaction, is central to cell biology. The answer is obtained by reference to a quantity called the *free energy*: the total change in free energy during a set of reactions determines whether or not the entire reaction sequence can occur. In this panel we shall explain some of the fundamental ideas—derived from a special branch of chemistry and physics called *thermodynamics*—that are required for understanding what free energy is and why it is so important to cells.

ENERGY RELEASED BY CHANGES IN CHEMICAL BONDING IS CONVERTED INTO HEAT
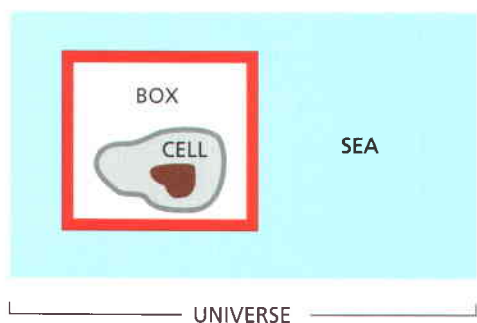
An *enclosed system* is defined as a collection of molecules that does not exchange matter with the rest of the universe (for example, the "cell in a box" shown above). Any such system will contain molecules with a total energy E. This energy will be distributed in a variety of ways: some as the translational energy of the molecules, some as their vibrational and rotational energies, but most as the bonding energies between the individual atoms that make up the molecules. Suppose that a reaction occurs in the system. The first law of thermodynamics places a constraint on what types of reactions are possible: it states that "in any process, the total energy of the universe remains constant." For example, suppose that reaction $A \rightarrow B$ occurs somewhere in the box and releases a great deal of chemical bond energy. This energy will initially increase the intensity of molecular motions (translational, vibrational, and rotational) in the system, which is equivalent to raising its temperature. However, these increased motions will soon be transferred out of the system by a series of molecular collisions that heat up first the walls of the box and then the outside world (represented by the sea in our example). In the end, the system returns to its initial temperature, by which time all the chemical bond energy released in the box has been converted into heat energy and transferred out of the box to the surroundings. According to the first law, the change in the energy in the box (ΔE_{box}, which we shall denote as ΔE) must be equal and opposite to the amount of heat energy transferred, which we shall designate as h: that is, $\Delta E = -h$. Thus, the energy in the box (E) decreases when heat leaves the system.

E also can change during a reaction as a result of work being done on the outside world. For example, suppose that there is a small increase in the volume (ΔV) of the box during a reaction. Since the walls of the box must push against the constant pressure (P) in the surroundings in order to expand, this does work on the outside world and requires energy. The energy used is $P(\Delta V)$, which according to the first law must decrease the energy in the box (E) by the same amount. In most reactions chemical bond energy is converted into both work and heat. *Enthalpy* (H) is a composite function that includes both of these ($H = E + PV$). To be rigorous, it is the change in enthalpy (ΔH) in an enclosed system, and not the change in energy, that is equal to the heat transferred to the outside world during a reaction. Reactions in which H decreases release heat to the surroundings and are said to be "exothermic," while reactions in which H increases absorb heat from the surroundings and are said to be "endothermic." Thus, $-h = \Delta H$. However, the volume change is negligible in most biological reactions, so to a good approximation

$$-h = \Delta H \cong \Delta E$$

THE SECOND LAW OF THERMODYNAMICS

Consider a container in which 1000 coins are all lying heads up. If the container is shaken vigorously, subjecting the coins to the types of random motions that all molecules experience due to their frequent collisions with other molecules, one will end up with about half the coins oriented heads down. The reason for this reorientation is that there is only a single way in which the original orderly state of the coins can be reinstated (every coin must lie heads up), whereas there are many different ways (about 10^{298}) to achieve a disorderly state in which there is an equal mixture of heads and tails; in fact, there are more ways to achieve a 50-50 state than to achieve any other state. Each state has a probability of occurrence that is proportional to the number of ways it can be realized. The second law of thermodynamics states that "systems will change spontaneously from states of lower probability to states of higher probability." Since states of lower probability are more "ordered" than states of high probability, the second law can be restated: "the universe constantly changes so as to become more disordered."

THE ENTROPY, S

The second law (but not the first law) allows one to predict the *direction* of a particular reaction. But to make it useful for this purpose, one needs a convenient measure of the probability or, equivalently, the degree of disorder of a state. The entropy (S) is such a measure. It is a logarithmic function of the probability such that the *change in entropy* (ΔS) that occurs when the reaction A → B converts one mole of A into one mole of B is

$$\Delta S = R \ln p_B / p_A$$

where p_A and p_B are the probabilities of the two states A and B, R is the gas constant (2 cal deg^{-1} mole^{-1}), and ΔS is measured in entropy units (eu). In our initial example of 1000 coins, the relative probability of all heads (state A) versus half heads and half tails (state B) is equal to the ratio of the number of different ways that the two results can be obtained. One can calculate that $p_A = 1$ and $p_B = 1000!(500! \times 500!) = 10^{299}$. Therefore, the entropy change for the reorientation of the coins when their container is vigorously shaken and an equal mixture of heads and tails is obtained is $R \ln (10^{298})$, or about 1370 eu per mole of such containers (6×10^{23} containers). We see that, because ΔS defined above is positive for the transition from state A to state B ($p_B/p_A > 1$), reactions with a large *increase* in S (that is, for which $\Delta S > 0$) are favored and will occur spontaneously.

As discussed in Chapter 2, heat energy causes the random commotion of molecules. Because the transfer of heat from an enclosed system to its surroundings increases the number of different arrangements that the molecules in the outside world can have, it increases their entropy. It can be shown that the release of a fixed quantity of heat energy has a greater disordering effect at low temperature than at high temperature, and that the value of ΔS for the surroundings, as defined above (ΔS_{sea}), is precisely equal to h, the amount of heat transferred to the surroundings from the system, divided by the absolute temperature (T):

$$\Delta S_{sea} = h / T$$

THE GIBBS FREE ENERGY, G

When dealing with an enclosed biological system, one would like to have a simple way of predicting whether a given reaction will or will not occur spontaneously in the system. We have seen that the crucial question is whether the entropy change for the universe is positive or negative when that reaction occurs. In our idealized system, the cell in a box, there are two separate components to the entropy change of the universe—the entropy change for the system enclosed in the box and the entropy change for the surrounding "sea"—and both must be added together before any prediction can be made. For example, it is possible for a reaction to absorb heat and thereby decrease the entropy of the sea ($\Delta S_{sea} < 0$) and at the same time to cause such a large degree of disordering inside the box ($\Delta S_{box} > 0$) that the total $\Delta S_{universe} = \Delta S_{sea} + \Delta S_{box}$ is greater than 0. In this case the reaction will occur spontaneously, even though the sea gives up heat to the box during the reaction. An example of such a reaction is the dissolving of sodium chloride in a beaker containing water (the "box"), which is a spontaneous process even though the temperature of the water drops as the salt goes into solution.

Chemists have found it useful to define a number of new "composite functions" that describe *combinations* of physical properties of a system. The properties that can be combined include the temperature (T), pressure (P), volume (V), energy (E), and entropy (S). The enthalpy (H) is one such composite function. But by far the most useful composite function for biologists is the *Gibbs free energy, G*. It serves as an accounting device that allows one to deduce the entropy change of the universe resulting from a chemical reaction in the box, while avoiding any separate consideration of the entropy change in the sea. The definition of G is

$$G = H - TS$$

where, for a box of volume V, H is the enthalpy described above (E + PV), T is the absolute temperature, and S is the entropy. Each of these quantities applies to the inside of the box only. The change in free energy during a reaction in the box (the G of the products minus the G of the starting materials) is denoted as ΔG and, as we shall now demonstrate, it is a direct measure of the amount of disorder that is created in the universe when the reaction occurs.

At constant temperature the change in free energy (ΔG) during a reaction equals $\Delta H - T\Delta S$. Remembering that $\Delta H = -h$, the heat absorbed from the sea, we have

$$-\Delta G = -\Delta H + T\Delta S$$
$$-\Delta G = h + T\Delta S, \text{ so } -\Delta G/T = h/T + \Delta S$$

But h/T is equal to the entropy change of the sea (ΔS_{sea}), and the ΔS in the above equation is ΔS_{box}. Therefore

$$-\Delta G/T = \Delta S_{sea} + \Delta S_{box} = \Delta S_{universe}$$

We conclude that the free-energy change is a direct measure of the entropy change of the universe. A reaction will proceed in the direction that causes the change in the free energy (ΔG) to be less than zero, because in this case there will be a positive entropy change in the universe when the reaction occurs.

For a complex set of coupled reactions involving many different molecules, the total free-energy change can be computed simply by adding up the free energies of all the different molecular species after the reaction and comparing this value with the sum of free energies before the reaction; for common substances the required free-energy values can be found from published tables. In this way one can predict the direction of a reaction and thereby readily check the feasibility of any proposed mechanism. Thus, for example, from the observed values for the magnitude of the electrochemical proton gradient across the inner mitochondrial membrane and the ΔG for ATP hydrolysis inside the mitochondrion, one can be certain that ATP synthase requires the passage of more than one proton for each molecule of ATP that it synthesizes.

The value of ΔG for a reaction is a direct measure of how far the reaction is from equilibrium. The large negative value for ATP hydrolysis in a cell merely reflects the fact that cells keep the ATP hydrolysis reaction as much as 10 orders of magnitude away from equilibrium. If a reaction reaches equilibrium, $\Delta G = 0$, the reaction then proceeds at precisely equal rates in the forward and backward direction. For ATP hydrolysis, equilibrium is reached when the vast majority of the ATP has been hydrolyzed, as occurs in a dead cell.

For each step, the part of the molecule that undergoes a change is shadowed in blue, and the name of the enzyme that catalyzes the reaction is in a yellow box.

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


glucose   hexokinase   glucose 6-phosphate
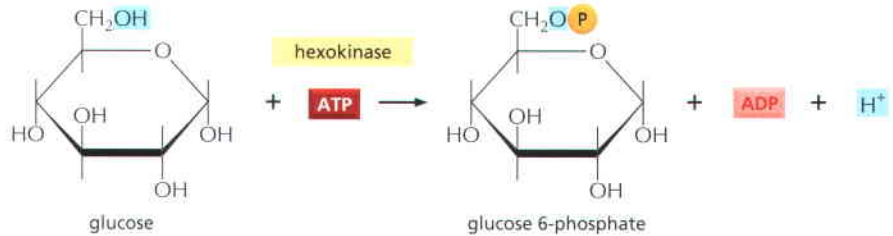
STEP 2   A readily reversible rearrangement of the chemical structure (isomerization) moves the carbonyl oxygen from carbon 1 to carbon 2, forming a ketose from an aldose sugar. (See Panel 2–4.)


glucose 6-phosphate   phosphoglucose isomerase   fructose 6-phosphate
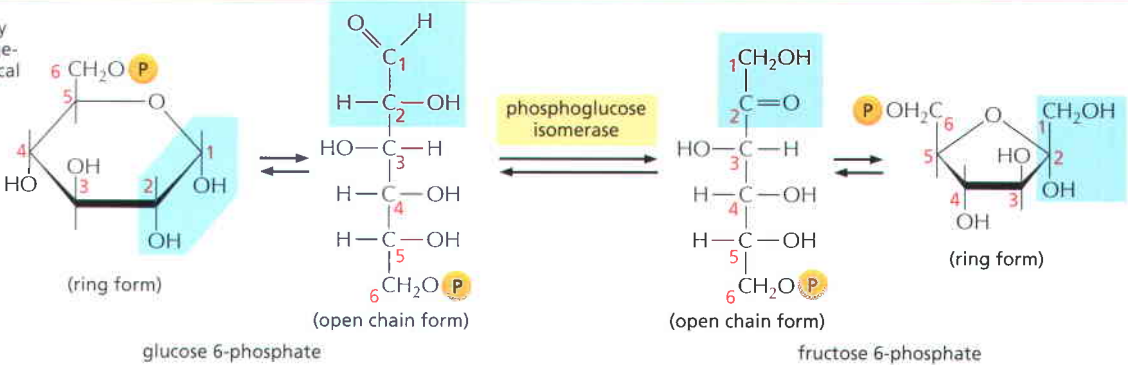
STEP 3   The new hydroxyl group on carbon 1 is phosphorylated by ATP, in preparation for the formation of two three-carbon sugar phosphates. The entry of sugars into glycolysis is controlled at this step, through regulation of the enzyme *phosphofructokinase*.


fructose 6-phosphate   phosphofructokinase   fructose 1,6-bisphosphate
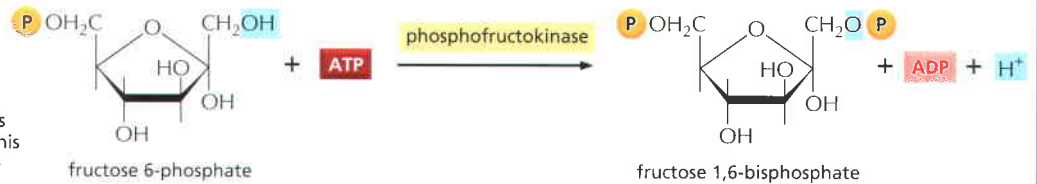
STEP 4   The six-carbon sugar is cleaved to produce two three-carbon molecules. Only the glyceraldehyde 3-phosphate can proceed immediately through glycolysis.


fructose 1,6-bisphosphate   aldolase   dihydroxyacetone phosphate   glyceraldehyde 3-phosphate
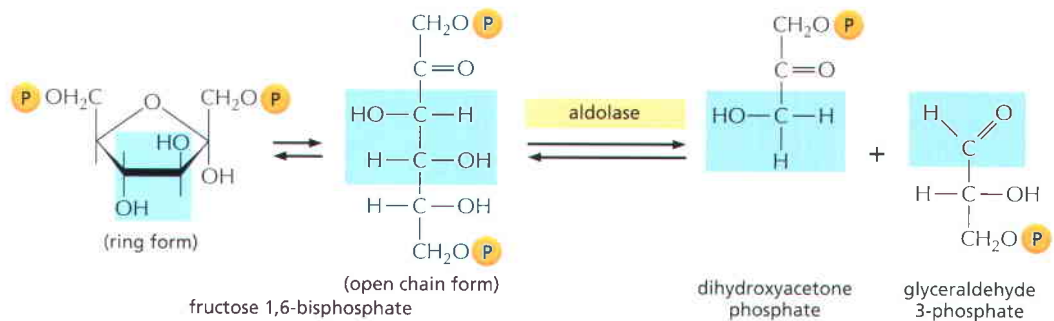
STEP 5   The other product of step 4, dihydroxyacetone phosphate, is isomerized to form glyceraldehyde 3-phosphate.

dihydroxyacetone phosphate   triose phosphate isomerase   glyceraldehyde 3-phosphate
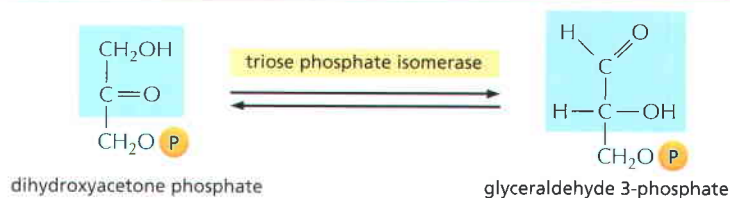
STEP 6 The two molecules of glyceraldehyde 3-phosphate are oxidized. The energy generation phase of glycolysis begins, as NADH and a new high-energy anhydride linkage to phosphate are formed (see Figure 2–73).


glyceraldehyde 3-phosphate

glyceraldehyde 3-phosphate dehydrogenase

1,3-bisphosphoglycerate

$+$ NADH $+$ H$^+$
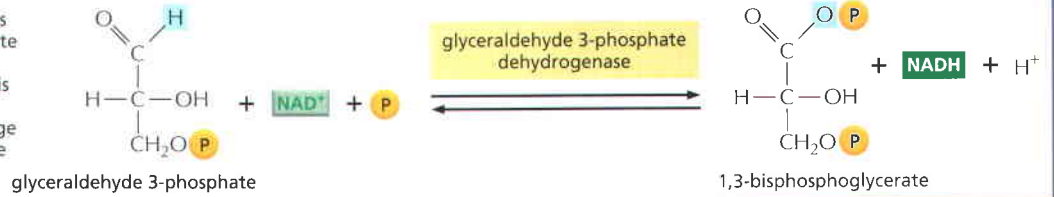
STEP 7 The transfer to ADP of the high-energy phosphate group that was generated in step 6 forms ATP.

1,3-bisphosphoglycerate

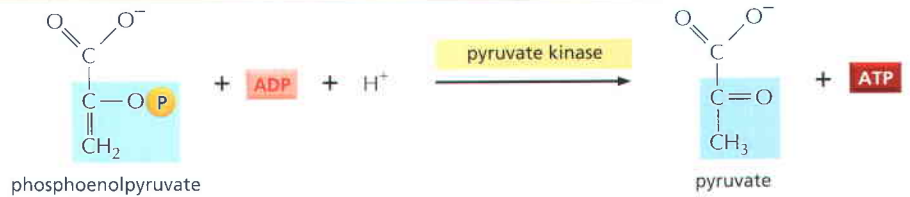phosphoglycerate kinase

3-phosphoglycerate

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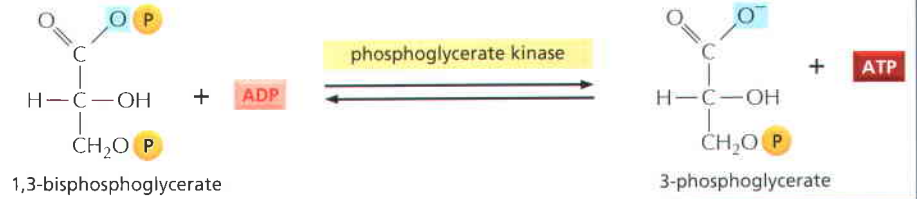
STEP 8 The remaining phosphate ester linkage in 3-phosphoglycerate, which has a relatively low free energy of hydrolysis, is moved from carbon 3 to carbon 2 to form 2-phosphoglycerate.
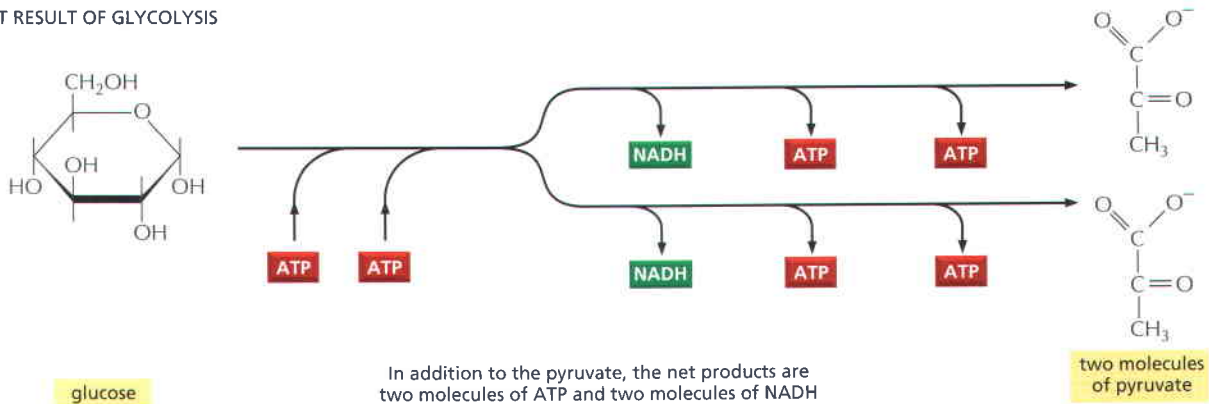

3-phosphoglycerate

phosphoglycerate mutase

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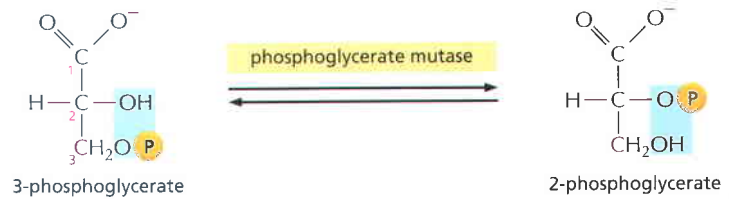
STEP 9 The removal of water from 2-phosphoglycerate creates a high-energy enol phosphate linkage.
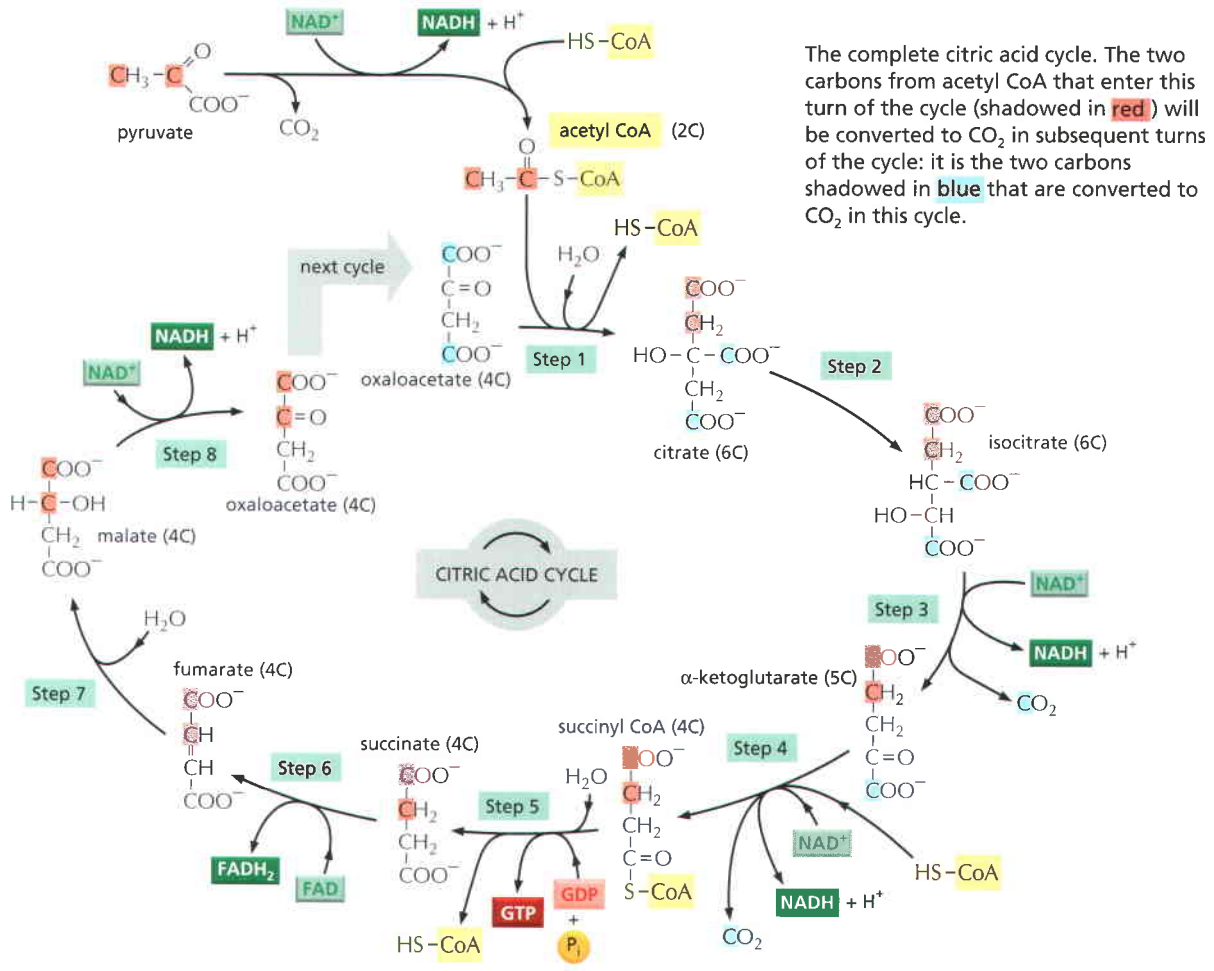

2-phosphoglycerate

enolase

phosphoenolpyruvate

$+$ H$_2$O
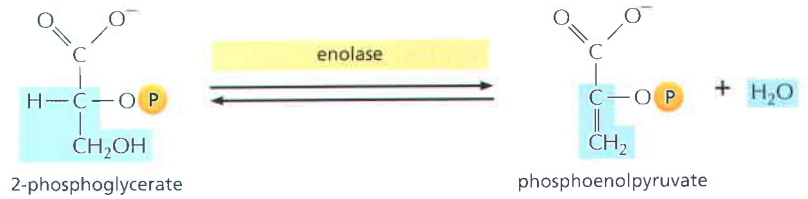
STEP 10 The transfer to ADP of the high-energy phosphate group that was generated in step 9 forms ATP, completing glycolysis.

phosphoenolpyruvate

$+$ ADP $+$ H$^+$

pyruvate kinase

pyruvate

$+$ ATP

NET RESULT OF GLYCOLYSIS

glucose

In addition to the pyruvate, the net products are two molecules of ATP and two molecules of NADH

two molecules of pyruvate

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

Details of the eight steps are shown below. For each step, the part of the molecule that undergoes a change is shadowed in blue, and the name of the enzyme that catalyzes the reaction is in a yellow box.
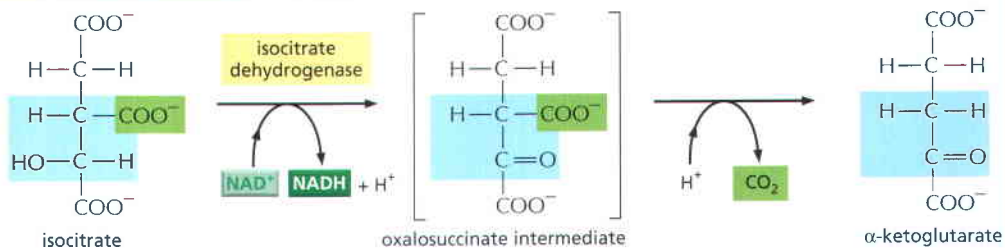
STEP 1   After the enzyme removes a proton from the CH_3 group on acetyl CoA, the negatively charged CH_2^- forms a bond to a carbonyl carbon of oxaloacetate. The subsequent loss by hydrolysis of the coenzyme A (CoA) drives the reaction strongly forward.
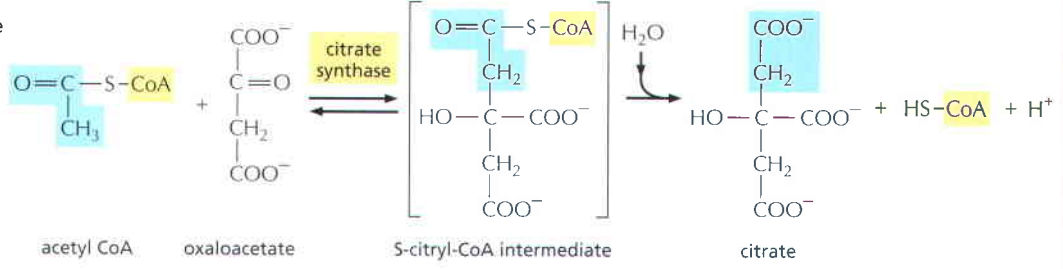

STEP 2   An isomerization reaction, in which water is first removed and then added back, moves the hydroxyl group from one carbon atom to its neighbor.
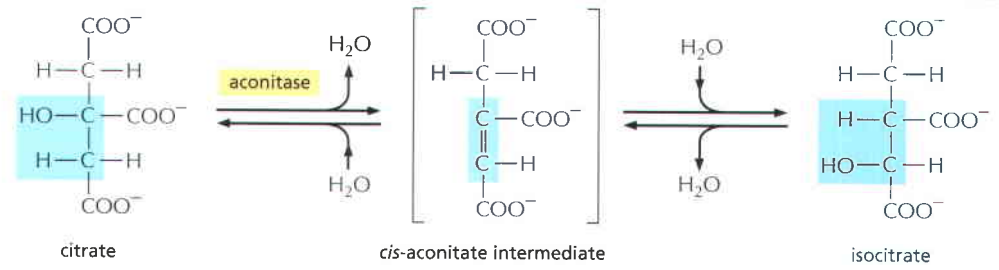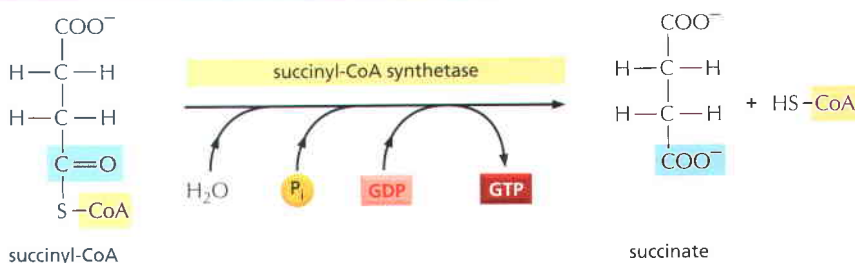
STEP 3 In the first of four oxidation steps in the cycle, the carbon carrying the hydroxyl group is converted to a carbonyl group. The immediate product is unstable, losing CO_2 while still bound to the enzyme.

isocitrate dehydrogenase

isocitrate → oxalosuccinate intermediate → α-ketoglutarate

NAD^+ $NADH + H^+$ H^+ CO_2

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

α-ketoglutarate + HS-CoA

α-ketoglutarate dehydrogenase complex

NAD^+ $NADH + H^+$ CO_2 → succinyl-CoA   S-CoA
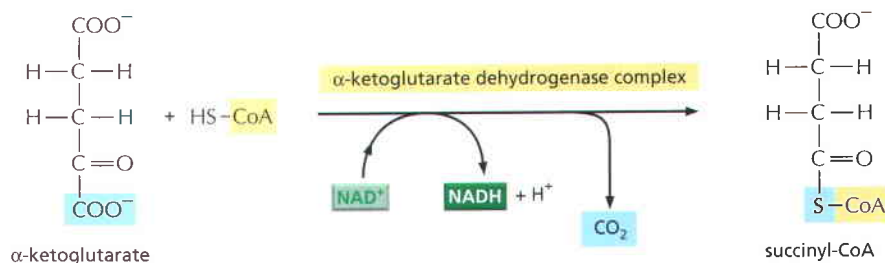
STEP 5 A phosphate molecule from solution displaces the CoA, forming a high-energy phosphate linkage to succinate. This phosphate is then passed to GDP to form GTP. (In bacteria and plants, ATP is formed instead.)

succinyl-CoA

succinyl-CoA synthetase

H_2O P_i GDP   GTP

succinate + HS-CoA

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

succinate

succinate dehydrogenase

FAD $FADH_2$
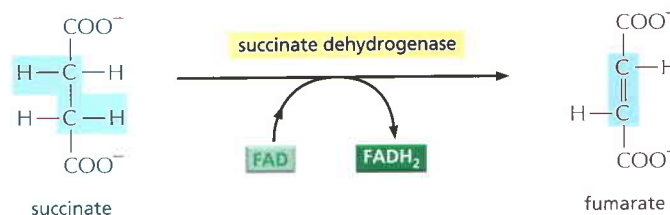
fumarate

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

fumarate

fumarase

H_2O

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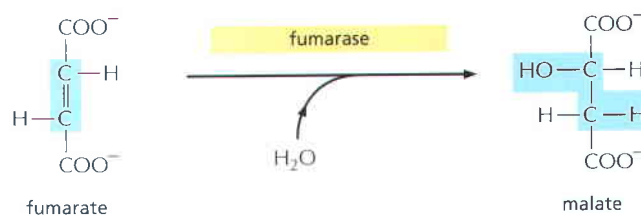
STEP 8 In the last of four oxidation steps in the cycle, the carbon carrying the hydroxyl group is converted to a carbonyl group, regenerating the oxaloacetate needed for step 1.

malate

malate dehydrogenase

NAD^+ $NADH + H^+$

oxaloacetate
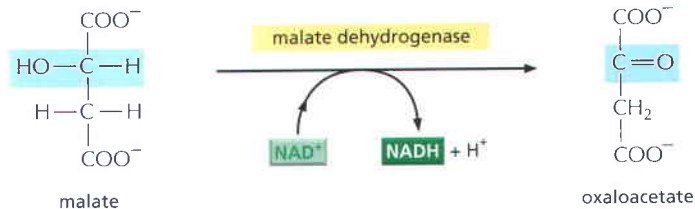
REFERENCES

General

Berg, JM, Tymoczko, JL & Stryer L (2006) Biochemistry, 6th ed. New York: WH Freeman.

Garrett RH & Grisham CM (2005) Biochemistry, 3rd ed. Philadelphia: Thomson Brooks/Cole.

Horton HR, Moran LA, Scrimgeour et al (2005) Principles of Biochemistry 4th ed. Upper Saddle River, NJ: Prentice Hall.

Nelson DL & Cox MM (2004) Lehninger Principles of Biochemistry, 4th ed. New York: Worth.

Nicholls DG & Ferguson SJ (2002) Bioenergetics, 3rd ed. New York: Academic Press

Mathews CK, van Holde KE & Ahern K-G (2000) Biochemistry, 3rd ed. San Francisco: Benjamin Cummings.

Moore JA (1993) Science As a Way of Knowing. Cambridge, MA: Harvard University Press.

Voet D, Voet JG & Pratt CM (2004) Fundamentals of Biochemistry, 2nd ed. New York: Wiley.

The Chemical Components of a Cell

Abeles RH, Frey PA & Jencks WP (1992) Biochemistry. Boston: Jones & Bartlett.

Atkins PW (1996) Molecules. New York: WH Freeman.

Branden C & Tooze J (1999) Introduction to Protein Structure, 2nd ed. New York: Garland Science.

Bretscher MS (1985) The molecules of the cell membrane. Sci Am 253:100–109.

Burley SK & Petsko GA (1988) Weakly polar interactions in proteins. Adv Protein Chem 39:125–189.

De Duve C (2005) Singularities: Landmarks on the Pathways of Life. Cambridge: Cambridge University Press.

Dowhan W (1997) Molecular basis for membrane phospholipid diversity: Why are there so many lipids? Annu Rev Biochem 66:199–232.

Eisenberg D & Kauzman W (1969) The Structure and Properties of Water. Oxford: Oxford University Press.

Fersht AR (1987) The hydrogen bond in molecular recognition. Trends Biochem Sci 12:301–304.

Franks F (1993) Water. Cambridge: Royal Society of Chemistry.

Henderson LJ (1927) The Fitness of the Environment, 1958 ed. Boston: Beacon.

Neidhardt FC, Ingraham JL & Schaechter M (1990) Physiology of the Bacterial Cell: A Molecular Approach. Sunderland, MA: Sinauer.

Pauling L (1960) The Nature of the Chemical Bond, 3rd ed. Ithaca, NY: Cornell University Press.

Saenger W (1984) Principles of Nucleic Acid Structure. New York: Springer.

Sharon N (1980) Carbohydrates. Sci Am 243:90–116.

Stillinger FH (1980) Water revisited. Science 209:451–457.

Tanford C (1978) The hydrophobic effect and the organization of living matter. Science 200:1012–1018.

Tanford C (1980) The Hydrophobic Effect. Formation of Micelles and Biological Membranes, 2nd ed. New York: John Wiley.

Catalysis and the Use of Energy by Cells

Atkins PW (1994) The Second Law: Energy, Chaos and Form. New York: Scientific American Books.

Atkins PW & De Paula JD (2006) Physical Chemistry for the Life Sciences. Oxford: Oxford University Press.

Baldwin JE & Krebs H (1981) The Evolution of Metabolic Cycles. Nature 291:381–382.

Berg HC (1983) Random Walks in Biology. Princeton, NJ: Princeton University Press.

Dickerson RE (1969) Molecular Thermodynamics. Menlo Park, CA: Benjamin Cummings.

Dill KA & Bromberg S (2003) Molecular Driving Forces: Statistical Thermo-dynamics in Chemistry and Biology. New York: Garland Science.

Dressler D & Potter H (1991) Discovering Enzymes. New York: Scientific American Library.

Einstein A (1956) Investigations on the Theory of Brownian Movement. New York: Dover.

Fruton JS (1999) Proteins, Enzymes, Genes: The Interplay of Chemistry and Biology. New Haven: Yale University Press.

Goodsell DS (1991) Inside a living cell. Trends Biochem Sci 16:203–206.

Karplus M & McCammon JA (1986) The dynamics of proteins. Sci Am 254:42–51.

Karplus M & Petsko GA (1990) Molecular dynamics simulations in biology. Nature 347:631–639.

Kauzmann W (1967) Thermodynamics and Statistics: with Applications to Gases. In Thermal Properties of Matter Vol 2. New York: WA Benjamin, Inc.

Kornberg A (1989) For the Love of Enzymes. Cambridge, MA: Harvard University Press.

Lavenda BH (1985) Brownian Motion. Sci Am 252:70–85.

Lawlor DW (2001) Photosynthesis, 3rd ed. Oxford: BIOS.

Lehninger AL (1971) The Molecular Basis of Biological Energy Transformations, 2nd ed. Menlo Park, CA: Benjamin Cummings.

Lipmann F (1941) Metabolic generation and utilization of phosphate bond energy. Adv Enzymol 1:99–162.

Lipmann F (1971) Wanderings of a Biochemist. New York: Wiley.

Nisbet EE & Sleep NH (2001) The habitat and nature of early life. Nature 409:1083–1091.

Racker E (1980) From Pasteur to Mitchell: a hundred years of bioenergetics. Fed Proc 39:210–215.

Schrödinger E (1944 & 1958) What is Life?: The Physical Aspect of the Living Cell and Mind and Matter, 1992 combined ed. Cambridge: Cambridge University Press.

van Holde KE, Johnson WC & Ho PS (2005) Principles of Physical Biochemistry, 2nd ed. Upper Saddle River, NJ: Prentice Hall.

Walsh C (2001) Enabling the chemistry of life. Nature 409:226–231.

Westheimer FH (1987) Why nature chose phosphates. Science 235:1173–1178.

Youvan DC & Marrs BL (1987) Molecular mechanisms of photosynthesis. Sci Am 256:42–49.

How Cells Obtain Energy from Food

Cramer WA & Knaff DB (1990) Energy Transduction in Biological Membranes. New York: Springer-Verlag.

Dismukes GC, Klimov VV, Baranov SV et al (2001) The origin of atmospheric oxygen on Earth: The innovation of oxygenic photosytheis. Proc Nat Acad Sci USA 98:2170–2175.

Fell D (1997) Understanding the Control of Metabolism. London: Portland Press.

Flatt JP (1995) Use and storage of carbohydrate and fat. Am J Clin Nutr 61, 952S–959S.

Friedmann HC (2004) From Butybacterium to E. coli: An essay on unity in biochemistry. Perspect Biol Med 47:47–66.

Fothergill-Gilmore LA (1986) The evolution of the glycolytic pathway. Trends Biochem Sci 11:47–51.

Heinrich R, Melendez-Hevia E, Montero F et al (1999) The structural design of glycolysis: An evolutionary approach. Biochem Soc Trans 27:294–298.

Huynen MA, Dandekar T & Bork P (1999) Variation and evolution of the citric-acid cycle: a genomic perspective. Trends Microbiol 7:281–291.

Kornberg HL (2000) Krebs and his trinity of cycles. Nature Rev Mol Cell Biol 1:225–228.

Krebs HA & Martin A (1981) Reminiscences and Reflections. Oxford/New York: Clarendon Press/Oxford University Press.

Krebs HA (1970) The history of the tricarboxylic acid cycle. Perspect Biol Med 14:154–170.

Martin BR (1987) Metabolic Regulation: A Molecular Approach. Oxford: Blackwell Scientific.

McGilvery RW (1983) Biochemistry: A Functional Approach, 3rd ed. Philadelphia: Saunders.

Morowitz HJ (1993) Beginnings of Cellular Life: Metabolism Recapitulates Biogenesis. New Haven: Yale University Press

Newsholme EA & Stark C (1973) Regulation of Metabolism. New York: Wiley.