8

The Biochemistry of Cells

Introduction

The purpose of this chapter is to present the structure of some of the molecules that make up a cell and to show how they are constructed under the supervision of hereditary elements of the cell. This will lead the way to a mathematical description of biological catalysis at the end of this chapter and is a necessary prelude to the discussion of the human immunodeficiency virus in Chapter 10. As a result, this chapter contains a lot of biological information.

We will see that biological molecules can be created outside of a cellular environment, but only very inefficiently. Inside a cell, however, the information for biomolecules is encoded in the genetic material called nucleic acid. Thus we will establish a direct relationship between the chemicals that constitute a cell and the cell's hereditary information.

The topical material of this chapter is organized along the lines of small to large. We begin by presenting a description of the atoms found in cells and then show how they are assembled into small organic molecules. Some of these small molecules can then be polymerized into large biochemical molecules, the biggest of which have molecular weights on the order of billions. These assembly processes are mediated by certain macromolecules which are themselves molecular polymers and whose own assembly has been mediated by similar molecular polymers. Thus we develop a key process in biology—self-replication.

8.1 Atoms and Bonds in Biochemistry

Most of the atoms found in a cell are of common varieties: hydrogen, carbon, nitrogen and oxygen. They are, in fact, major components of air and dirt. What is it then that makes them so fundamental to life? To answer this question we must examine the ways that these atoms form bonds to one another—because it is through molecular organization that we will characterize living systems.

A living system is a highly organized array of atoms, attached to one another by chemical bonds. The bonds may be strong, requiring considerable energy for their

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rearrangement. This leads to structures that are somewhat permanent and which can be changed only under special biochemical conditions. These bonds are said to be covalent, and they result from a process of "electron sharing." Carbon, nitrogen, and oxygen atoms can form a practically unlimited array, held together by covalent bonds.

Alternatively, some chemical bonds are weak, the heat energy available at room temperature being sufficient to break them. Because of their weakness, the structures they form are highly variable, leading to material movement and regional uniformity (among other things). The most important weak bond is called a hydrogen bond: it is the electrical attraction between a hydrogen nucleus on one molecule and an asymmetrically oriented electron on nitrogen or oxygen atoms of the same molecule or another one.

Organization is the key to living systems.

In Section 3.3, we pointed out that the individual processes found in living systems are also found in nonbiological situations. We emphasized that the "signature" of life was the organization, or integration, of those processes into a unified system. We now extend that concept to physical organization at the atomic and molecular levels.

Calcium, phosphorus, potassium, sulfur, sodium, and chlorine account for about 3.9% of the atoms in our bodies.[1] Just four other elements make up the other 96%; they are hydrogen, carbon, nitrogen, and oxygen. These four elements most abundant in our bodies are also found in the air and earth around us—as H_2O, CO_2, N_2, O_2, and H_2. Thus if we want to explain why something has the special quality we call "life," it does not seem very fruitful to look for exotic ingredients; they aren't there. Where else might the explanation be?

An important clue can be found in experiments in which living systems are frozen to within a few degrees of 0°K, so that molecular motion is virtually halted. Upon reheating, these living systems resume life processes. The only properties conserved in such an experiment are static structural ones. We can conclude that a critical property of life lies in the special ways that the constituent atoms of living systems are organized into larger structures. We should therefore suspect that the atoms most commonly found in our bodies have special bonding properties, such that they can combine with one another in many ways. This is indeed the case: carbon, nitrogen, oxygen, and hydrogen are capable of a virtually infinite number of different molecular arrangements. In fact, it has been estimated that the number of ways that the atoms C, H, O, N, P, and S can be combined to make low-molecular-weight compounds (MW $<$ 500) is in the billions [1]!

Of the large number of possible arrangements of C, N, O, and H, the forces of evolution have selected a small subset, perhaps a thousand or so, on which to base life. Members of this basic group have then been combined into a vast array of biomacromolecules. For example, the number of atoms in a typical biomacromolecule might range from a few dozen up to millions, but those with more than a few hundred atoms are always polymers of simpler subunits.

[1] About 15 more elements are present in trace amounts.

Living systems are assemblages of common atoms, each part of the system having a very specific organization at all size levels. In other words, all living things can be thought of as regions of great orderliness, or organization. Death is marked by the disruption of this organization—either suddenly, as in the case of an accident, or slowly, as in the case of degenerative disease. In any case, death is followed by decompositional processes that convert the body to gases, which are very disorganized.

Physicists use *entropy* as a measure of disorder; there is an important empirical rule, the *second law of thermodynamics*, which states that entropy in the universe increases in the course of every process. Living systems obey this rule, as they do all other natural chemical and physical principles. As an organism grows, it assembles atoms into an orderly, low-entropy arrangement; at the same time, the entropy of the organism's surroundings increases by even more, to make the net entropic change in the universe positive. This net increase is to be found in such effects as the motion of air molecules induced by the organism's body heat, in the gases it exhales, and in the natural waste products it creates.

Nature is full of good examples of the critical role played by organization in living systems. Consider that a bullfighter's sword can kill a 600-pound bull and that 0.01 micrograms of the neurotoxin *tetrodotoxin* from a puffer fish can kill a mouse. The catastrophic effects of the sword and the toxin seem out of proportion to their masses. In light of the discussion above, however, we now understand that their effects are not based on mass at all, but instead on the disruption of critically-organized structures, e.g., the nervous system [2].

Covalent bonds are strong interactions involving electron sharing.

A very strong attraction between two atoms results from a phenomenon called "*electron sharing*"; it is responsible for binding atoms into biochemical molecules. One electron from each of two atoms becomes somewhat localized on a line between the two nuclei. The two nuclei are electrostatically attracted to the electrons and therefore remain close to one another.

Figure 8.1.1 shows simple planetary models of two hydrogen atoms. (Later we will generalize our discussion to other atoms.) The radius of this orbit is about 0.05 nm, and so the nuclei are about 0.1 nm apart. At some time each of the electrons will find itself at a point immediately between the two nuclei. When this happens, each of the two nuclei will exert the same electrical attraction on the electron, meaning that the electron can no longer be associated with a particular nucleus. There being no reason to "choose" either the right or the left nucleus, the electron will spend more time directly in between the two than in any other location.[2] The two electrons in the center then act like a kind of glue, attracting the nuclei to themselves and thus

[2] The idea that an electron is more likely to be found in one region of space than in another is built into the quantum-mechanical formulation, which is outside the scope of this book. In the quantum-mechanical formulation, there are no orbits and the electron is represented as a probability cloud. The denser the cloud, the greater the probability of finding the electron there.

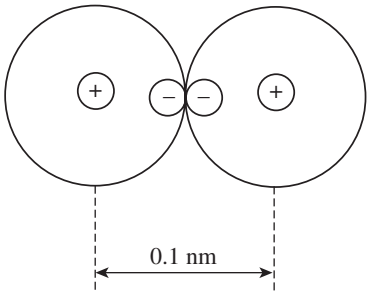


Fig. 8.1.1. A model of the hydrogen molecule. Planetary orbits are shown, but the electrons are equally attracted to both nuclei and therefore spend most of their time in the region directly between the two nuclei. This interaction is called a covalent bond.

toward each other. A stable molecule is thereby formed; the attraction between its constituent atoms is called a *covalent bond*.

A covalent bond always contains two electrons because of an unusual electronic property: An electron spins on its own axis. For quantum-mechanical reasons, an electron always pairs up with another electron having the opposite spin direction, leading to "*spin pairing*" in covalent bonds and in certain other situations. An atom or molecule with an odd number of electrons is called a *radical*; it is unstable, quickly pairing up with another radical via a covalent bond. For example, atomic hydrogen has a very transitory existence, quickly forming the diatomic hydrogen molecule H_2, in which the electrons' spins are paired. Thus electrons in stable chemicals appear in pairs. For a further discussion of this topic, see Yeargers [3].

Covalent bonds are very stable. In order to break one, i.e., to dissociate a biomolecule, it would require at least four electron volts of energy. For comparison, that much energy is contained by quanta in the ultraviolet region of the electromagnetic spectrum and exceeds that of the visible region of the solar spectrum. In passing, this helps us to understand why sunlight is carcinogenic—its ultraviolet component alters the chemistry of chemical components of our skin. If not for the fact that most of the sun's ultraviolet radiation is filtered out by the earth's atmosphere, life on earth would have to be chemically quite different from what it is.

Each kind of atom forms a fixed number of covalent bonds to its atomic neighbors; this number is called the *valence*. Table 8.1.1 gives the atomic numbers and valences of hydrogen, carbon, nitrogen, and oxygen.

Table 8.1.1.

Atom	Symbol	Atomic number	Valence
Hydrogen	H	1	1
Carbon	C	6	4
Nitrogen	N	7	3
Oxygen	O	8	2

Figure 8.1.2 shows the structures and names of several common organic molecules. The bond angles are shown; they may fluctuate by several degrees depending on the exact composition of the molecule. In each case the length of the bond is about 0.1 nm, again depending on the constituent atoms. Note also that double bonds are possible, but only if they are consistent with the valences given above.

You can see from Figure 8.1.2 that there are only two basic bonding schemes: If the molecule has only single bonds, the bond angles are 109°, and if there is a double bond the bond angles are 120°. Note that the former leads to a three-dimensional shape and the latter to a planar shape. This should become evident if you compare the structures of ethane and ethene.

Hydrogen bonds are weak interactions.

Figure 8.1.3 shows some more molecular models, containing oxygen and nitrogen. These molecules are electrically neutral: Unless ionized, they will not migrate toward either pole of a battery. Unlike hydrocarbons, however, their charges are not uniformly distributed. In fact, nitrogen and oxygen atoms in molecules have pairs of electrons (called lone pairs) that are arranged in a highly asymmetrical way about the nucleus. Figure 8.1.3 shows the asymmetrically oriented electrons of nitrogen and oxygen. There are three important points to be noted about these pictures: First, the reason that lone pair electrons are "paired" is that they have opposite spin directions from one another, as was described earlier. Second, the angles with which the lone pairs project outward are consistent with the 109° or 120° bond angles described earlier. Third, it must be emphasized that these molecules are electrically neutral—their charges are not uniformly distributed in space, but they total up to exactly zero for each complete molecule. The presence of lone pairs has important structural consequences to molecules that contain nitrogen or oxygen. Consider the water molecule shown in Figure 8.1.3. Two lone pairs extend toward the right and bottom of the picture, meaning that the right and lower ends of the molecule are negative. The entire molecule is neutral, and therefore the left and upper ends must be positive. We associate the negative charge with the lone pairs and the positive charge with the nuclei of the hydrogen atoms at the other end. Such a molecule is said to be *dipolar*.

Dipolar molecules can electrically attract one another, the negative end of one attracting the positive end of the other. In fact, a dipolar molecule might enter into several such interactions, called *hydrogen bonds* (H-bonds). Figure 8.1.4 shows the H-bonds in which a water molecule might participate. Note carefully that the ensemble of five water molecules is not planar.

Hydrogen bonds are not very strong, at least compared to covalent bonds. They can be broken by energies on the order of 0.1 eV, an energy that is thermally available at room temperature. There are two mitigating factors, however, that make H-bonds very important in spite of the ease with which they can be broken. The first is their sheer numbers. Nitrogen and oxygen are very common atoms in living systems, as mentioned earlier, and they can enter into H-bonding with neighboring, complementary H-bonding groups. While each H-bond is weak, there are so many of them that they can give considerable stability to systems in which they occur.

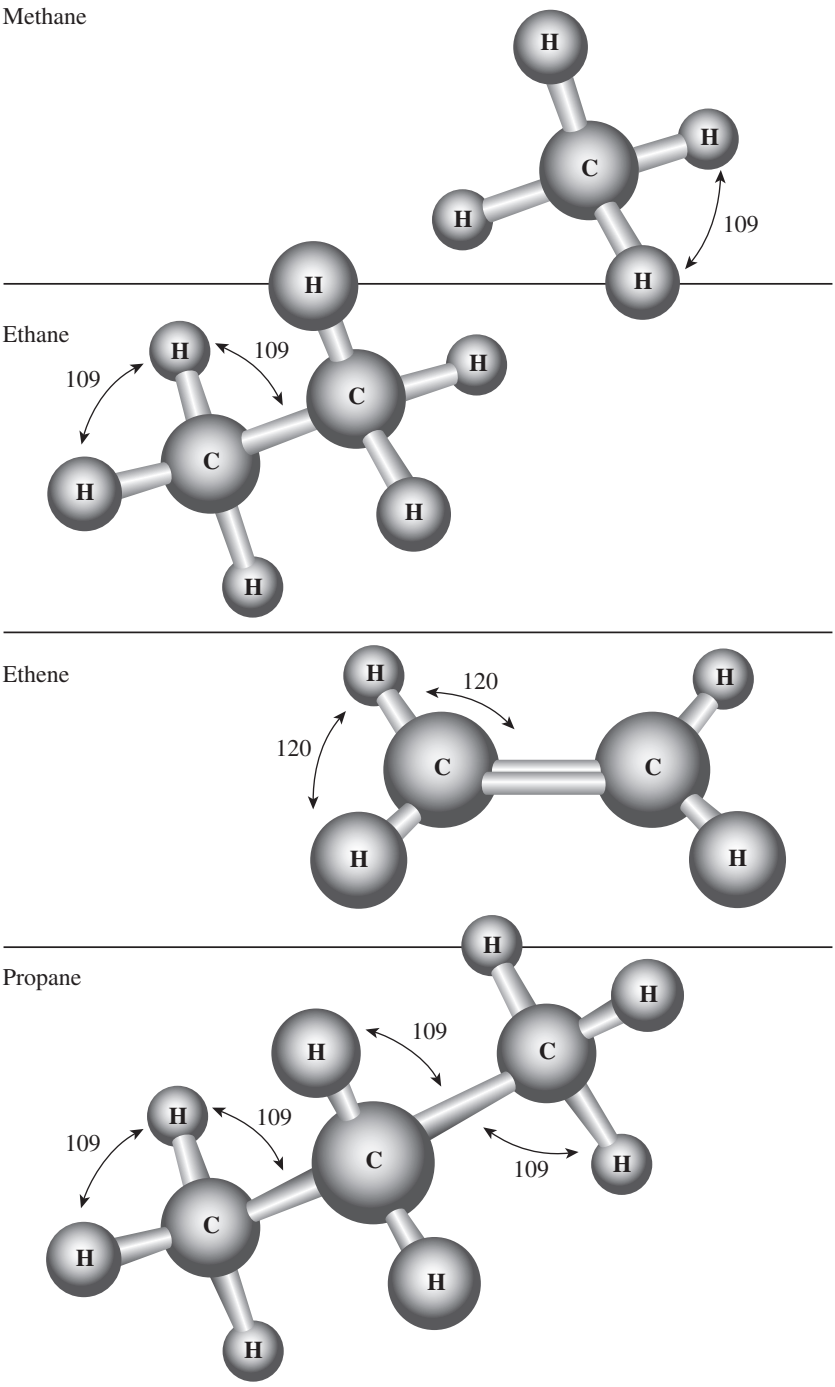


Fig. 8.1.2. Three-dimensional models of several small hydrocarbons (containing only hydrogen and carbon atoms). Bond angles are shown.

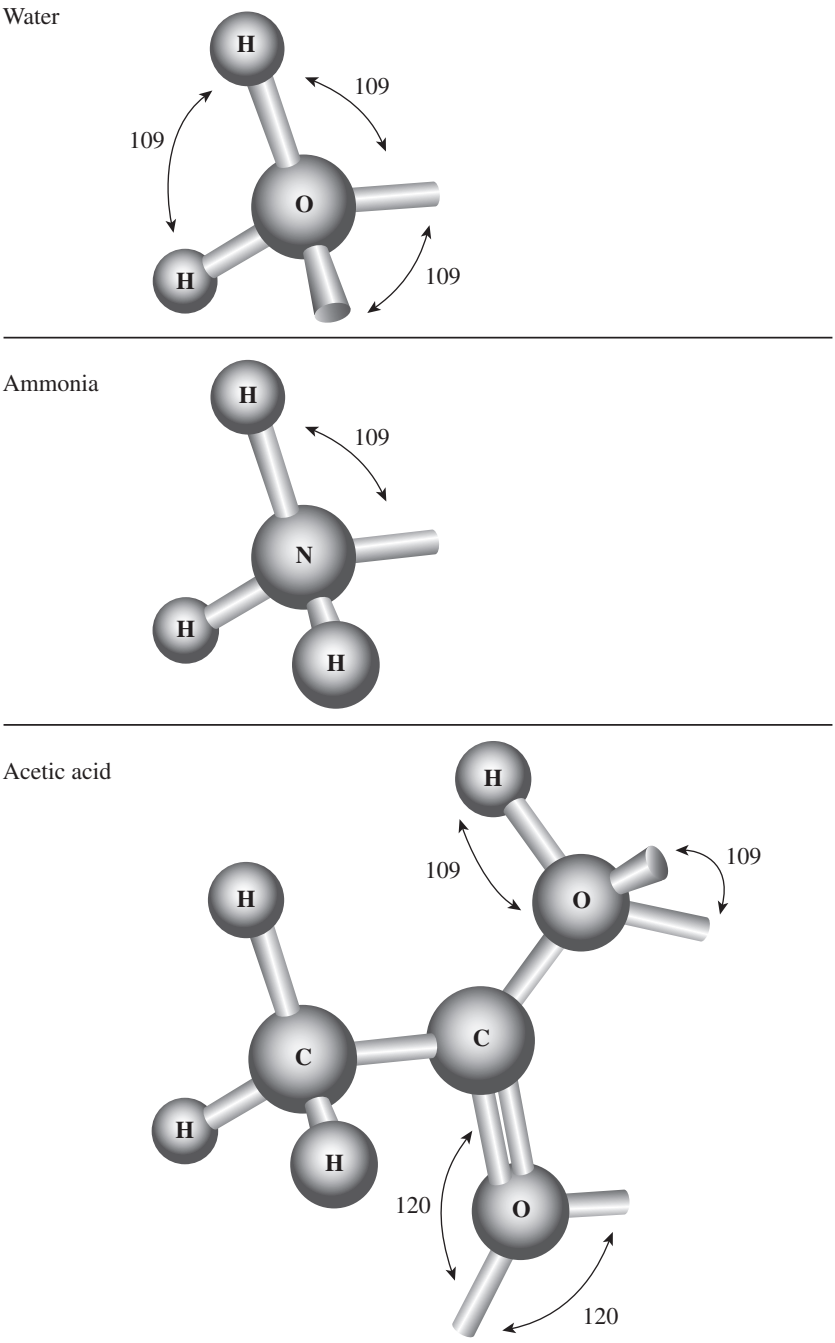


Fig. 8.1.3. Three-dimensional models of molecules containing oxygen and nitrogen. The stubs originating on the oxygen and the nitrogen atoms, but not connected to any other atom, represent lone pairs, or asymmetrically oriented electrons.

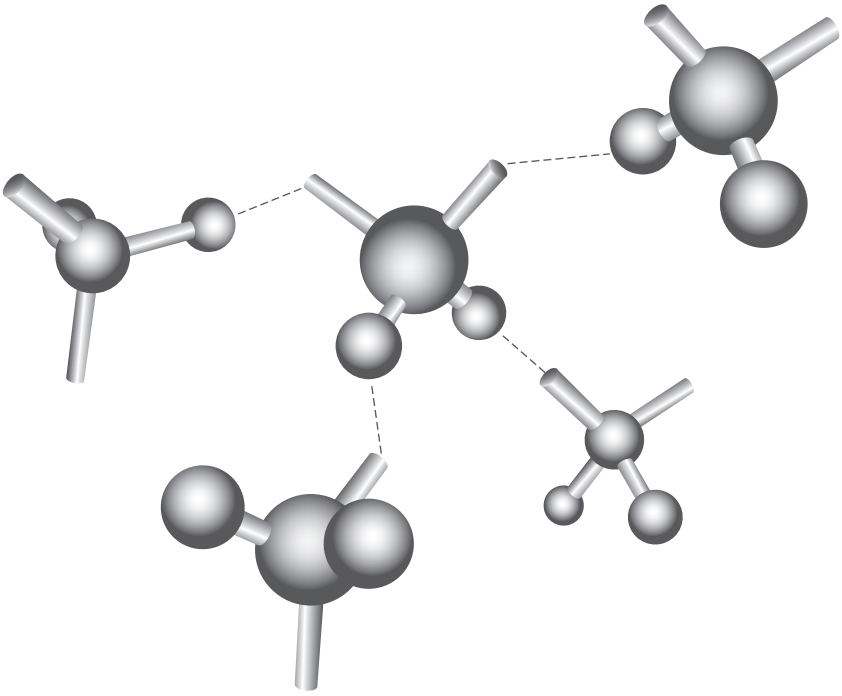


Fig. 8.1.4. Three-dimensional model of one possible transient arrangement of water molecules in the liquid phase. The central molecule is hydrogen-bonded to four other molecules, each of which is in turn hydrogen-bonded to four. The hydrogen bonds are represented by dotted lines between the lone pairs and hydrogen protons. This configuration will break up quickly at room temperature, and the molecules will re-form into other, similar configurations.

The second factor complements the first: The weakness of H-bonds means that the structures they stabilize can be altered easily. For example, every water molecule can be held by H-bonds to four other water molecules (see Figure 8.1.4). At 20–30° Celsius there is just enough heat energy available to break these bonds. Thus H-bonds between water molecules are constantly being made and broken, causing water to be a liquid at room temperature. This allows biological chemistry to be water-based at the temperatures prevailing on earth. As a second example, we shall see later that the genetic chemical DNA is partly held together by H-bonds that have marginal stability at body temperature, a considerable chemical convenience for genetic replication, which requires partial disassembly of the DNA.

Hydrogen-bonding plays a critical role in a number of biological phenomena. Solubility is an example: A molecule that is capable of forming hydrogen bonds tends to be water soluble. We can understand this by substituting any other dipolar molecule (containing one or more lone pairs) for the central water molecule of Figure 8.1.4. On the other hand, a molecule lacking lone pair electrons is not water soluble. Look at the propane molecule in Figure 8.1.2 and note that such *hydrocarbons* lack the ability

to dissolve in water because they lack the necessary asymmetrical charges needed for H-bonding. We shall return to the topic of H-bonding when nucleic acids and heat storage are discussed later in this chapter. These topics, as well as other kinds of chemical bonding interactions, are discussed by Yeargers [3].

8.2 Biopolymers

At the beginning of this chapter, it was pointed out that the attribute we call "life" is due to the organization, not the rarity, of constituent atoms. Figures 8.1.2 *and* 8.1.3 *showed that sequences of carbon, oxygen, and nitrogen atoms, with their many bends and branches, can potentially combine to form elaborate three-dimensional macromolecules. What happens is that atoms combine to form molecular monomers having molecular weights on the order of a few hundred. In turn, these monomers are chained into linear or branched macromolecular polymers having molecular weights of up to a billion. The ability to create, organize, and maintain these giant molecules is what distinguishes living things from nonliving things.*

Polysaccharides are polymers of sugars.

A typical sugar is *glucose*, shown in Figure 8.2.1(a). The chemical characteristics that make glucose a sugar are the straight chain of carbons, the multiple −OH groups, and the double-bonded oxygen. Most of the other sugars we eat are converted to glucose, and the energy is then extracted via the conversion of glucose to carbon dioxide. This process is called *respiration*; it will be described below. A more common configuration for a sugar is exemplified by the ring configuration of glucose, shown in Figure 8.2.1(b).

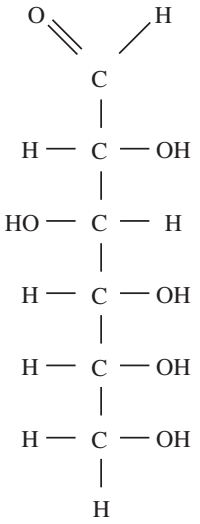
The polymerization of two glucose molecules is a *condensation* reaction, shown in Figure 8.2.2. Its reverse is *hydrolysis*. We can extend the notion of sugar polymerization into long linear or branched chains, as shown by the arrows in Figure 8.2.2. The actual function of a polysaccharide, also called a *carbohydrate*, will depend on the sequence of component sugars, their orientations with respect to each other, and whether the chains are branched.

Polysaccharides serve numerous biological roles. For example, plants store excess glucose as *starch*, a polysaccharide found in seeds such as rice and wheat (flour is mostly starch). The structural matter of plants is mainly *cellulose*; it comprises most of what we call wood. When an animal accumulates too much glucose, it is polymerized into *glycogen* for storage in the muscles and liver. When we need glucose for energy, glycogen is hydrolyzed back to monomers. These and other functions of sugars will be discussed later in this and subsequent chapters.

Lipids are polymers of fatty acids and glycerol.

Fatty acids, exemplified in Figure 8.2.3, are distinguished from each other by their lengths and the positions of their double bonds. Note the organic acid group (-COOH) at one end. Fatty acids with double bonds are said to be *unsaturated*; *polyunsaturated*

(a)



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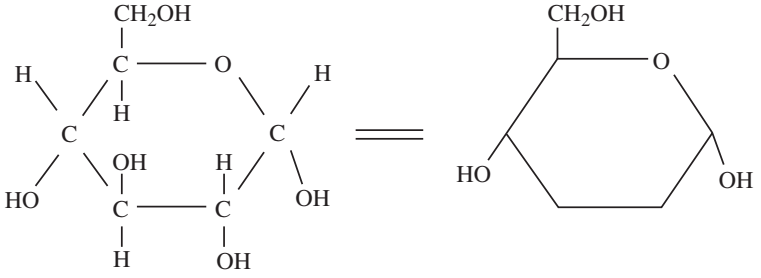


Fig. 8.2.1. (a) A model of the linear form of the glucose molecule. (b) A model of the ring form of the glucose molecule. The right-hand version, which omits many of the identifying symbols, is the more common representation.

fatty acids are common in plants, whereas saturated fatty acids, lacking double bonds, are common in animals. *Glycerol* and three fatty acids combine to form a *lipid*, or *fat*, or *triglyceride*, as pictured in Figure 8.2.4. The reverse reaction is again called hydrolysis.

Lipids are efficient at storing the energy of excess food that we eat; a gram of lipid yields about four times the calories of other foods, e.g., carbohydrates and proteins. Lipids are fundamental components of cell membranes: A common lipid of cell membranes is a phospholipid, pictured in Figure 8.2.5. You should now be able to put Figure 8.2.5 into the context of Figure 6.1.1. Note how the hydrocarbon regions of

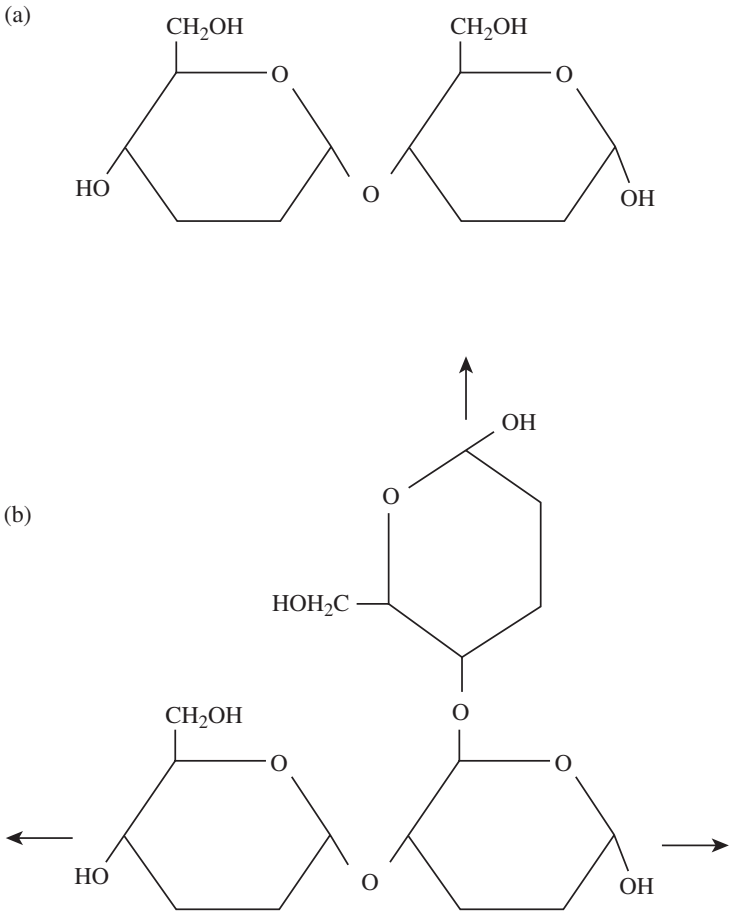


Fig. 8.2.2. (a) A model of a disaccharide, consisting of two glucose molecules. (b) A model showing the three possible directions that the polysaccharide of (a) could be extended. A large polysaccharide, with many such branches, would be very complex.

the phospholipid are in the interior of the membrane and how the hydrophilic oxygen groups (having lone pair electrons) are on the membrane's exterior, where they can hydrogen-bond to the surrounding water.

Nucleic acids are polymers of nucleotides.

Nucleic acids contain the information necessary for the control of a cell's chemistry. This information is encoded into the sequence of monomeric units of the nucleic acid, called *nucleotides*, and is expressed as chemical control through a series of processes called the *central dogma of genetics*—to be described below. When a cell reproduces asexually, its nucleic acids are simply duplicated and the resultant molecules are partitioned equally among the subsequent daughter cells, thus ensuring

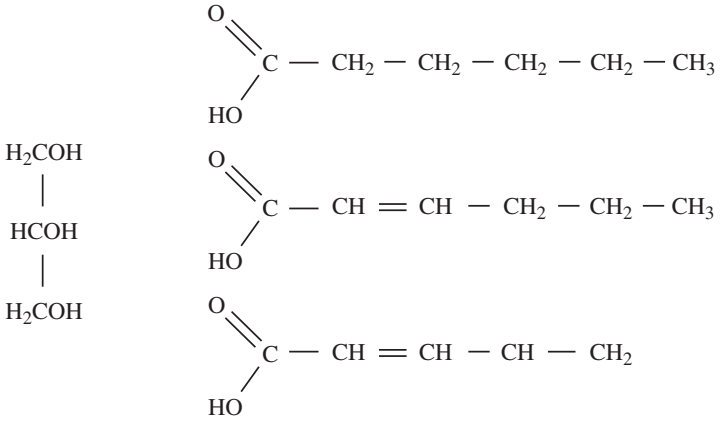


Fig. 8.2.3. A model of a glycerol molecule (left) and three arbitrary fatty acids (right).

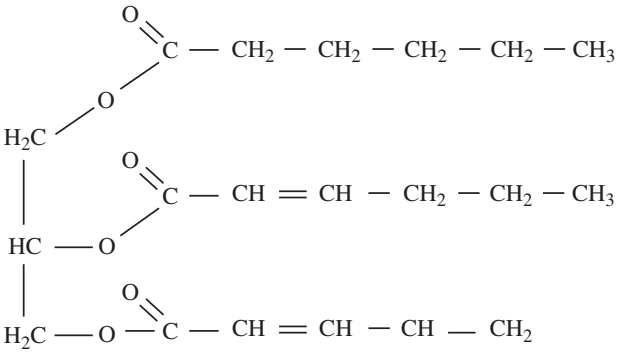


Fig. 8.2.4. A model of a fat, or triglyceride. It consists of a glycerol and three fatty acids. Compare Figure 8.2.3.

that the daughter cells will have the same chemical processes as the original cell. In sexual reproduction, nucleic acids from two parents are combined in fertilization, resulting in an offspring whose chemistry is related by sometimes complex rules to that of its parents.

There are two kinds of nucleic acids: Their names are *deoxyribonucleic acid* (DNA) and *ribonucleic acid* (RNA). The monomer of a nucleic acid is a *nucleotide*, which is composed of three parts: a sugar, one or more phosphate groups, and a nitrogenous base. Figure 8.2.6 shows the components of a typical nucleotide.

DNA is a double helix. Figure 8.2.7 shows a model of the macromolecule, partially untwisted to reveal its underlying structure. Note that it is formed from two covalently linked linear polymers, which are wrapped around each other. The two single strands are H-bonded to one another, as shown by dotted lines in the figure. Figure 8.2.8 shows the details of the H-bonding between DNA nucleotides.

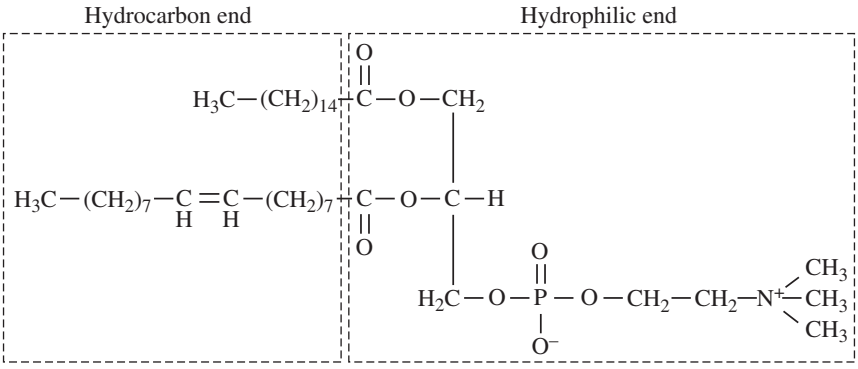


Fig. 8.2.5. A phospholipid, or phosphoglyceride, found in cell membranes. Note that it has a hydrophilic end that is attracted to water and a hydrocarbon (hydrophobic) end that is repelled by water. The hydrophilic end faces the aqueous outside world or the aqueous interior of the cell. The hydrophobic end of all such molecules is in the interior of the membrane, where there is no water. This picture should be compared to the schematic lipids shown in Figure 6.1.1: The circles on the phospholipids of Figure 6.1.1 correspond to the right-hand box of this figure, and the two straight lines of Figure 6.1.1 correspond to the two hydrocarbon chains in the left-hand box of this figure.

The DNA molecule is very long compared to its width. The double helix is 2.0×10^{-9} m wide, but about 10^{-3} m long in a bacterium and up to 1 m long in a human. There are ten base pairs every 3.6×10^{-7} m of length of double helix. Thus a 1-meter-long DNA molecule has about 3×10^{8} base pairs. If any of the four nucleotides can appear at any position, there could exist $4^{3\times10^{8}}$ possible DNA molecules of length 1 m. Obviously, an incredible amount of information can be encoded into such a complex molecule. Note that DNA uses only a four-letter "alphabet" but can compensate for the small character set by writing very long "words."

There are some important structural details and functional consequences to be noted about Figures 8.2.7 and 8.2.8:

1. *Each of the two single-stranded polymers of a DNA molecule is a chain of covalently linked nucleotides.* All four possible nucleotides are shown, but there are no restrictions on their order in natural systems; any nucleotide may appear at any position on a given single strand. It is now possible experimentally to determine the sequences of long DNA chains; see Section 14.1.

2. *Once a particular nucleotide is specified at a particular position on one strand, the nucleotide opposite it on the other strand is completely determined.* Note that *A* and *T* are opposite one another, as are *C* and *G*; no other base pairs are allowed in DNA. (From now on, we shall indicate the names of the nucleotides by their initials, i.e., *A*, *T*, *C*, and *G*.) There are very important physical and biological reasons for this *complementary* property. The physical reason can be seen by a close examination of the H-bonds between an *A* and a *T* or between a *C* and a *G* in Figure 8.2.8. Recall that an H-bond is formed between a lone pair of

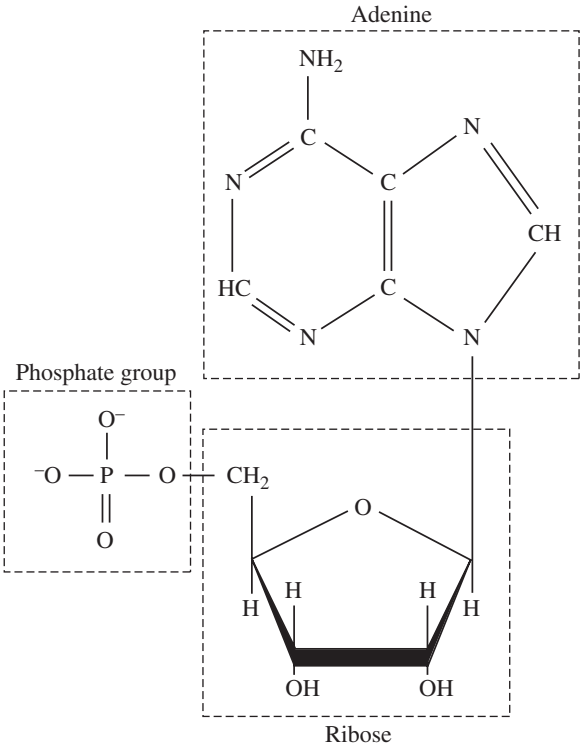


Fig. 8.2.6. A typical nucleotide, consisting of a nitrogenous base (adenine), a sugar (ribose), and a phosphate group. Other nucleotides can have other nitrogenous bases, a different sugar, and more phosphates.

electrons and a hydrogen nucleus, and note that two such bonds form between *A* and *T* and that three form between *C* and *G*. There are no other ways to form two or more strong H-bonds between any of these nucleotides; thus the ways shown in Figure 8.2.7 are the only possibilities. For example, *A* cannot effectively H-bond to *C* or *G*. We should note that the property of complementary H-bonding requires that the two single strands have different nucleotide sequences, but that the sequence of one strand be utterly determined by the other.

3. *The helical configuration is a spontaneous consequence of H-bonding the two single strands together.* Helicity disappears if the H-bonds are disrupted. Recall from the discussion of the structure of water in Section 8.1 that H-bonds have marginal stability at room temperature. We should therefore expect that the two strands of helical DNA can be separated, i.e., the helix can be *denatured*, without expending much energy. In fact, DNA becomes denatured at around 45–55°C, only about 8 to 18 degrees above body temperature. Once thermal denaturation has occurred, however, the two strands can often spontaneously reassociate into their native double helical configuration if the temperature is then slowly reduced.

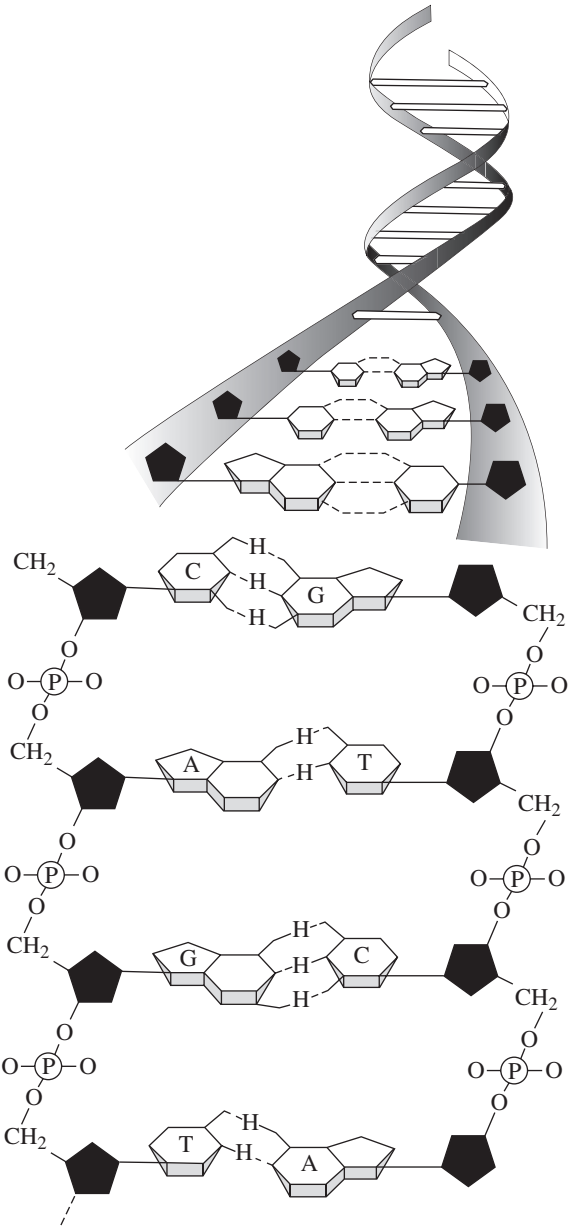


Fig. 8.2.7. A DNA molecule, showing the arrangement of the nucleotide components into two covalent polymers, each of which is hydrogen-bonded to the other. Note that *A* (adenine) and *T* (thymine) are hydrogen-bonded to each other, and *C* (cytosine) and *G* (guanine) are hydrogen-bonded to each other. The hydrogen bonds are indicated by the dashes. (Redrawn from C. Starr and R. Taggart, *Biology: The Unity and Diversity of Life*, 6th ed., Wadsworth, Belmont, CA, 1992. Used with permission.)

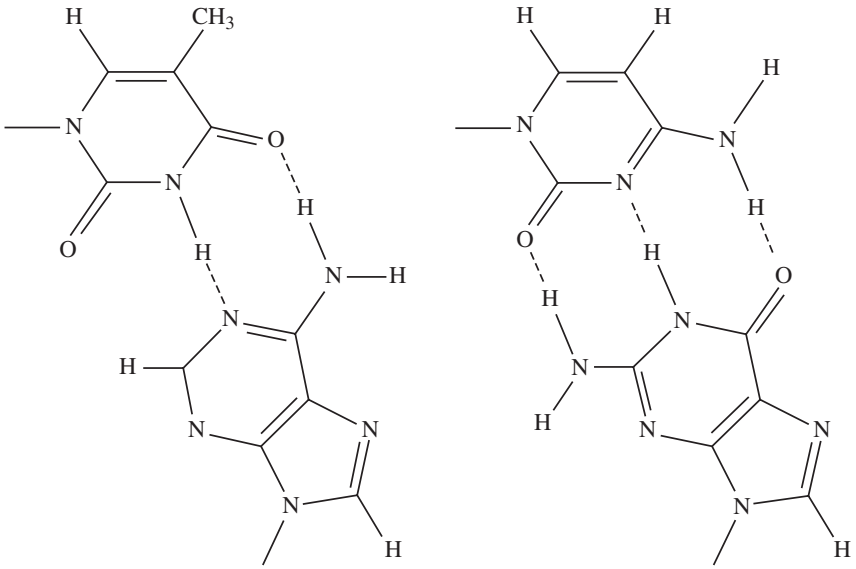


Fig. 8.2.8. A detailed picture of the complementary hydrogen bonds between *A* and *T* (left pair), and between *C* and *G* (right pair). Compare this figure to the hydrogen-bonded groups in Figure 8.2.7. See the text for details.

This should be expected in light of complementary H-bonding between the two strands.

There is another important structural feature related to double helicity: Look at Figure 8.2.7 and note that each nucleotide is fitted into the polynucleotide in such a way that it points in the same direction along the polymer. It is therefore possible to associate directionality with any polynucleotide. In order for the two strands of any nucleic acid to form a double helix, they must have opposite directionalities, i.e., they must be antiparallel to each other.[3]

4. *Complementary hydrogen-bonding provides a natural way to replicate DNA accurately.* This is the biological reason for complementary H-bonding and is illustrated in Figure 8.2.9. The two strands of DNA are separated, and each then acts as a *template* for a new, complementary strand. In other words, the sequence information in each old strand is used to determine which nucleotides should be inserted into the new, complementary strand. *This mechanism allows DNA to code for its own accurate replication*, which is a necessary requirement for a genetic chemical.

Occurring just prior to cell division, the process of DNA self-replication yields two double-stranded DNA molecules that are exact copies of the original. Then, during

[3] For example, look at the location of the methyl group (-CH_2-) between the phosphate group and the ribose group. Note how it is in a different position on the two strands.

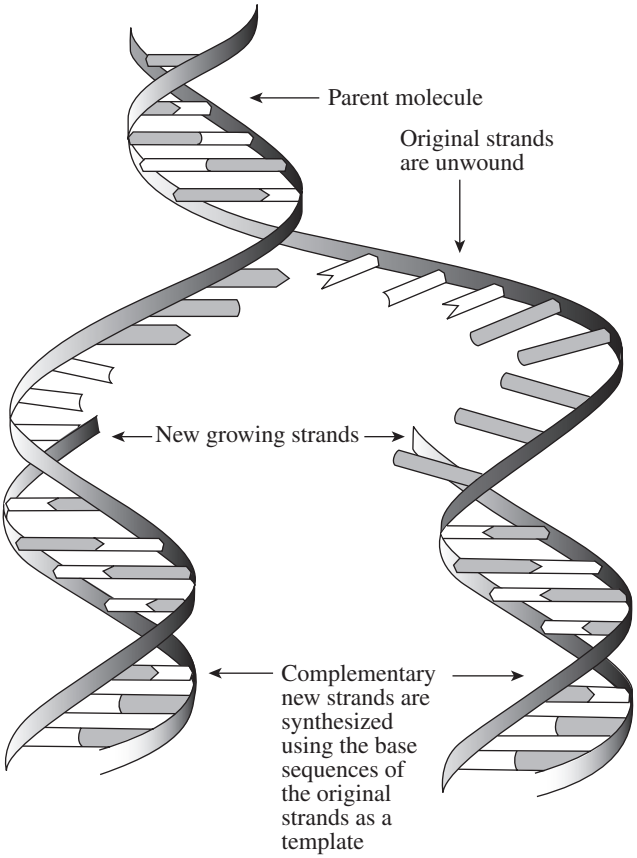


Fig. 8.2.9. A model of a replicating DNA molecule. The two strands of the parent double helix separate, and each one acts as a template for a new strand. Complementary hydrogen-bonding ensures that the two resulting double helices are exact copies of the original molecule. (Redrawn from J. Levine and K. Miller, *Biology*, 1st ed., D. C. Heath, Lexington, MA, 1991.)

cell division, each of the daughter cells gets one of the copies. The two daughter cells thus each end up with the same genetic material that the original cell had and should therefore also have the same life properties.

There are three classes of RNA molecules: The first is called *messenger RNA*, or mRNA. Each piece of mRNA averages about a thousand bases in length, but is quite variable. It is single stranded and nonhelical. The second kind of RNA is *transfer RNA*, or tRNA. There are several dozen distinguishable members of this class; they contain in the range of 75 to 95 bases, some of which are not the familiar A, T, C, and G. tRNA is single stranded but is double helical. This unexpected shape is due to the folding over of the tRNA molecule, as shown in Figure 8.2.10. The third kind of RNA is *ribosomal RNA*, or rRNA. This molecule accounts for most of a cell's RNA. It appears in several forms in cellular organelles associated with protein synthesis,

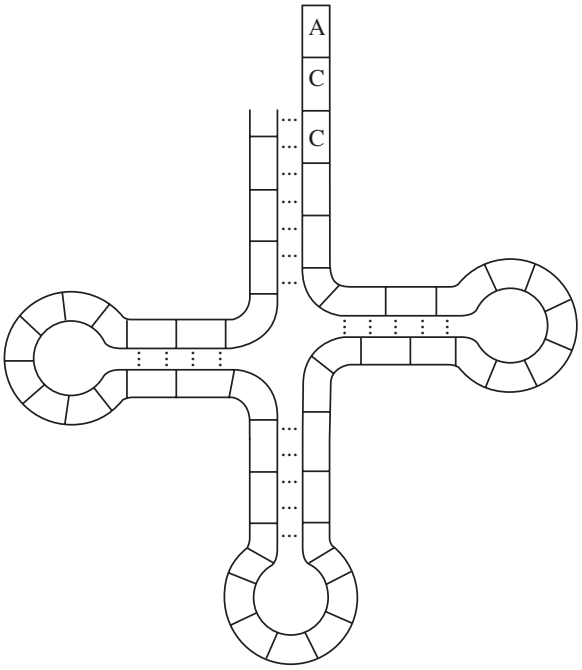


Fig. 8.2.10. A model of a transfer RNA molecule. A single-stranded tRNA molecule folds back on itself and becomes double helical in the regions shown by the dotted hydrogen bonds. (The actual helicity is not shown in the figure.) Note that there are several nonhelical (non-hydrogen-bonded) turns, at the bottom, right, and left sides. The anticodon is in the nonhelical region at the bottom; see the text.

and it has molecular weights ranging from around a hundred up to several thousand. The functions of the various RNAs will be discussed shortly.

Proteins are polymers of amino acids.

The monomer of a protein is an amino acid, a synonym for which is *residue*. A protein polymer is often called a *polypeptide*. While many amino acids can exist, only twenty are found in proteins. They share the general structure shown in Figure 8.2.11. The group labeled *R* can take on twenty different forms, thus accounting for all members of the group.[4] The right end (-COOH) is the *carboxyl* and the bottom ($-NH_2$) is the *amino* end.

Figure 8.2.12 shows how two amino acids are polymerized into a dipeptide (two residues). Note that the attachment takes place by combining the amino end of one residue with the carboxyl end of the other. The covalent bond created in this process is called a *peptide bond*, as shown in Figure 8.2.12.

An interesting feature of a dipeptide is that, like an individual amino acid, it has both a carboxyl end and an amino end. As a result, it is possible to add other residues

[4] We will ignore the fact that one of the amino acids is a slight exception.

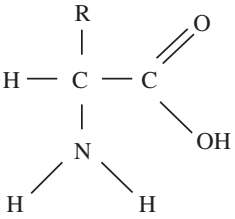


Fig. 8.2.11. A model of an amino acid, which is the monomer of a protein. The label *R* stands for any one of twenty different groups. (The text mentions a slight exception.) Thus twenty different amino acids may be found in proteins.

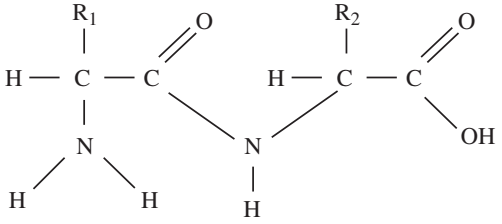


Fig. 8.2.12. A pair of amino acids bonded covalently into a dipeptide. The labels R_1 and R_2 can be any of the twenty groups mentioned in the caption of Figure 8.2.11. Thus there are 400 different dipeptides.

to the two ends of the dipeptide and thereby to extend the polymerization process as far as we like. It is quite common to find natural polypeptides of hundreds of residues and molecular weights over a hundred thousand. Figure 8.2.13 is an idealized picture of a polypeptide "backbone''; the individual amino acids are represented as boxes. Note that the polymer has a three-dimensional structure that includes helical regions and sheetlike regions, and that the whole three-dimensional shape is maintained by H-bonds and disulfide (-S-S-) bonds. The disulfide bonds are covalent, and the two amino acids that contribute the sulfur atoms are generally far from one another as measured along the polymer. They are brought into juxtaposition by the flexibility of the polymer and held there by the formation of the disulfide bond itself.

Our model of a protein is that of a long polymer of amino acids, connected by peptide bonds and folded into some kind of three-dimensional structure. At any location any of twenty different amino acids may appear. Thus there are 20^{100} possible polypeptides of 100 amino acids in length. Nowhere near this number have actual biological functions, but the incomprehensibly large number of possible amino acid sequences allows living systems to use proteins in diverse ways. Some of these ways will be described next.

Some proteins are catalysts.

There exists a very important class of proteins, called *enzymes*, whose function it is to to speed up the rate of biochemical reactions in cells (see [2] and [4]). In

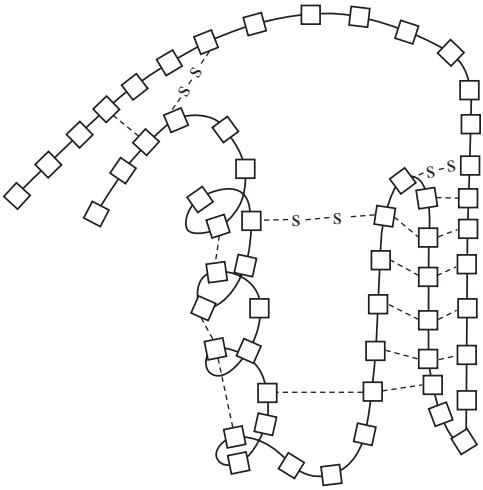


Fig. 8.2.13. A model of a single protein, or polypeptide, molecule. Each box corresponds to an amino acid. The resultant chain is held in a roughly ovate shape by sulfur-to-sulfur covalent bonds and by many hydrogen bonds, a few of which are indicated by dashed lines.

Increasing time	A	B	
↓	100	0	← Initial state concentrations
	75	25	
	50	50	
	10	90	← Final (equilibrium) state concentrations

Fig. 8.2.14. The progress of the reaction $A \leftrightarrow B$. The numbers give the amounts of the compounds A and B at various times. At the outset, there is no B, but as time passes, the amount of B increases until A and B reach equilibrium at a ratio of $B : A = 9 : 1$.

order to understand this function we must understand what is meant by "reaction rate": Suppose there is a chemical reaction described by $A \longleftrightarrow B$, as shown in Figure 8.2.14. Let us suppose that initially there is lots of A and no B. As time passes, some A is converted to B, and also some B back to A. Eventually, the relative amounts of A and B reach steady values, i.e., do not change with time. This final state is called an *equilibrium state*. The speed with which A is converted to B is the rate of the *reaction*. The observed rate evidently changes with time, starting out fast and reaching a net of zero at equilibrium, and therefore it is usually measured at the outset of the experiment, when there is lots of A and no B.

There are several very important biological consequences of enzymatic catalysis. First, the essential effect of a catalyst is to speed up the rate of a reaction. A biochemical catalyst, i.e., an enzyme, can speed up the rate of a biochemical reaction by as much as 10^{13} times. This enormous potential increase has some very important consequences to cellular chemistry: First, catalyzed biochemical reactions are fast enough to sustain life, but uncatalyzed reactions are not. Second, if a reaction will not

proceed at all in the absence of a catalyst, then no catalyst can ever make it proceed. After all, speeding up a rate of zero by 10^{13} still gives a rate of zero. Third, catalysts have no effect whatsoever on the relative concentrations of reactants and products at equilibrium, but they do affect the time the system takes to reach that equilibrium. Thus enzymes do not affect the underlying chemistry or net energetic requirements of the system in which they participate. Fourth, enzymes are very specific as to the reactions that they catalyze, their activity usually being limited to a single kind of reaction. This observation can be combined with the first one above (enzymatic increase in reaction rate) to yield an important conclusion: Whether a particular biochemical reaction goes at a high enough rate to sustain life depends entirely on the presence of specific enzyme molecules that can catalyze that particular reaction. Thus enzymes act like valves, facilitating only the reactions appropriate to a particular cell. No other reactions proceed fast enough to be significant and so they can be ignored.

The valvelike function of enzymes explains why a human and a dog can eat the same kind of food, drink the same kind of water, and breathe the same air, yet not look alike. The dog has certain enzymes that are different from those of the human (and, of course, some that are the same). Thus many biochemical reactions in a dog's cells proceed in a different direction from those in a human—in spite of there being the same initial reactants in both animals. Figure 8.2.15 shows how different metabolic paths can originate from the same starting point because of different enzyme complements.

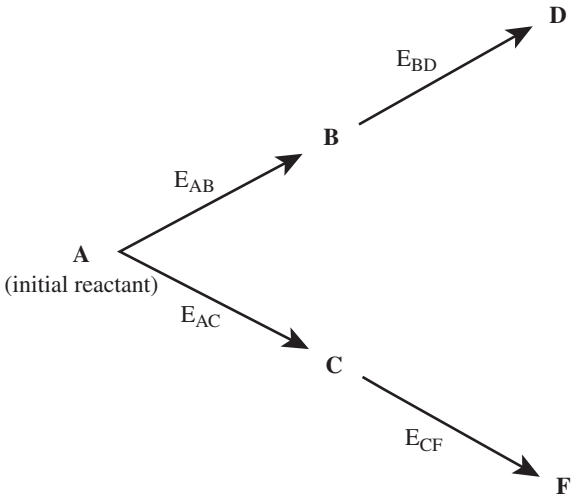


Fig. 8.2.15. A diagram showing how enzymes can direct sequences of reactions. A is the initial reactant, and the pair of enzymes E_{AB} and E_{BD} would catalyze the conversion of A to D. Alternatively, the enzymes E_{AC} and E_{CF} would catalyze the conversion of A to F. It is the enzymes, not the initial reactant, that determine what the end product will be. Of course, this does not mean that there will always exist an enzyme that can catalyze a particular reaction; rather, there will almost always exist an enzyme that can catalyze the particular reactions needed by a given cell.

The same reasoning explains why two people have different hair color, or numerous other differences.

The nature of the specificity of an enzyme for a single chemical reaction can be understood in terms of a "lock and key" mechanism: Suppose that we are again dealing with the reaction $A \longleftrightarrow B$, catalyzed by the enzyme E_{AB}. The catalytic event takes place on the surface of the enzyme at a specific location, called the *active site*, as shown in Figure 8.2.16. The compound A, or *substrate*, has a shape and electrical charge distribution that are complementary to the active site. This ensures that only the reaction $A \longleftrightarrow B$ will be catalyzed. Note that this reaction is reversible, and that the enzyme catalyzes in both directions.

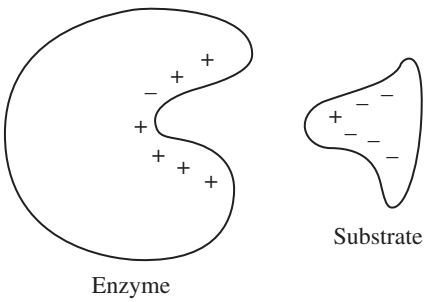


Fig. 8.2.16. A model of the "lock and key" mechanism for enzyme–substrate specificity. The enzyme and the substrate are matched to each other by having complementary shapes and electrical charge distributions.

Now we are in a position to understand why the three-dimensional structure of an enzyme is so important. Refer back to Figure 8.2.13 and recall that H-bonds and disulfide bonds hold together amino acids that are far from one another in the primary amino acid sequence. Therefore, the active site may be composed of several amino acids that are separated along the polymeric chain by a hundred or more intervening amino acids, but which are held close together by virtue of the folded three-dimensional polypeptide structure. This means that anything that disturbs, or denatures, the folded structure may disrupt the active site and therefore destroy enzymatic activity. All that is necessary is to break the hydrogen and disulfide bonds that maintain the three-dimensional structure. We can now see why cells are sensitive to heat: Heating to about 50°C inactivates their enzymes, quickly reducing the rates of their reactions to almost zero. Later in this chapter, we will return to the topic of enzymatic function.

Noncatalytic proteins.

The immense diversity of possible protein structures allows these macromolecules to be used for many biological purposes. Many of these have nothing to do with catalysis. We will divide these noncatalytic proteins into two somewhat arbitrary, but customary, categories and discuss them next.

Category 1: *Fibrous proteins*. These are called "fibrous" because they consist of large numbers of polypeptides arranged in parallel to yield long, stringlike arrays. Collagen, for example, is a fibrous protein found in skin and other organs. It consists of shorter protein molecules, each staggered one quarter length from the next one and thus linked into very long strings. Collagen acts as a binder, the long fibers helping to hold our bodies together.

Other examples of fibrous proteins are found in muscle tissue. Each muscle cell contains large numbers of fibrous proteins that are capable of sliding past one another and exerting force in the process. Our muscles can then move our skeletons and, therefore, our bodies. What we call "meat" is just muscle cut from an animal, and of course, it contains a lot of protein.

Another example of a fibrous protein is *keratin*, which appears in several forms in hair and nails, among other places. Some keratins form ropes of multiple strands, held together by disulfide bonds. Other keratins form sheetlike structures. One important form of keratin is silk, a threadlike exudation used in the wrapping of the cocoon of the silkworm *Bombyx mori*.

Category 2: *Globular proteins*. These proteins tend to be spherical or ovate and are often found dispersed, e.g., dissolved in solution. If aggregated, they do not form fibers. Enzymes are globular proteins, but we have already discussed them, and we will therefore restrict our discussion here to noncatalytic globular proteins.

As an example, the polypeptides *hormones* are typical noncatalytic globular proteins. They were introduced in Chapter 7. Hormones are biochemical communicators: They are manufactured in *endocrine glands* in one part of the body and are moved by the bloodstream to another part of the body, where they exert their effects on *target tissues*. At their target tissues hormones change the production and activity of enzymes and alter membrane permeability.

Insulin, a globular protein hormone, is produced by an organ called the pancreas and is released into the blood, to be carried throughout the body. The function of insulin is to regulate the metabolism of glucose in the body's cells. Lack of insulin has powerful metabolic consequences: The disorder *diabetes mellitus* is associated with the loss of insulin-producing cells of the pancreas, increases in the glucose levels of blood and urine, malaise, and even blindness.

Another class of noncatalytic globular proteins, introduced in Chapter 6, determines the selectivity of material transport by membranes. These proteins recognize and regulate the intercellular movements of specific compounds such as amino acids and various sugars and ions such as Na^+ and Cl^-. Called *transport proteins*, or *permeases*, they penetrate through membranes and have a sort of active site on one end to facilitate recognition of the material to be transported. They are, however, not catalysts in that the transported matter does not undergo a permanent chemical change as a result of its interaction with the transport protein.

Globular proteins are used to transport material in the body. One example, *hemoglobin*, which is discussed in Chapter 9, contains four polypeptide chains and four heme groups, the latter being organic groups with an iron atom. Hemoglobin is

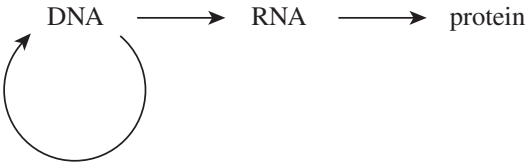
found in red blood cells, or *erythrocytes*. The principal use of hemoglobin is to carry oxygen from the lungs to the sites of oxygen-utilizing metabolism in the body.

Globular proteins are key molecules in our immune systems. A group of blood cells, called *lymphocytes*, are able to distinguish between "self" and "nonself" and therefore to recognize foreign material, such as pathogens, in our bodies. These foreign substances are often proteins but may be polysaccharides and nucleic acids; in any case, if they stimulate immune responses they are called *antigens* (Ag). Antigens stimulate lymphocytes to produce a class of globular proteins, called *antibodies* (Ab) or *immunoglobulins*, that can preferentially bind to Ag, leading to the inactivation of the Ag. The immune response will be discussed in some detail in Chapter 10.

Of particular importance to us in that chapter will be the globular proteins found in a covering, or *capsid*, of viruses. Viruses have very elementary structures, the simplest being a protein coat surrounding a core of genetic material. Viruses are so small that the amount of genetic material they can contain is very limited. Thus as an information-conserving mechanism, they use multiple copies of the same one or two polypeptides to build their protein coverings. Thus a typical virus may have an outer coat consisting of hundreds of copies of the same globular protein.

8.3 Molecular Information Transfer

This section is a discussion of molecular genetics. The ability of DNA to guide its own self-replication was described in an earlier section. In this section, we will see how genetic information of DNA, coded into its polymeric base sequence, can be converted into base-sequence information of RNA. The base-sequence information of RNA can then be converted into amino acid–sequence information of proteins. The amino acid sequence of a protein determines its three-dimensional shape and therefore its function, i.e., participation in O_2 *transport in erythrocytes, selection of material to cross a membrane, or catalysis of a specific biochemical reaction. The net process is contained in the following statement: DNA is the hereditary chemical because it provides an informational bridge between generations via self-replication, and it ultimately determines cellular chemistry. These processes are schematically condensed into the central dogma of genetics:*



It is very important to recognize that the arrows of the central dogma show the direction of information flow, not the direction of chemical reactions. Thus DNA passes its information on to RNA—the DNA is not chemically changed into RNA.[5]

[5] We will modify this "dogma" somewhat in Chapter 10.

Information flow from DNA to RNA is called transcription.

Recall that enzymes determine which reactions in a cell effectively take place. For organisms other than certain viruses, DNA is the source of the information that determines which enzymes will be produced. In any case, there is an intermediary between DNA and proteins—it is RNA. This is expressed in the central dogma presented above (see [5] and [6]).

RNA production is shown schematically in Figure 8.3.1. The sequence of the single covalent strand of RNA nucleotides is determined by complementary H-bonding with one strand of a DNA molecule; in other words, the single, or coding, strand of DNA acts as a template for RNA production. Note the similarity between the use of a single-stranded DNA template for DNA production and the use of a single-stranded DNA template for RNA production. The differences are that RNA uses a different sugar and substitutes uracil in place of thymine.

The process of RNA production from DNA, called *transcription*, requires that the DNA molecule become denatured over a short portion of its length, as shown in Figure 8.3.1. This is a simple matter energetically because all that is required is to break a small number of H-bonds. The O-shaped denatured region moves along the DNA molecule, the double helix opening up at the leading edge of the "O" and closing at its trailing edge. RNA molecules, as mentioned earlier, are usually less than a thousand or so nucleotides long. Thus RNA replication normally begins at many sites in the interior of the DNA molecule, whose length may be on the order of millions of nucleotides.

Information flow from RNA to enzymes is called translation.

The process of protein production from RNA code brings together, one by one, all three kinds of RNA: ribosomal, messenger, and transfer. The three varieties are transcribed from the DNA of the cell and exported to sites away from the DNA. Here subcellular structures called *ribosomes* are constructed, in part using the rRNA. Ribosomes are the sites of protein synthesis, but the actual role of the rRNA is not well understood.

Several dozen different kinds of transfer RNA are transcribed from DNA. They all have a structure similar to that shown in Figures 8.2.10 and 8.3.2, which shows that tRNA is single stranded, but is helical by virtue of the folding of the polymer on itself. This requires that some regions on the strand have base sequences that are complementary to others, but in reverse linear order. (Recall from Figure 8.2.7 that a nucleic acid double helix requires that the two strands be antiparallel.) The various kinds of tRNA differ in their constituent bases and overall base sequences; the most important difference for us, however, is the base sequence in a region called the *anticodon*, at the bottom of the figure. The anticodon is actually a loop containing three bases that, because of the looping, are not H-bonded to any other bases in the tRNA molecule.

Let us consider the anticodon more closely. It contains three nucleotides that are not hydrogen-bonded to any other nucleotides. The number of such trinucleotides, generated at random, is $4^3 = 64$, so we might expect that there could be 64 different

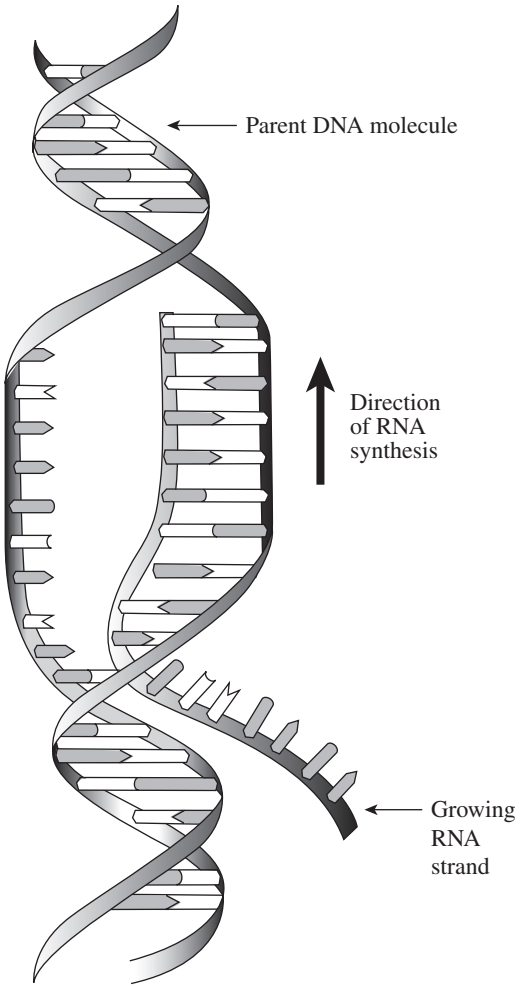


Fig. 8.3.1. A model showing the polymerization of RNA, using a DNA template. The DNA opens up to become temporarily single stranded over a short section of its length, and one of the two DNA strands then codes for the RNA. Complementary hydrogen-bonding between the DNA nucleotides and the RNA nucleotides ensures the correct RNA nucleotide sequence. (Redrawn from J. Levine and K. Miller, *Biology*, 1st ed., D. C. Heath, Lexington, MA, 1991. Used with permission.)

kinds of tRNA, if we considered only the anticodons. Actually, fewer than that seem to exist in nature, for reasons to be discussed shortly. The anticodon bases are not H-bonded to any other bases in the tRNA molecule but are arranged in such a three-dimensional configuration that they can H-bond to three bases on *another* RNA molecule.

All tRNA molecules have a short "pigtail" at one end that extends beyond the opposite end of the polymer. This pigtail always ends with the sequence CCA. An

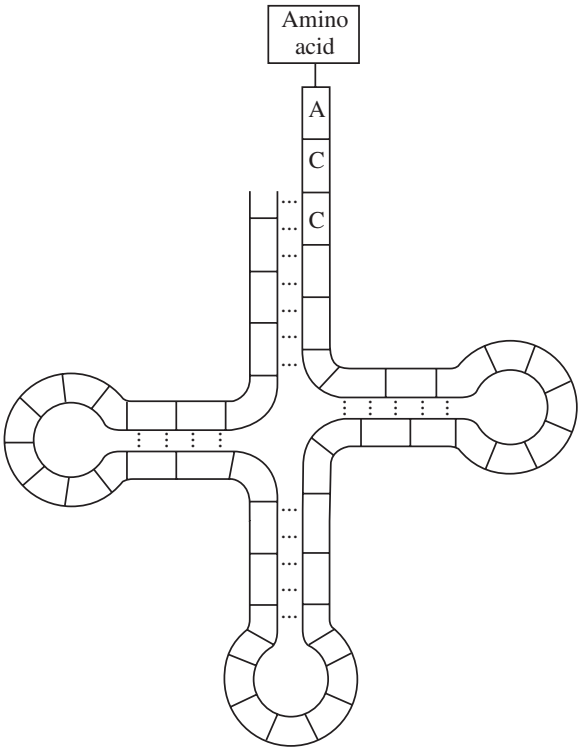


Fig. 8.3.2. A model of a tRNA molecule, with an amino acid attached to one end. An enzyme ensures that the tRNA molecule becomes covalently attached to its correct amino acid. The anticodon region is at the bottom. Compare this figure with Figure 8.2.10.

amino acid can be covalently attached to the terminal adenine, giving a tRNA amino acid molecule, as shown in Figure 8.3.2. A given type of tRNA, identified by its anticodon, can be attached to one, and only one, specific type of amino acid. No other pairings are possible. When we see such specificity in biochemistry, we should always suspect that enzymes are involved. In fact, there are enzymes whose catalytic function is to link up an amino acid with its correct tRNA. A tRNA molecule that is attached to its correct amino acid is said to be "charged."

Messenger RNA consists of strings of about 1000 or so nucleotides, but that is only an average figure—much mRNA is considerably longer or shorter. The reason for this variability is that each piece of mRNA is the transcription product of one or a few genes on DNA. Thus the actual length of a particular piece of mRNA corresponds to an integral number of DNA genes, and of course, that leads to a great deal of variability in length. After being exported from the DNA, the mRNA travels to a ribosome, to which it becomes reversibly attached.

The next part of this discussion is keyed to Figure 8.3.3:

(a) One end of a piece of mRNA is attached to a ribosome, the area of association covering at least six mRNA nucleotides.

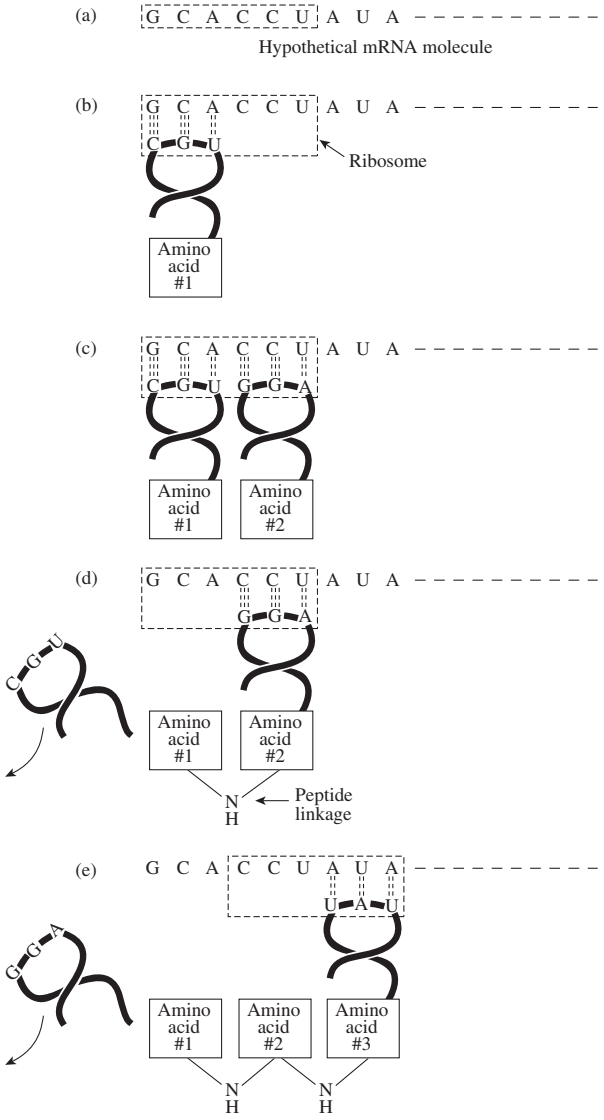


Fig. 8.3.3. The polymerization of a polypeptide, using DNA information and RNA intermediaries. (a) A ribosome attaches to the end of an mRNA molecule. (b) A molecule of tRNA, with its correct amino acid attached, hydrogen-bonds to the mRNA. The hydrogen-bonding is between the first three nucleotides of the mRNA (a codon) and the three tRNA nucleotides in a turn of tRNA (an anticodon). Each tRNA has several such turns, as depicted in Figures 8.2.10 and 8.3.2, but only one is the anticodon. (c) A second tRNA then hydrogen-bonds to the mRNA, thus lining up two amino acids. (d) The two amino acids are joined by a covalent bond, a process that releases the first tRNA. (e) The ribosome moves down the mRNA molecule by three nucleotides and a third tRNA then becomes attached to the mRNA. The process continues until an intact protein is formed. Note how the amino acid sequence is ultimately dictated by the nucleotide sequence of the DNA.

(b) A tRNA molecule, with its correct amino acid attached, forms complementary H-bonds between its anticodon and the first three nucleotides of the mRNA. The latter trinucleotide is called a *codon*. Note that codon–anticodon recognition mates up not only the correct anticodon with its correct codon, but in the process also matches up the correct amino acid with its codon.
(c) Next, a second charged tRNA hydrogen-bonds to the second mRNA codon.
(d) A peptide linkage forms between the two amino acids, detaching the first amino acid from its tRNA in the process.

Let us review what has happened so far: A sequence of DNA nucleotides comprising a small integral number of genes has been transcribed into a polymer of mRNA nucleotides. The sequence of the first six of these nucleotides has subsequently been translated into the sequence of two amino acids. There is a direct informational connection mapping the sequence of the original six DNA nucleotides into the sequence of the two amino acids. The correctness of this mapping is controlled by two physical factors: First, complementarity between DNA and mRNA and between mRNA and tRNA, and second, specific enzymatic attachment of tRNA to amino acids.

Now returning to Figure 8.3.3, the ribosome moves three nucleotides down the mRNA and a third charged tRNA attaches to the mRNA at the third codon:

(e) A third amino acid is then added to the growing polypeptide chain. The translation process continues and eventually a complete polypeptide chain is formed. The nucleotide sequence of the DNA has been converted into the primary structure of the polypeptide. Note how the conversion of nucleotide sequence to amino acid sequence was a transfer of information, not a chemical change of DNA to protein.

Figure 8.3.3 is really a pictorial representation of the central dogma. The overall process yields proteins, including enzymes of course. These enzymes determine what chemical reactions in the cell will proceed at a rate consistent with life. Two very important observations come out of this discussion: First, the chemistry of a cell is ultimately determined by the sequence of DNA nucleotides, and second, because of this, the replication and partitioning of DNA during cell division ensures that daughter cells will have the same chemistry as the parent cell. We can extend the latter conclusion: The union of a sperm and an egg in sexual reproduction combines genetic material from two parents into a novel combination of DNAs in a new organism, thus ensuring that the offspring has both chemical similarities to, and chemical differences from, each of the parents.

A gene is enough nucleic acid to code for a polypeptide.

The word "gene'' is often loosely used to mean "a site of genetic information.'' A more exact definition from molecular biology is that a gene is a sequence of nucleotides that codes for a complete polypeptide. This definition, however, requires the elaboration of several points:

1. If a functioning protein's structure contains two separately created polypeptides, then by definition, two genes are involved.

2. As will be discussed below, some viruses eliminate DNA from their replicative cycle altogether. Their RNA is self-replicating. In those cases, their genes are made of RNA.
3. The nucleotide sequence for any one complete gene normally lies entirely on one strand of DNA, called the *coding strand*. Note that coding segments for a gene, called *exons*, are not generally contiguous. Noncoding stretches between coding segments are *introns*; see Section 14.6. Furthermore, not all genes need lie on the same one strand; transcription may jump from one strand to another between gene locations. There may even be overlapping genes on the same strand.

The concept of coding.

The aptly named *genetic code* can be presented in a chart showing the correspondence between RNA trinucleotides (codons) and the amino acids they specify, the *codon translation* table; see Table 8.3.1.

Table 8.3.1. Codon translation table.

	T	C	A	G
T	TTT Phe(F)	TCT Ser(S)	TAT Tyr(Y)	TGT Cys(C)
	TTC Phe(F)	TCC Ser(S)	TAC Tyr(Y)	TGC Cys(C)
	TTA Leu(L)	TCA ”	TAA stop	TGA stop
	TTG ”	TCG ”	TAG stop	TGG Trp(W)
C	CTT Leu(L)	CCT Pro(P)	CAT His(H)	CGT Arg(R)
	CTC ”	CCC ”	CAC ”	CGC ”
	CTA	CCA ”	CAA Gln(Q)	CGA ”
	CTG ”	CCG ”	CAG ”	CGG ”
A	ATT Ile(I)	ACT Thr(T)	AAT Asn(N)	AGT Ser(S)
	ATC ”	ACC ”	AAC ”	AGC ”
	ATA ”	ACA ”	AAA Lys(K)	AGA Arg(R)
	ATG Met(M)	ACG ”	AAG ”	AGG ”
G	GTT Val(V)	GCT Ala(A)	GAT Asp(D)	GGT Gly(G)
	GTC ”	GCC ”	GAC ”	GGC ”
	GTA ”	GCA ”	GAA Glu(E)	GGA ”
	GTG ”	GCG ”	GAG ”	GGG ”

Several interesting features emerge from considering such a table. There are 64 codons potentially available to specify 20 amino acids. It turns out, however, that there are only about half that many distinctive tRNA molecules, indicating that some tRNAs can bind to more than one codon. This redundancy is explained by the *wobble hypothesis*: Examination of tRNA structure shows that the nucleotide at one end of the anticodon has only a loose fit to the corresponding codon nucleotide—it wobbles. Thus H-bonding specificity is relaxed at this position and some tRNAs can bind to more than one codon.[6]

[6] Recall that polynucleotides have directionality; thus the two ends of a codon or anticodon are distinct. Only the one drawn at the right-hand end wobbles.

Not all possible codons specify an amino acid. Three of them are *termination codons*, or *stop codons*. They do not specify any amino acid; rather, they signal the ribosome to cease translation and to release the completed polypeptide. This is especially useful if one piece of mRNA codes for two adjacent genes: termination codons signal the translation machinery to release the first polypeptide before starting on the translation of the second one. Without the termination codons, the ribosome would continue to add the amino acids of the second polypeptide to the end of the first one, negating the biological functions of both.

The nature of mutations.

Mutations are changes in the nucleotide sequence of DNA. A base change in a codon would probably result in a new amino acid being coded at that point. For example, sickle-cell anemia results from a single incorrect amino acid being inserted into the protein fraction of hemoglobin. Suppose a nucleotide pair were deleted: Virtually every amino acid encoded thereafter (downstream) would be incorrect. Evidently, the severity of a *deletion error*, or an *insertion error*, for that matter, depends on how close to the start of transcription it occurs.

8.4 Enzymes and Their Function

Two important concepts that have been presented in this chapter are the central dogma of genetics and the role of enzymes in facilitating specific chemical reactions in a cell. DNA, via RNA, codes for a specific set of cellular enzymes (*among other proteins*). *Those enzymes can catalyze a specific set of chemical reactions and thereby determine the biological nature of the cell.*

In this section, we will take a closer look at the way that enzymes work. Our approach will be a thermodynamic one, following the path of solar energy into biological systems, where it is used to create orderly arrangements of atoms and molecules in a cell. We will show how enzymes select from among the many possible configurations of these atoms and molecules to arrive at those that are peculiar to that type of cell.

The sun is the ultimate source of energy used by most biological systems.

The sun is the ultimate source of energy available to drive biological processes. (We ignore the tiny amounts of energy available from geothermal sources.) Its contributions are twofold: First, solar energy can be captured by green plants and incorporated into chemical bonds, from which it can be then obtained by animals that eat the plants and each other. Second, solar energy heats the biosphere and thus drives biochemical reactions, virtually all of whose rates are temperature-dependent. Both of these considerations will be important in the discussion to follow.

Entropy is a measure of disorder.

A highly disordered configuration is said to have high *entropy*. The most disordered

of two configurations is the one that can be formed in the most ways. To show how this definition conforms to our everyday experience, consider the possible outcomes of tossing three coins: HHH, HHT, HTH, THH, HTT, THT, TTH, TTT. There is only one way to get all heads, but there are six ways to get a mixture of heads and tails. Thus a mixture of heads and tails is the more disordered configuration. The condition of mixed heads and tails has high entropy (is a disorderly outcome), and the condition of all heads has low entropy (is an orderly outcome). Note that all eight specific configurations have the same probability ($\frac{1}{8}$), but that six of them contain at least one head and one tail.

Given that there generally are more disordered outcomes than there are ordered outcomes, we would expect that disorder would be more likely than order. This, of course, is exactly what we see in the case of the coins: Throw three coins and a mixture of heads and tails is the most common result, whereas all heads is a relatively uncommon result.

The universe is proceeding spontaneously from lower to higher entropy.

An empirical rule, the *second law of thermodynamics*, states that the entropy of the universe increases in every process. For instance, if a drop of ink is placed in a beaker of water, it will spontaneously spread throughout the water. There are few ways to put all the ink into one spot in the water and many ways to distribute it throughout the water, so we see that the entropy of the water/ink mixture increases. As other examples, consider what happens when the valve on a tank of compressed gas is opened or when a neatly arranged deck of cards is thrown up into the air. In each case, entropy increases.

The second law does not preclude a decrease in entropy in some local region. What it does require is that if entropy decreases in one place it must increase somewhere else by a greater absolute amount. There is no reason why the ink, once dispersed, cannot be reconcentrated. The point is that reconcentration will require some filtration or adsorption procedure that uses energy and generates heat. That heat will cause air molecules to move, and rapidly moving air molecules have more entropy (are more disordered) than slowly moving molecules. Likewise, the air can be pumped back into the tank and the cards can be picked up and resorted, both of which processes require work, which generates heat and therefore entropy.

Living systems are local regions of low entropy; their structures are highly organized, and even small perturbations in that organization can mean the difference between being alive and not being alive. From the earlier discussion, we can see that nothing in the second law forbids the low entropy of living systems, as long as the entropy of the universe increases appropriately during their formation.

Entropy increases in a process until equilibrium is reached.

Recall the examples of the previous section: The ink disperses in the water until it is uniformly distributed; the gas escapes the tank until the pressure is the same inside and outside of the tank; the cards flutter helter-skelter until they come to rest on a

surface. In each case, the process of entropy-increase continues to some endpoint and then stops. That endpoint is called an *equilibrium state*.

Any equilibrium can be disrupted; more water can be added to the ink, the room containing the gas can be expanded, and the table bearing the cards can drop away. In each case, the system will then find a new equilibrium. Thus we can regard equilibria as temporary stopping places along the way to the maximal universal entropy predicted by the second law.

Free energy is energy available to do useful work.

Every organism needs energy for growing, moving, reproducing, and all the other activities we associate with being alive. Each of these activities requires organized structures. To maintain this organization, or low entropy, requires that the living system expend energy, much as energy was required to reconcentrate the ink or to re-sort the cards in the earlier examples.

Free energy is energy that can do useful work. In living systems, "useless" work is that which causes a volume change or which increases entropy. Whatever energy is left over is "free" energy. Living systems do not change their volume much, so entropy is the only significant thief of free energy in a cell. Therefore, free energy in a cell decreases when entropy increases. To a good approximation, we can assume that a living system begins with a certain amount of potential energy obtained from sunlight or food; some energy will then be lost to entropy production, and the remainder is free energy.[7]

To a physical chemist, the convenient thing about free energy is that it is a property of the system alone, thus excluding the surroundings. In contrast, the second law requires that one keep track of the entropy of the entire universe. As a result, it is usually easier to work with free energy than with entropy. We can summarize the relationship between the two quantities as they pertain to living systems by saying that entropy of the universe always increases during processes and that a system in equilibrium has maximized its entropy, whereas the free energy of a system decreases during processes and, at equilibrium, the system's free energy is minimized.

Free energy flows, with losses, through biological systems.

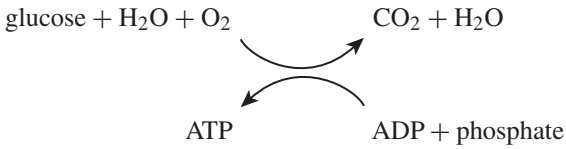
Thermonuclear reactions in the sun liberate energy, which is transmitted to the earth as radiation, which is absorbed by green plants. Some of the sun's radiation then heats the plant and its surroundings, and the rest is incorporated into glucose by *photosynthesis*. In photosynthesis, some of the free energy of the sun is used to create covalent bonds among parts of six carbon dioxide molecules, forming glucose, the six-carbon sugar, as shown in the following (unbalanced) reaction:[8]

[7] If you have studied physical chemistry, you will recognize this quantity specifically as Gibbs's free energy [4].

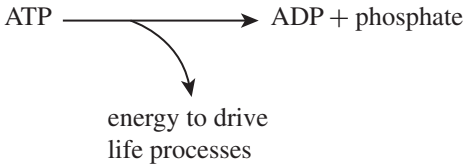
[8] The reason that water appears on both sides of the reaction equation is that the two water molecules are not the same: One is destroyed and the other is created in the reaction. The reaction shown is a summary of the many reactions that constitute photosynthesis.

$$CO_2 + H_2O \xrightarrow{\text{light energy}} \text{glucose} + H_2O + O_2$$

The plant, or an animal that eats the plant, then uses some of the free energy of the glucose to add a phosphate group to adenosine diphosphate (ADP) in the process called *respiration*:



The resultant adenosine triphosphate now has some of the energy that originated in the sun. The ATP can then move around the cell by diffusion or convection and drive various life processes (moving, growing, repair, driving Na/K pumps, etc.):



To recapitulate: Sunlight drives photosynthesis, in which carbon dioxide is combined to make glucose. The latter thus contains some of the energy that originated in the sun. In respiration, the plant or animal that eats the plant then converts some of the free energy in the glucose into free energy of ATP. Finally, at a site where it is needed, the ATP gives up its free energy to drive a biological process, e.g., contraction of a muscle.

At every step along the way from sun to, e.g., muscle movement, entropy is created and free energy is therefore lost. By the time an animal moves its muscle, only a small fraction of the original free energy the green plant got from the sun remains. If a subsequent carnivore should eat the herbivore, still more free energy would be lost. After the carnivore dies, decomposing organisms get the last of whatever free energy is available to living systems.

The heat generated in biochemical reactions can help to drive other reactions.

The earlier discussion pointed out that free energy, ultimately derived from the sun, is used to drive the processes we associate with being alive. As these processes occur, entropy is generated. Although the resultant heat energy will eventually be lost to the surroundings, it can be stored for a short while in the water of the cell and thus be used to maintain or increase the rates of cellular chemical reactions.

In order to understand how heat energy can promote chemical reactions, we need to digress a bit. If a process were able to occur spontaneously (increasing entropy; decreasing free energy), why would it not have already occurred? Water should spontaneously flow from a lake to the valley below, as shown in Figure 8.4.1(a). This has not happened because there is a dam in the way, but a siphon would take

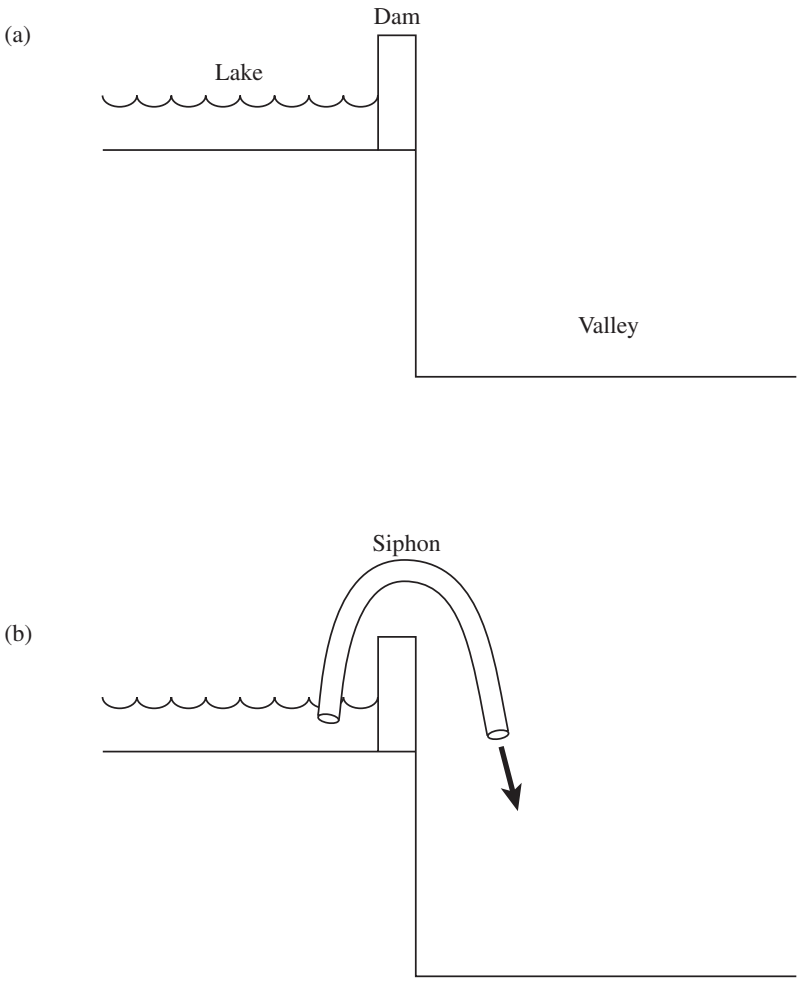


Fig. 8.4.1. (a) A lake holds back water above a valley; thus the water has a certain amount of potential energy with respect to the valley. (b) The water can get past the dam via a siphon, but the energy of the water with respect to the valley is not changed by the trip through the siphon. In other words, the energy yielded by the water in falling to the valley is independent of the path it takes. (We are assuming that friction is negligible.)

care of that without any net outlay of energy (Figure 8.4.1(b)). The latter point is critical: The water going up the siphon requires the same amount of energy that it gets back in going down the siphon.[9] From that point on, the water can fall to the valley, developing exactly as much kinetic energy as it would have if the dam had not existed in the first place.

[9] We are ignoring friction here.

The example of the dam is a macroscopic analogue to biochemical processes. For example, in respiration a cell takes up glucose, a high-free-energy compound, and converts it to CO_2, a low-free-energy compound. This process, on thermodynamic grounds, should therefore be spontaneous. In fact, we can demonstrate a spontaneous change of glucose to CO_2 by putting some glucose into an open dish, from which it will disappear over a period of days to weeks, via conversion to CO_2 and H_2O. The reason the process in the dish takes so long is that there is an intermediate state (in reality, several) between glucose and CO_2 and H_2O, as shown in the free energy diagram in Figure 8.4.2. The intermediate state is called a *transition state*, and it is the analogue of the dam in Figure 8.4.1. Before the sugar can change to the gas, releasing its free energy, the transition state must be overcome, i.e., a certain amount of *activation energy* is needed to move the system into the transition state.[10]

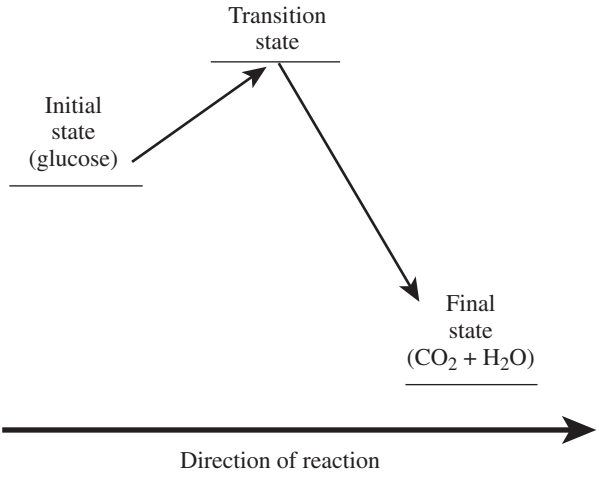


Fig. 8.4.2. A free energy diagram of the following conversion: glucose $\rightleftarrows CO_2 + H_2O$. There is a transition state between the initial and final states. Even though the conversion of glucose to CO_2 and H_2O is energetically downhill, it will not be a spontaneous conversion because of the transition state.

This energy is returned on the other side of the transition state, after which the chemical system behaves as if the transition state were not there. The examples of the dam and the glucose suggest a general conclusion: The net change in free energy between two states is independent of any intermediate states.

Transition states are the rule, not the exception, and the biochemical reactions of living systems are typical in that those that release free energy must first be activated.

[10] Figure 8.4.2 is, of course, only a model. The actual conversion of glucose to carbon dioxide in an open dish would involve numerous intermediate compounds, some of which would be real transition states and some of which would be more-or-less stable compounds. For instructive purposes, we represent the system as having a single transition state.

There are two sources of activation energy available to cells, however: First, most cells exist at 0–40°C, and second, heat energy is generated by the normal inefficiency of cellular processes.[11] This heat energy is stored in H-bond vibrations in the water of the cell, at least until it is finally lost to the external environment. While this heat energy is in the cell it is available to push systems into their transition states, thus promoting chemical reactions. After serving its activation function, the heat energy is returned unchanged.

The preceding discussion explains how heat serves a vital cellular function in providing activation energy to drive cellular biochemical reactions. This, however, does not close the subject, because activation energy is tied in with another observation: The glucose in a dish changes to CO_2 and H_2O over a period of months, and the same change can occur in a cell in seconds or less. Yet the temperatures in the dish and in the cell are the same, say 37°C. The difference is that the reactions in the cell are catalyzed by enzymes.

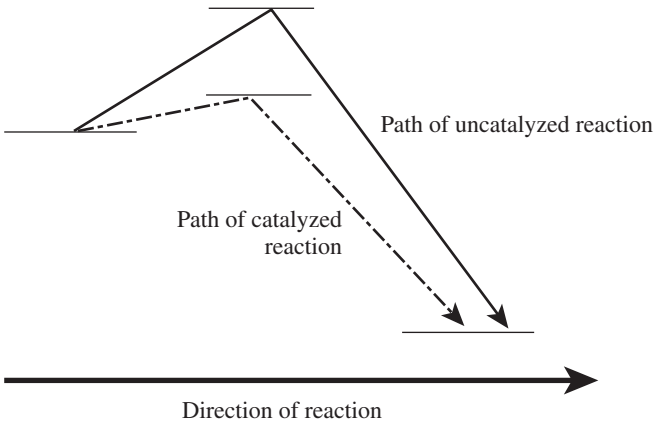


Fig. 8.4.3. The effect of enzymatic catalysis on the height of a transition state. The enzyme lowers the energy of the transition state, but as in Figure 8.4.1, the overall change in energy is independent of the path. Lowering the transition state does, however, permit the reaction to proceed spontaneously in the presence of a little thermal energy.

In brief, the catalytic function of an enzyme is to reduce the energy of the transition state and thereby to lessen the amount of heat energy needed by the system to meet the activation energy requirement. In this manner, the enzyme speeds up the rate at which the reaction proceeds from the initial state (100% reactant) toward the final, equilibrium state (almost 100% product). Figure 8.4.3 is a free-energy diagram for a biochemical system in its catalyzed and uncatalyzed conditions. The enzyme catalyst lowers the activation energy and makes it much easier for the initial state to be converted into the transition state and thus into the final state. The dependence of reaction rate on activation energy is exponential; thus a small change in activation

[11] Direct sunlight is also used by many "cold-blooded" animals to heat up their bodies.

energy can make a very big difference in reaction rate. For comparison, enzymatic catalysis potentially can speed up the rates of reactions by as much as 10^{13} times.

How much energy is actually available? At 30°C the average amount of heat energy available is about 0.025 eV per molecule, but the energy is unevenly distributed, and some substrate molecules will have more and some will have less. Those that have more will often have enough to get to the transition states made accessible by enzymatic catalysis.

8.5 Rates of Chemical Reactions

Stoichiometric rules are not sufficient to determine the equilibrium position of a reversible chemical reaction; but adding reaction rate principles makes the calculation possible. Primarily, rate equations were designed to foretell the speed of specific reactions, and in this capacity, they predict an exponential decaying speed, as reactants are consumed, characterized by the reaction's rate constant. But in fact, the equilibrium position of a reversible reaction is reached when the rate of formation equals the rate of dissociation. Therefore, equilibrium positions, as well as reaction rates, are determined by a combination of the forward and reverse rate constants.

Irreversible (*unidirectional*) *reactions are limited by the first reactant to be exhausted.*

Consider the irreversible bimolecular reaction

$$A + B \longrightarrow X + Y, \tag{8.5.1}$$

in which one molecule each of reactants A and B chemically combine to make one molecule each of products X and Y. It follows that the rate of disappearance of reactants equals the rate of appearance of products. The *conservation of mass* principle takes the form

$$\frac{dX}{dt} = \frac{dY}{dt} = -\frac{dA}{dt} = -\frac{dB}{dt}.$$

If M_0 denotes the initial number of molecules of species M, by integrating each member of this chain of equalities from time zero to time t, we get

$$X(t) - X_0 = Y(t) - Y_0 = -A(t) + A_0 = -B(t) + B_0. \tag{8.5.2}$$

Equation (8.5.2) gives the amount of each species in terms of the others, so if any one of them is known, then they all are. But in order to know the amount of any one of them, we must know how fast the reaction occurs. This is answered by the *law of mass action* (due to Lotka): The rate at which two or more chemical species simultaneously combine is proportional to the product of their concentrations. Letting $[M]$ denote the concentration of species M, the mass action principle states that the rate at which product is formed is equal to

$$k[A][B], \tag{8.5.3}$$

where the constant of proportionality k is characteristic of the reaction.

So far, our considerations have been completely general, but now we must make some assumptions about where the reaction is occurring. We suppose this to occur in a closed reaction vessel, such as a beaker with a fixed amount of water. In this case, concentration of a given species is the number of its molecules divided by the (fixed) volume of the medium.[12] There is the possibility that one or more of the products, X or Y, is insoluble and precipitates out of solution. This is one of the main reasons that a bimolecular reaction may be irreversible. In what follows, we use the notation $m(t)$ to denote the concentration of species M. In case some species, say X, precipitates out of solution, we can still interpret $x(t)$ to mean the number of its molecules divided by the volume of the medium, but it will no longer be able to participate in the reaction. In this way, we can calculate the amount of X that is produced. While a product may precipitate out without disturbing the (forward) reaction, the reactants must remain dissolved.

Combining the mass action principle with (8.5.2), we get

$$\frac{dx}{dt} = kab = k(a_0 + x_0 - x)(b_0 + x_0 - x) \tag{8.5.4}$$

with initial value $x(0) = x_0$. The stationary points of (8.5.4) are given by setting the right-hand side to zero and solving to get (see Section 2.4)

$$x = a_0 + x_0 \quad \text{or} \quad x = b_0 + x_0. \tag{8.5.5}$$

The first of these says that the amount of X will be its original amount plus an amount equal to the original amount of A. In other words, A will be exhausted. The second equation says the reaction stops when B is exhausted.

Suppose, just for argument, that $a_0 < b_0$; then also $a_0 + x_0 < b_0 + x_0$. While $x(t) < a_0 + x_0$, the right-hand side of (8.5.4) is positive; therefore, the derivative is positive and so x increases. This continues until x asymptotically reaches $a_0 + x_0$, whereupon the reaction stops. The progression of the reaction as a function of time is found by solving (8.5.4), which is variables separable:

$$\frac{dx}{(a_0 + x_0 - x)(b_0 + x_0 - x)} = kdt.$$

Note the similarity of this equation to the Lotka–Volterra system of Section 4.4. The left-hand side can be written as the sum of simpler fractions:

$$\frac{1}{(a_0 + x_0 - x)(b_0 + x_0 - x)} = \frac{1}{b_0 - a_0}\frac{1}{a_0 + x_0 - x} - \frac{1}{b_0 - a_0}\frac{1}{b_0 + x_0 - x}.$$

Thus (8.5.4) may be rewritten as

$$\left[\frac{1}{a_0 + x_0 - x} - \frac{1}{b_0 + x_0 - x}\right] dx = (b_0 - a_0)kdt.$$

[12] By contrast, for an open reaction vessel, such as the heart or a chemostat, the concentrations are determined by that of the inflowing reactants.

Integrating gives the solution

$$-\ln(a_0 + x_0 - x) + \ln(b_0 + x_0 - x) = (b_0 - a_0)kt + q,$$
$$\ln\left(\frac{b_0 + x_0 - x}{a_0 + x_0 - x}\right) = (b_0 - a_0)kt + q,$$

where q is the constant of integration. Now this may be solved for in terms of x,

$$x = \frac{(a_0 + x_0)Qe^{(b_0 - a_0)kt} - (b_0 + x_0)}{Qe^{(b_0 - a_0)kt} - 1}, \tag{8.5.6}$$

where $Q = e^q$ is a constant. This equation is graphed in Figure 8.5.1. The procedure described above can be performed by the computer using code such as the following:

```
  MAPLE
> k:=1; a0:=2; b0:=3; x0:=1/2;
> dsolve({diff(x(t),t)=k*(a0+x0-x(t))*(b0+x0-x(t)),x(0)=x0},{x(t)});
> simplify(%);
> x:=unapply(rhs(%),t);
> plot([t,x(t),t=0..4],t=-1..3,tickmarks=[3,3],labels=['t','x(t)']);

  MATLAB
  % make up an m-file, chemRate.m, with
  %   function xprime=chemRate(t,x);
  %   k=1; a0=2; b0=3; x0=0.5; xprime = k*(a0+x0-x)*(b0+x0-x);
> x0=0.5; [t,x]=ode23('chemRate',[0 4],x0);
> plot(t,x)
```

The result is

$$x(t) = \frac{1}{2}\frac{-15 + 14e^{-t}}{-3 + 2e^{-t}}.$$

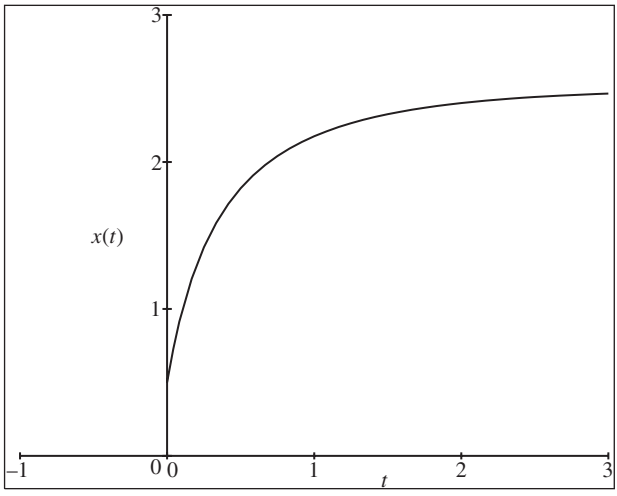


Fig. 8.5.1. A typical solution to (8.5.4).

Example. Suppose 2 moles of silver nitrate ($AgNO_3$) are mixed with 3 moles of hydrochloric acid (HCl). A white precipitate, silver chloride, is formed and the reaction tends to completion:

$$AgNO_3 + HCl \longrightarrow AgCl \downarrow + HNO_3.$$

From above, asymptotically, the reaction stops when the 2 moles of silver nitrate have reacted, leaving 2 moles of silver chloride precipitate and 1 mole of hydrochloric acid unreacted.

Kinetics for reversible reactions work the same way.

Now assume that reaction (8.5.1) is reversible,

$$A + B \rightleftarrows X + Y, \tag{8.5.7}$$

with the reverse reaction also being bimolecular. This time there is a backward rate constant, k_{-1}, as well as a forward one, k_1. From the mass action principle applied to the reverse reaction, we have

$$\text{rate of conversion of } X + Y = k_{-1}[X][Y].$$

Under normal circumstances, the forward and backward reactions take place independently of each other, and consequently the net rate of change of any species, say X, is just the sum of the effects of each reaction separately. It follows that the net rate of change in X is given by

$$\begin{aligned}\frac{dx}{dt} &= (\text{conversion rate of } A + B) - (\text{conversion rate of X+Y}) \\ &= k_1[A][B] - k_{-1}[X][Y] \\ &= k_1(a_0 + x_0 - x)(b_0 + x_0 - x) - k_{-1}x(y_0 - x_0 + x),\end{aligned} \tag{8.5.8}$$

where (8.5.2) has been used in the last line. Circumstances under which the forward and backward reactions are not independent include precipitation of one of the species, as we have seen above. Another occurs when one of the reactions is highly exothermic. In that case, conditions of the reaction radically change, such as the temperature.

The analysis of (8.5.8) goes very much like that of (8.5.4). The stationary points are given as the solutions of the $\frac{dx}{dt} = 0$ equation

$$\begin{aligned}0 &= k_1(a_0 + x_0 - x)(b_0 + x_0 - x) - k_{-1}x(y_0 - x_0 + x) \\ &= (k_1 - k_{-1})x^2 - (k_1(a_0 + b_0 + 2x_0) + k_{-1}(y_0 - x_0))x + k_1(a_0 + x_0)(b_0 + x_0).\end{aligned} \tag{8.5.9}$$

As one can see, if $k_1 \neq k_{-1}$, this is a quadratic equation and therefore has two roots, say $x = \alpha$ and $x = \beta$, which may be found using the quadratic formula, $\frac{1}{2a}(-b \pm \sqrt{b^2 - 4ac})$. The right-hand side of (8.5.8) thus factors into the linear factors

$$\frac{dx}{dt} = (k_1 - k_{-1})(x - \alpha)(x - \beta). \tag{8.5.10}$$

Again, just as above, this variable-separable differential equation is easily solved, but the nature of the solution depends on whether the roots are real or complex, equal or distinct. To decide about that, we must examine the discriminant of the quadratic formula, $b^2 - 4ac$. By direct substitution of the coefficients from (8.5.9) into the discriminant and simplifying, we get

$$b^2 - 4ac = k_1^2(a_0 - b_0)^2 + 2k_1k_{-1}(a_0 + b_0 + 2x_0)(y_0 - x_0) + k_{-1}^2(y_0 - x_0)^2. \tag{8.5.11}$$

The first and last terms are squares and so are positive (or zero). We see that if $y_0 \geq x_0$, then the discriminant is always positive or zero and the two roots are real. Since X was an arbitrary choice, we can always arrange that $y_0 \geq x_0$, and so we assume that this is so.

Unless the initial concentrations are equal, $a_0 = b_0$ and $y_0 = x_0$, the roots will be distinct. We assume without loss of generality that

$$\alpha < \beta. \tag{8.5.12}$$

Then in a similar way to the derivation of (8.5.6), the solution of (8.5.10) is

$$\ln\left(\frac{x - \beta}{x - \alpha}\right) = (\beta - \alpha)(k_1 - k_{-1})t + q,$$

where q is the constant of integration. This may be solved in terms of x,

$$x = \frac{\beta - Qe^{rt}}{1 - Qe^{rt}}, \tag{8.5.13}$$

where Q is a constant and

$$r = (\beta - \alpha)(k_1 - k_{-1}).$$

If the discriminant is zero, then $\beta = \alpha$, and in that case the solution is

$$\frac{-1}{x - \alpha} = (k_1 - k_{-1})t + q,$$

or

$$x = \alpha - \frac{1}{(k_1 - k_{-1})t + q},$$

where q is again the constant of integration.

Exercises/Experiments

1. Suppose that $A + B \to C$, that the initial concentrations of A, B, and C are $\frac{1}{2}$, $\frac{1}{3}$, and 0, respectively, and that the rate constant is k.

(a) Show that this leads to the differential equation in $z(t) = [C(t)]$ given by

$$z' = k\left(\frac{1}{2} - z\right)\left(\frac{1}{3} - z\right), \quad z(0) = 0.$$

(b) Solve this equation.
(c) Show that the corresponding equation for $x(t) = [A(t)]$ is

$$x' = kx\left(\frac{1}{6} - x\right), \quad x(0) = \frac{1}{2}.$$

(d) Solve this equation. Show by adding the solutions x and z that the sum is constant.
(e) At what time is 90% of the steady-state concentration of C achieved?
(f) Suppose that k is increased 10%. Now rework question (e).

2. Suppose that $A + B \leftrightarrow C + D$ is a reversible reaction, that the initial concentrations of A and B are $\frac{4}{10}$ and $\frac{5}{10}$, respectively, and that the initial concentrations of C and D are 0. Take $k_1 = 10$ and $k_{-1} = \frac{5}{2}$.
(a) Show that this leads to the differential equation

$$y' = 10(0.4 - y)(0.5 - y) - \frac{5y^2}{2}, \quad y(0) = 0.$$

(b) What is the equilibrium level of $[C]$. Draw two graphs: one where $k_{-1} = \frac{5}{2}$ and one where $k_{-1} = \frac{5}{4}$.

8.6 Enzyme Kinetics

Enzymes serve to catalyze reactions in living systems, enabling complex chemical transformations to occur at moderate temperatures, many times faster than their uncatalyzed counterparts. Proteins, serving as the catalysts, are first used and then regenerated in a multistep process. Overall, the simplest enzyme-catalyzed reactions transform the enzyme's specific substrate into product, possibly with the release of a by-product. Referred to as enzyme saturation, these reactions are typically rate limited by the amount of enzyme itself. The degree to which saturation occurs relative to substrate concentration is quantified by the Michaelis–Menten constant of the enzyme–substrate pair.

Enzyme-catalyzed reactions are normally rate limited by enzyme saturation.

The importance of enzyme-catalyzed reactions along with a general description of the biochemical principles of enzyme catalysis was given in Section 8.4. Here we will consider an enzyme, E, that acts on a single substrate, S, and converts it to an

alternative form that is regarded as the product P. The enzyme performs this function by temporarily forming an enzyme–substrate complex, C, which then decomposes into product plus enzyme:

$$\begin{aligned} S + E &\rightleftarrows C \\ C &\longrightarrow P + E. \end{aligned} \tag{8.6.1}$$

The regenerated enzyme is then available to repeat the process.[13] Here we will work through the mathematics of enzyme kinetics. The general principles of chemical kinetics discussed in the previous section apply to enzyme kinetics as well. However, due to the typically small amount of enzyme compared to substrate, the conversion rate of substrate to product is limited when the enzyme becomes *saturated* with substrate as enzyme–substrate complex.

As in the previous section, we let m denote the concentration of species M. The forward and reverse rate constants for the first reaction will be denoted by k_1 and k_{-1}, respectively, while the rate constant for the second will be taken as k_2. The rate equations corresponding to the reactions (8.6.1) are[14]

$$\begin{aligned} \frac{dc}{dt} &= k_1 es - k_{-1} c - k_2 c, \\ \frac{ds}{dt} &= -k_1 es + k_{-1} c, \\ \frac{de}{dt} &= -k_1 es + k_{-1} c + k_2 c, \\ \frac{dp}{dt} &= k_2 c. \end{aligned} \tag{8.6.2}$$

Note that complex C is both formed and decomposed by the first reaction and decomposed by the second. Similarly, enzyme E is decomposed and formed by the first reaction and formed by the second. The first three equations are independent of the formation of product P, and so for the present, we can ignore the last equation. As before, we denote by subscript 0 the initial concentrations of the various reactants. In particular, e_0 is the initial, and therefore total, amount of enzyme, since it is neither created nor destroyed in the process.

By adding the first and third equations of system (8.6.2), we get

$$\frac{dc}{dt} + \frac{de}{dt} = 0.$$

Integrating this and using the initial condition that $c_0 = 0$, we get

$$e = e_0 - c. \tag{8.6.3}$$

[13] Compare this scheme to Figure 8.4.3; $S + E$ constitutes the initial state, C is the transition state, and $P + E$ is the final state.

[14] The units of k_1 are different from those of k_{-1} and k_2, since the former is a bimolecular constant, while the latter are unimolecular.

We may use this to eliminate e from system (8.6.2) and get the following reduced system:

$$\begin{aligned} \frac{dc}{dt} &= k_1 s(e_0 - c) - (k_{-1} + k_2)c, \\ \frac{ds}{dt} &= -k_1 s(e_0 - c) + k_{-1}c. \end{aligned} \tag{8.6.4}$$

In Figure 8.6.1, we show some solutions of this system of differential equations.

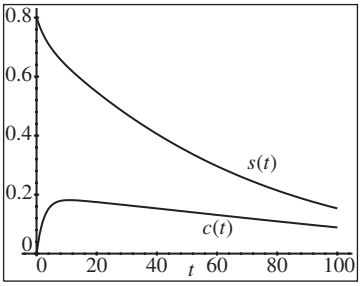


Fig. 8.6.1. Solutions to (8.6.2).

For the purpose of drawing the figure, we take the constants to be the following:

MAPLE
```
> k1:=1/10; km1:=1/10; k2:=1/10; e0:=4/10; (km1+k2)/k1;
```

The equations are nonlinear and cannot be solved in closed form. Consequently, we use numerical methods to draw these graphs. It should be observed that the level of S, graphed as $s(t)$, drops continuously toward zero. Also, the intermediate substrate C, graphed as $c(t)$, starts at zero, rises to a positive level, and gradually settles back to zero. In the exercises, we establish that this behavior is to be expected.

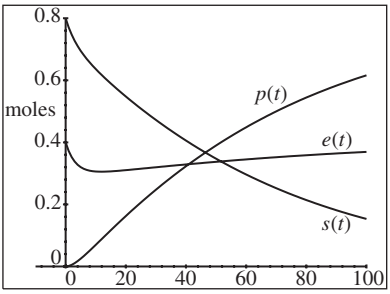


Fig. 8.6.2. Solutions to (8.6.1).

Also in the exercises we provide techniques to draw what may be a more interesting graph: Figure 8.6.2. In particular, we draw graphs of $s(t)$, $p(t)$, and $e(t)$. The first

two of these are, in fact, the most interesting, since they demonstrate how much of S is left and how much of P has been formed. The addition of a graph for $e(t)$ illustrates that during the intermediate phase, some of the enzyme is tied up in the enzyme–substrate complex, but as the reaction approaches equilibrium, the value of $e(t)$ returns to its original value.

MAPLE
```
> with(plots): with(DEtools): # recall the parameters assigned on the previous page
> enz:=diff(c(t),t)=k1*s(t)*(e0-c(t))-(km1+k2)*c(t),diff(s(t),t)=-k1*s(t)*(e0-c(t))+km1*c(t);
> sol:=dsolve({enz,c(0)=0,s(0)=8/10},{c(t),s(t)}, type=numeric, output=listprocedure);
> csol:=subs(sol,c(t)); ssol:=subs(sol,s(t));
> J:=plot(csol,0..100): K:=plot(ssol,0..100):
> display({J,K});
```

MATLAB
```
% make up an m-file, enzymeRate.m, with
%   function Yprime=enzymeRate(t,Y);
%   k1=0.1; km1=0.1; k2=0.1; e0=0.4;
%   Yprime=[k1*Y(2)*(e0-Y(1))-(km1+k2)*Y(1);-k1*Y(2)*(e0-Y(1))+km1*Y(1)];
> [t,Y]=ode23('enzymeRate',[0 100],[0; 0.8]); plot(t,Y)
```

From Figure (8.6.1), notice that the concentration of complex rises to a relatively invariant ("effective") level, which we denote by c_{Eff}. This is found by setting $\frac{dc}{dt} = 0$ in system (8.6.4) and solving for c,

$$0 = k_1 s(e_0 - c) - (k_{-1} + k_2)c,$$

or

$$s(e_0 - c) = \frac{k_{-1} + k_2}{k_1} c.$$

The combination k_M of rate constants

$$k_M = \frac{k_{-1} + k_2}{k_1} \tag{8.6.5}$$

is known as the *Michaelis–Menten constant*; it has units moles per liter. Solving for c above, we get

$$c = \frac{s e_0}{k_M + s}, \tag{8.6.6}$$

which is seen to depend on the amount of substrate S. But if s is much larger than k_M, then the denominator of (8.6.6) is approximately just s, and we find the invariant level of complex to be

$$c_{\text{Eff}} \approx e_0. \tag{8.6.7}$$

Thus most of the enzyme is tied up in enzyme–substrate complex.

By the *velocity* v of the reaction, we mean the rate, $\frac{dp}{dt}$, at which product is formed. From system (8.6.2), this is equal to $k_2 c$. When the concentration of substrate is large, we may use c_{Eff} as the concentration of complex and derive the maximum reaction velocity,

$$v_{\max} = k_2 e_0. \tag{8.6.8}$$

MAPLE

```
> fcn:=s->vmax*s/(kM+s); vmax:=10: kM:=15:
> crv:=plot([x,fcn(x),x=0..150],x=-20..160,y=-2..12,tickmarks=[0,0]):
> asy:=plot(10,0..150,tickmarks=[0,0]):
> midline:=plot(5,0..15.3,tickmarks=[0,0]):
> vertline:=plot([15.3,y,y=0..5],tickmarks=[0,0]):
> a:=0: A:=0: b:=13: B:=13*vmax/kM:
> slope:=x->A*(x-b)/(a-b)+B*(x-a)/(b-a):
> slopeline:=plot(slope,a..b):
> with(plots):
> display({crv,asy,midline,vertline,slopeline});
```

MATLAB

```
> vmax=10; kM=15;
> s=0:.1:150;
> v=vmax.*s./(kM+s);
> plot(s,v)
> hold on
> asy=vmax*ones(size(s));
> plot(s,asy)
> x=[0 13]; y=[0 13*vmax/kM];
> plot(x,y)
> x=[0 15.3]; y=[vmax/2 vmax/2];
> plot(x,y)
> x=[15.3 15.3];
y=[0 vmax/2];
> plot(x,y)
```

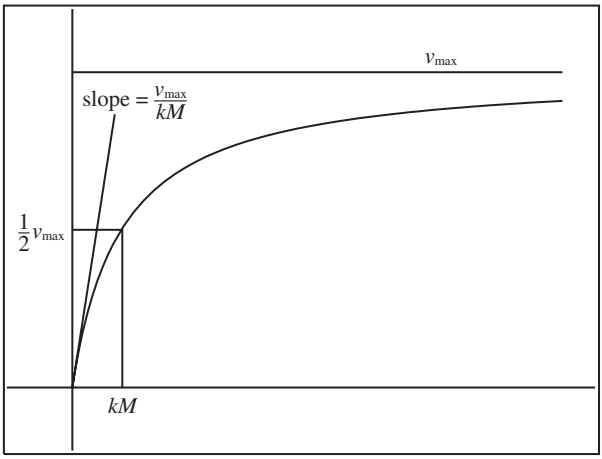


Fig. 8.6.3. Michaelis–Menten plot.

Likewise, from (8.6.6) and (8.6.8), the initial reaction velocity, v_0, is given by

$$v_0 = \left.\frac{dp}{dt}\right|_{t=0} = k_2 \frac{se_0}{k_M + s} = \frac{v_{\max} s}{k_M + s}. \tag{8.6.9}$$

This is the *Michaelis–Menten equation*, the rate equation for a one-substrate, enzyme-catalyzed reaction. Its graph is shown in Figure 8.6.3.

The value of k_M for an enzyme can be experimentally found from a plot of initial velocity vs. initial substrate concentration at fixed enzyme concentrations. This graph has the form of a rectangular hyperbola because at low substrate concentrations, v_0 is

nearly proportional to substrate concentration $[S]$. On the other hand, at high substrate concentrations the reaction rate approaches $v_{\max}$ asymptotically because at these concentrations, the reaction is essentially independent of substrate concentration. By experimentally measuring the initial reaction rate for various substrate concentrations, we can make a sketch of the graph. Working from the graph, the substrate level that gives $\frac{1}{2}v_{\max}$ initial velocity is the value of k_M, seen as follows: From (8.6.9) with $v_0 = \frac{v_{\max}}{2}$,

$$\frac{1}{2}v_{\max} = \frac{v_{\max}s}{k_M + s},$$

and solving for k_M gives

$$k_M = s.$$

Thus we interpret k_M as the substrate concentration at which the reaction rate is half-maximal. By inverting the Michaelis–Menten equation (8.6.9), we get

$$\frac{1}{v_0} = \frac{k_M + s}{v_{\max}s} = \frac{k_M}{v_{\max}}\frac{1}{s} + \frac{1}{v_{\max}}. \tag{8.6.10}$$

This is the *Lineweaver–Burk equation*, and it shows that a least squares fit may be made to this *double reciprocal plot* of $\frac{1}{v_0}$ vs. $\frac{1}{s}$. This has the advantage of allowing an accurate determination of $v_{\max}$. Recall this example in Section 2.3 leading to (2.3.1). The intercept b of the plot will be $\frac{1}{v_{\max}}$ and the slope m will be $\frac{k_M}{v_{\max}}$. From these, both $v_{\max}$ and k_M can be determined.

Another transform of the Michaelis–Menten equation that allows the use of least squares is obtained from (8.6.10) by multiplying both sides by $v_0 v_{\max}$; this yields

$$v_0 = -k_M\frac{v_0}{s} + v_{\max}. \tag{8.6.11}$$

A plot of v_0 against $\frac{v_0}{[S]}$ is called the *Eadie–Hofstee plot*; it allows the determination of k_M as its slope and $v_{\max}$ as its intercept.

Exercises/Experiments

1. Our intuition for the long-range forecast for (8.6.1) is that some of the reactants that move from S to C move on to P. But the assumption is that the second reaction is only one-way, so that the products will never move back toward S.[15] This suggests that S will be depleted. We conjecture that $s_\infty = 0$ and $c_\infty = 0$. We confirm this with the notions of stability that we studied in Section 2.5.

(a) Find all the stationary solutions by observing that setting $\frac{ds}{dt} = 0$ and $\frac{dc}{dt} = 0$ leads to the equations

$$k_1 s(e_0 - c) - (k_{-1} + k_2)c = 0,$$

[15] In the context of a free-energy diagram (Figures 8.4.2 and 8.4.3), the one-way nature of the process $C \to P$ is due to a lack of sufficient free energy in the environment to cause the reaction $P \to C$.

$$-k_1 s(e_0 - c) + k_{-1} c = 0.$$

While it is clear that $s = 0$ and $c = 0$ is a solution, establish that this is the only solution for the equations as follows:

MAPLE
```
> restart;
> solve(k1*s*(e0-c)-(km1+k2)*c=0,c);
> subs(c=%, -k1*s*(e0-c)+km1*c); normal(%);
> numer(%)/denom(%%)=0;
```

MATLAB
```
% MATLAB cannot symbolically solve the system but we can proceed this way: add the two
% equations and notice that the first terms cancel and the second terms nearly cancel, leaving
% -k2*c = 0. This shows that c=0. With c=0 in either equation it is easy to see that s=0 too. Now
% we find the Jacobian numerically.
% Make an m-file, enzyme96.m, with
%   k1=1; k2=2; km1=1.5; e0=5;
%   csPrime=[k1*s.*(e0-c)-(km1+k2)*c; -k1*s.*(e0-c)+km1*c];
%
% The Jacobian = the matrix whose first column is the derivative of the component functions
% with respect to c and the second column is with respect to s. Take derivatives at c=s=0.
> J1=(enzyme96(0+eps,0)-enzyme96(0,0))/eps; J2=(enzyme96(0,0+eps)-enzyme96(0,0))/eps;
> J=[J1 J2]; % Jacobian at (0,0)
> eig(J) % both values negative real, so (0,0) stable
```

Substitute this into the second equation and set the resulting equation equal to zero. Argue that s must be zero and c must be zero.

(b) Establish that $s = c = 0$ is an attracting stationary point, by finding the linearization about this one and only stationary point. (Recall Section 4.4.)

MAPLE
```
> with(LinearAlgebra): with(VectorCalculus):
> Jacobian([k1*s*(e0-c)-(km1+k2)*c,-k1*s*(e0-c)+km1*c],[c,s]);
> subs({c=0,s=0},%);
> Eigenvalues(%);
> expand((km1+k2+k1*e0)^2-4*k2*k1*e0);
```

(c) Verify that the eigenvalues of the linearization are

$$-\frac{1}{2}\left((k_{-1} + k_2 + k_1 e_0) \pm \sqrt{(k_{-1} + k_2 + k_1 e_0)^2 - 4k_2 k_1 e_0}\right)$$

and that both these are negative. Argue that this implies that $\{0, 0\}$ is an attracting stationary point for $\{c(t), s(t)\}$.

2. Draw the graph of Figure (8.6.3). Here is the syntax that does the job:

MAPLE
```
> restart;
> k1:=1/10: k2:=1/10: km1:=1/10: s0:=8/10: e0:=4/10:
> sol:=dsolve({diff(s(t),t)=-k1*e(t)*s(t)+km1*c(t),diff(c(t),t)=k1*e(t)*s(t)-(km1+k2)*c(t),
                diff(p(t),t)=k2*c(t),diff(e(t),t)=-k1*e(t)*s(t)+(km1+k2)*c(t),
                s(0)=s0, c(0)=0, p(0)=0, e(0)=e0},{s(t),c(t),p(t),e(t)},numeric,output=listprocedure);
> es:=subs(sol,e(t)); ps:=subs(sol,p(t)); ss:=subs(sol,s(t));
> plot({es,ps,ss},0..100,color=[red,green,black]);
```

MATLAB
```
% For problem 2 and 3
% Make an m-file, exer962.m, with
%   function Yprime=exer962(t,Y); % Y(1)=c, Y(2)=s, Y(3)=e, Y(4)=p
%   k1=0.1; k2=0.1; km1=0.1; % problem 2
%   k1=1; k2=0.1; km1=0.025; % problem 3
% Yprime=[k1*Y(3).*Y(2)-(km1+k2)*Y(1);-k1*Y(3).*Y(2)+km1*Y(1);
%              -k1*Y(3).*Y(2)+(km1+k2)*Y(1); k2*Y(1)];
```

```
> s0=0.8; e0=0.4; [t,Y]=ode23('exer962',[0 100],[0;s0; e0; 0]);
> plot(t,Y)
```

3. Draw the graph of the solution $c(t)$ in system (8.6.2) with constants chosen so that $k_M \approx 1$ and $S = 10$. The point to observe is that $c(t) \approx e_0$ for large values of t.

MAPLE

```
> restart;
> k1:=1: k2:=1/10: km1:=1/40: s0:=10: e0:=4/10: (km1+k2)/k1: s0/(%+s0);
> sol:=dsolve({diff(s(t),t)=-k1*e(t)*s(t)+km1*c(t),diff(c(t),t)=k1*e(t)*s(t)-(km1+k2)*c(t),
                diff(p(t),t)=k2*c(t),diff(e(t),t)=-k1*e(t)*s(t)+(km1 + k2)*c(t),
                s(0)=s0,c(0)=0,p(0)=0,e(0)=e0},{s(t),c(t),p(t),e(t)},numeric,output=listprocedure);
> cs:=subs(sol,c(t));
> plot(cs,0..150);
```

MATLAB

```
% continued from previous problem and rerun for the second part change k1 and km1 above to k1=1;
% km1=0.025;
> s0=10; e0=0.4;
> [t,Y]=ode23('exer962',[0 100],[0;s0; e0; 0]);
> plot(t,Y(:,1)) % graph of c
```

4. Suppose that $A + B \to C$, that the initial concentrations of A, B, and C are 2, 3, and 0, respectively, and that the rate constant is k.

(a) The concentration of C is sampled at $t = \frac{3}{2}$ and is found to be $\frac{3}{5}$. What is an approximation for k?

(b) Instead of determining the concentration of C at just $t = \frac{3}{2}$, the concentration of C is found at five times (see Table 8.6.1).

Table 8.6.1.

Time	Concentration
$\frac{1}{2}$	0.2
1.0	0.4
$\frac{3}{2}$	0.6
2.0	0.8
$\frac{5}{2}$	1.0

Estimate k. Plot your data and the model that your k predicts on the same graph.

5. We have stated in this chapter that the addition of an enzyme to a reaction could potentially speed the reaction by a factor of 10^{13}. This problem gives a glimpse of the significance of even a relatively small increase in the reaction rate.
Suppose that we have a reaction

$$A \leftrightarrow B \to C.$$

Suppose also that $k_{-1} = k_2 = 1$, that the initial concentration of A is $a_0 = 1$, and the initial concentrations of B and C are zero.

(a) Show that the differential equations model for this system is

$$\frac{da}{dt} = -k_1 a(t) + k_{-1} b(t),$$

$$\frac{db}{dt} = k_1 a(t) - (k_{-1} + k_2) b(t),$$
$$\frac{dc}{dt} = k_2 b(t).$$

(b) Find $a(t)$, $b(t)$, and $c(t)$ for $k_1 = 1$ and for $k_1 = 10$. Plot the graphs for the three concentrations in both situations.

MAPLE

```
> with(LinearAlgebra):
> k1:=1; km1:=1; k2:=1;
> A:=Matrix([[-k1,km1,0],[k1,-km1-k2,0],[0,k2,0]]);
> u:=evalm(MatrixExponential(A,t) &* [1,0,0]):
> a:=unapply(u[1],t); b:=unapply(u[2],t); c:=unapply(u[3],t):
> plot({a(t),b(t),c(t)},t=0..7,color=[red,blue,black]);
> fsolve(c(t)=.8,t,1..7);
```

MATLAB

```
% contents of m-file exer964.m:
%   function Yprime=exer964(t,Y); km1=1; k2=1; k1=1;
%   Yprime=[-k1*Y(1)+k1*Y(2); k1*Y(1)-(km1+k2)*Y(2); k2*Y(2)];
%
> a0=1; b0=0; c0=0;
> [t1,Y1]=ode23('exer964',[0 7],[a0; b0; c0]);
> plot(t1,Y1);
  % now change k1 above to k1=10; note the y-axis scale
> [t10,Y10]=ode23('exer964',[0 7],[a0; b0; c0]);
> plot(t10,Y10)
  % compare product c directly; be sure to check the y-axis scale
> plot(t1,Y1(:,3),'b')
> hold on
> plot(t10,Y10(:,3),'r')
```

(c) Take $k_1 = 1$, 10, 20, 30, 40, and 50. Find T_k such that $c(T_k) = 0.8$ for each of these ks. Plot the graph of the pairs $\{k, T_k\}$. Find an analytic fit for these points.

Questions for Thought and Discussion

1. Draw the structural formulas ("stick models") for
   (a) butane, the four-carbon hydrocarbon, having all carbons in a row, and no double bonds;
   (b) isopropanol, having three carbons, an -OH group on the middle carbon, and no double bonds;
   (c) propene, with three carbons and one double bond.

2. Relate this set of reactions to a free-energy level diagram: $A + E \leftrightarrow B \rightarrow C + E$, where E is an enzyme. What effect does E have on the energy levels?

3. Assume the reaction $A \leftrightarrow C \leftrightarrow B$, where the intermediate state C has a *lower* free energy than A or B. Knowing what you do about the behavior of free energy, what would happen to the free energy difference between A and B if the free energy of C were changed?

4. A mechanical analogue of the situation in Question 3 is a wagon starting at A, rolling downhill to C, and then rolling uphill to B. There is a frictional force on the wagon wheels. What do you think will be the effect of varying the depth of C?

5. Describe the chemical differences between RNA and DNA. What are their biological (functional) differences?
6. Outline the process of information flow from DNA to the control of cellular chemistry.
7. Name six kinds of proteins and describe their functions.

References and Suggested Further Reading

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