

Foundations

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Biochemistry is the study of the chemical nature of biology. Biochemists use chemical ideas and tools to understand living systems. We begin with a review of the chemical concepts of equilibrium, kinetics, steady state, and energy. Additional chemical principles are discussed in the appendix. Some foundational ideas of biology complete the chapter, namely, the cell theory, evolution, and species hierarchies.

1.1 Origins of Biochemistry

Compared to its component sciences, biochemistry is a young discipline. The word *biochimie* was introduced by the German scientist Hoppe-Seyler in 1877. He also edited the first biochemistry journal, *Biological Chemistry*, still in existence today. It was during this period, the later part of the 19th century, in which two erroneous ideas—**spontaneous generation** and **vitalism**—were dispelled, clearing the way for a discipline that required new thinking.

According to the hypothesis of spontaneous generation, living organisms arise from nothingness, such as bacteria appearing in a nutrient-rich broth. In the 1860s, however, Louis Pasteur demonstrated that bacteria exist in the air; no bacteria appear in the broth if the container is isolated from the atmosphere. This led to the **cell theory**, which states that cells are the fundamental unit of living systems, arising from other cells. Despite this advance, Pasteur was himself a **vitalist**; that is, he believed that living systems do not obey the same chemical principles as inert materials.

Two challenges to vitalism bracketed the work of Pasteur. In 1828, Wohler discovered that urea (formed in living systems) could be synthesized in the laboratory from ammonia and bicarbonate. This *in vitro* synthesis of an organic compound therefore did not need the “aid of a kidney” as Wohler put it (**BOX 1.1**). In 1897, the German chemists (and brothers) Eduard and Hans Buchner showed that fermentation could exist in an extract from ruptured cells, thus dispelling the notion that cellular organization is required for processes that occur in living systems.

In the 20th century, biochemistry was dominated first by organic chemistry, as metabolic pathways were discovered, then by enzymology, then bioenergetics, and later by molecular biology as the role of DNA and information molecules emerged. As biochemistry plays an increasingly significant role in both the physical and chemical sciences today, all of these disciplines overlap to some extent. What remains, however, is the distinctive viewpoint of perceiving biology from a chemical perspective. While both the chemical and the biological understanding of many areas of study have greatly expanded in recent times, principles of biochemistry have emerged that are unique to this discipline and have been invaluable in elucidating a large number of biological phenomena. These underpinnings are presented in subsequent chapters. Presently, though, we consider some fundamental concepts of chemistry and biology.

BOX 1.1: WORD ORIGINS

Organic

Among its rich meanings, the word *organic* implies a sense of the whole, retained in the word *organism* or *organ*. Historically, however, it was used by vitalists to identify substances that could be produced only by a living organism. After vitalism was disproved, *organic* was redefined, rather than cast aside. Today, *organic chemistry* simply identifies a branch of chemistry that specifically deals with compounds of carbon. Strictly speaking, *inorganic chemistry* refers to the study of all other types of molecules. Yet another meaning for *organic* has emerged that is closer to its historical roots. That is, it describes farming methods that do not use synthesized chemicals (e.g., fertilizers and pesticides for plants and hormones for animals). Thus, growing plants and raising animals in this way is said to produce *organic foods*. Whether this is a definite health benefit is debatable; for example, the absence of pesticides can lead to a greater bacterial content in food. Moreover, this use of *organic* is less strict and may vary from one producer to another. Despite the three distinct definitions of *organic*, we are concerned in this book only with the middle one, the chemistry of carbon compounds.

1.2 Some Chemical Ideas

In order to determine whether you need a basic review, or can instead skip to the next section, take the following self-test:

- Without specifying a value, what is the meaning of Avogadro's number?
- Distinguish between atoms, electrons, molecules, and moles.
- When is it appropriate to use mol units as opposed to grams?
- Why is the equilibrium constant for a reaction equal to the products multiplied together, divided by the substrates multiplied together?
- How are equilibrium and kinetics related?

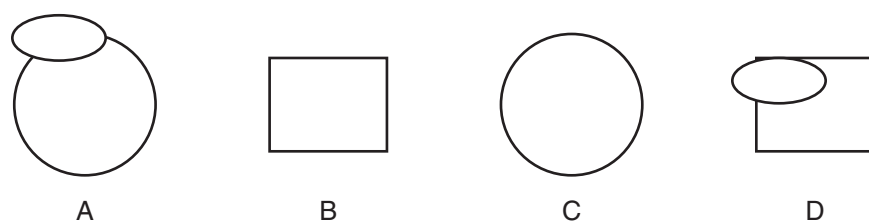
Mastering the ideas of the mole, Avogadro's number, atoms, and molecules is the first step in understanding more complex information in biochemistry. We consider here the notions of kinetics and thermodynamics with the assumption that the more elementary ideas are well in hand.

Reactions and Their Kinetic Description

If substances A and B react to form substances C and D, then the generic reaction for this transformation can be written as follows:



A and B are called *substrates*, whereas C and D are called *products*. Each is a molecule, but each can also be called a compound or a metabolite. In order to visualize what is happening, let us relax our molecular thinking and represent the molecules schematically as shown below.



Based on this representation, the reaction involves removing a piece of molecule A and placing it onto molecule B, thus creating molecules C and D. This is a “mechanistic” view. To more fully characterize the reaction, we need to know the rates in both the forward and reverse directions. Consider first the *forward* direction, which proceeds from left to right as the reaction is written:



Suppose there are three A and four B molecules, as shown in **FIGURE 1.1**. Each A can combine with any of 4 B's, so there are 12 possible collisions (3×4) between the three A molecules and the four B molecules.

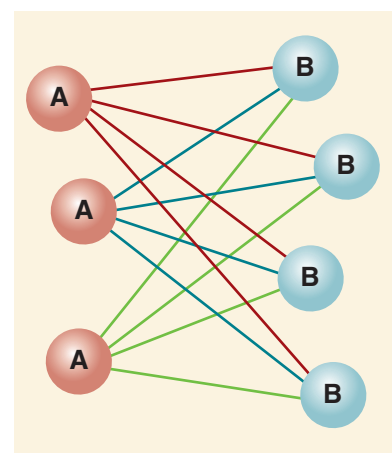


FIGURE 1.1 Collision Theory of Reaction Rates. Three A molecules react with four B molecules and each possible interaction of an A with a B is indicated. There are a total of $3 \times 4 = 12$ possible collisions. In general, the rate is proportional to the number of A molecules times the number of B molecules in a fixed volume; that is, rate is proportional to $[A] \times [B]$.

According to **collision theory**, the rate of a reaction is proportional to the number of collisions. In this example, the rate is proportional to 3×4 . In general, the rate is proportional to $[A] \times [B]$, where $[A]$ and $[B]$ are the concentrations of A and B, respectively.

The rate under consideration, the forward rate, can be designated as rate_f . This rate is equal to a constant multiplied by the product of $[A]$ and $[B]$:

$$\text{rate}_f = k_f[A][B], \quad (1.3)$$

where k_f is the constant of proportionality between the rate and the concentrations and is called the **rate constant**.

For the reverse reaction, $C + D \rightarrow A + B$, we can follow the same derivation and arrive at a similar expression:

$$\text{rate}_r = k_r[C][D] \quad (1.4)$$

where rate_r represents the reverse reaction rate. The expressions for reaction rate shown in Equations 1.3 and 1.4 can be generalized to any reaction. The rate constant, while independent of changes in concentration, can vary with conditions such as temperature or ionic strength. Experimentally, reaction rates are measured by determining the decrease in substrate concentration or the increase in product concentration with time. Equations 1.3 and 1.4 summarize the results of those experimental studies. Thus, the reaction rate has units of concentration per time.

A rate, by definition, has only one direction, yet chemical reactions are commonly written with double arrows between substrates and products. This notation, which indicates that both forward and reverse reactions occur, is commonly used when a reaction attains a position of *equilibrium*.

Equilibrium

Perhaps the most important fundamental idea in chemistry is that of **equilibrium**. A state of equilibrium exists when the forward and reverse rates of a reaction are equal. Once a reaction achieves equilibrium, no further observable change occurs in the substrate or product concentrations, unless there is a change in external conditions. Reactions not yet at equilibrium have a driving force toward the equilibrium state, just as a rolling ball eventually comes to rest as the forces acting on it balance. To become familiar with the state of equilibrium, we examine the origins of the equilibrium expression and the equilibrium constant.

According to the definition of equilibrium, $\text{rate}_f = \text{rate}_r$, so we can equate Equations 1.3 and 1.4:

$$k_f[A][B] = k_r[C][D] \quad (1.5)$$

Sometimes to emphasize the fact that we are describing an equilibrium, an “eq” subscript is added to the concentration terms (i.e., $[A]_{\text{eq}}$,

[B]_{eq}, etc.). In Equation 1.5, though, we will just assume the concentrations are those at equilibrium under our conditions. Rearranging,

$$k_f/k_r = [C][D]/[A][B] = K_{eq}, \quad (1.6)$$

where K_{eq} is the equilibrium constant. Thus, the rate constants (k_f and k_r) and the equilibrium constant (K_{eq}) are related.

Equation 1.6 relates kinetics (a rate process intrinsically tied to a time element) to equilibrium (a process independent of a time element). A single reaction may exist in equilibrium, but so, too, may multiple reactions:



Under biological conditions, though, multiply connected reactions are better represented using the *steady state*.

The Steady State

One problem with applying the equilibrium model to living cells is that living cells are never actually at equilibrium. Thus, a more elaborate system must be used to model metabolism: the **steady state**. The steady state applies when the intermediates of a process are constant with time, yet the overall process is changing with time. To understand this concept, consider just two components, a substrate (S) and a product (P), which are the absolute minimum requirements for an equilibrium:



The equilibrium constant for this reaction is:

$$K_{eq} = [P]/[S] \quad (1.9)$$

To describe a steady state, we need a minimum of three components: a substrate (S), an intermediate (I), and a product (P):



Equation 1.10 is really two reactions, $S \rightarrow I$ and $I \rightarrow P$, much in the same way that an equilibrium reaction is really two reactions (i.e., a forward reaction and a reverse reaction). In the steady state, there is a net flow of material from S to P in Equation 1.10, so the system overall is *not* constant with time. Commonly, multiple intermediate species are present in a steady state rather than just one. In Equation 1.11, for example,



all of the individual rates (e.g., $S \rightarrow I$, $I \rightarrow J$, etc.) are equal. Not only that, but the overall rate, $S \rightarrow P$, is the same. The result is that the intermediate

concentrations (i.e., [I], [J], [K], and [L]) do not vary with time. Instead, the concentrations of the intermediates are constant because each one is formed at the same rate that it is removed.

The concentrations of S and P need not be constant, however. Suppose, for example, that S is **satürating** for the first reaction. Saturation with a substrate means that its concentration is very high, effectively unchanging during the reaction, thus fixing the rate of the reaction $S \rightarrow I$. According to Equation 1.11, the product P cannot revert back to L. As a result, P can accumulate and its concentration has no effect on the rate of its formation from L.

Metabolic pathways that are linear in structure, as in Equation 1.11, are readily modeled by a steady state. In order to clearly picture the situation, consider an everyday example of a line of people forced to move single-file through a gate. Suppose, as diagrammed in **FIGURE 1.2**, that people are admitted at a rate that enables them to flow without backup through an entry gate to a building, and then allowed to exit through a gate that is also controlled to allow no buildup at the exit. The number of people entering is analogous to [S], whereas the number of those leaving is analogous to [P]. We can view the steady-state intermediates (analogous to I, J, K, and L in Equation 1.11) by imagining a window in the building that lets us look at the line. At any given time there are eight people visible through the window. Each time we look, there will be a different eight people in view, but the number is the same every time. This is the essence of the steady state.

While the steady state is distinct from an equilibrium, equating the two is a common scientific error. Often, an equilibrium is used for all situations where there is an element of balance, not recognizing that this is inappropriate when a series of chemical reactions produces a net flow. In that situation, the steady-state model is more appropriate.

Finally, it is important to stress that both equilibrium and steady state are models. Thus, while they often are appropriate to the situation, they are always approximations. In some instances they are wildly inaccurate ones. For example, prior to an enzymatic reaction or series of reactions reaching constant intermediate concentrations,

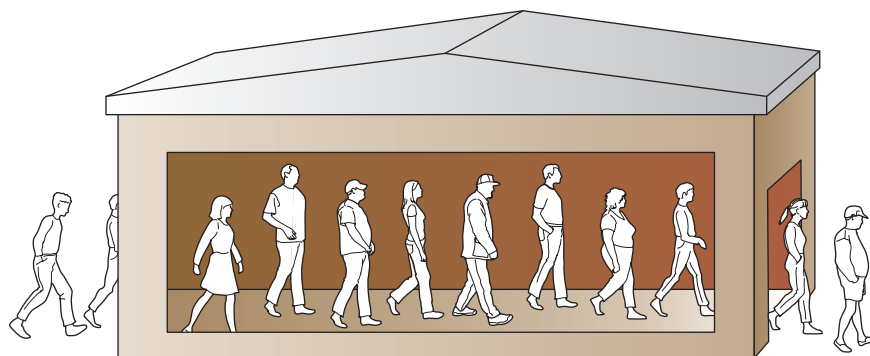


FIGURE 1.2 The Steady-State Window. People are moving through a building at a constant rate. As long as the rate of entry and exit is the same, the number of people visible through the window (the intermediate state) will be the same (8). At any instant in time, though, the eight people in this intermediate state will be different.

neither model applies. This is called a *pre-steady state condition*, which requires a different model entirely.

1.3 Energy

Energy is a concept that is at once simple and complex. It is simple because it is part of everyday parlance: a child knows about “having a lot of energy,” the relative difficulty of walking uphill, and the existence of friction. It is complex because all forms of energy, such as electrical, gravitational, pressure–volume work, and heat flow, can be interconverted. The energy of a waterfall, a galloping horse, a chemical reaction, or within a single molecule can be understood through a study of thermodynamics, the science of energy changes.

Thermodynamics has two faces: one that is classical and one that is statistical. The *classical* approach uses a few postulates and definitions but makes no assumptions about the exact nature of the systems under investigation; even the existence of molecules is unnecessary. The *statistical* approach considers the behavior of large collections of molecules and allows more mechanistic conclusions, but it is more narrowly applicable. Both approaches lead to a consistent set of equations and together they provide a reasonable understanding of the nature of energy. To start, we need strict definitions of three entities: **internal energy**, **enthalpy**, and **entropy**.

Internal energy is the energy of the system under study, such as a chemical reaction or a pot of boiling water. It is the sum of the **work** and **heat** of the system under study. Work is an energy of motion, the product of force and distance.

Enthalpy (from the Greek *enthalpos*, meaning “putting heat in”) is closely related to internal energy. Enthalpy is the heat released or absorbed by a reaction at constant pressure, a common condition in the laboratory as well as in the chemistry of living systems. As a result, values for reaction enthalpies rather than internal energies are listed in tables of thermodynamic data.

The statistical approach to thermodynamics reveals entropy as the number of ways that energy can be distributed as a result of a process. For example, if we suddenly apply brakes to a speeding car, the energy of motion will be redistributed to particles of tire rubber on the street, as well as into frictional heat. Entropy *increases* with an increase in energy dispersion.

When discussing energy in a biochemical context, we are invariably referring to a combination of changes in enthalpy and entropy, called **free energy**. The free energy change for a system can be used to determine whether a reaction can proceed in the direction written. Issues of thermodynamics, free energy, and the relationship between free energy and equilibrium are discussed elsewhere in this book. Here, we will just assume that chemical energy is equivalent to free energy.

We commonly speak of both molecules and portions of molecules as having *high energy*. Two **high-energy molecules** of particular importance in biochemistry are the reduced form of nicotinamide adenine

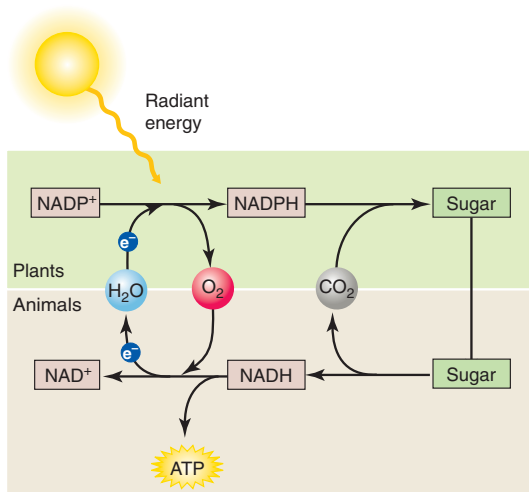


FIGURE 1.3 The Global CO₂ Cycle. Radiation from the Sun is used to energize electrons in the chloroplasts of plants, thus producing high-energy electrons in the form of NADPH. These electrons can be used to drive the photosynthesis of sugar from atmospheric CO₂ in plants. Subsequently, the plants are eaten by animals, and the sugars (and other molecules) are broken down to CO₂, which is released to the atmosphere, completing the cycle. Energy is trapped as electrons in NADH and is used to produce ATP for cell processes.

dinucleotide (NADH) and adenosine triphosphate (ATP). Both NADH and ATP are mobile cofactors that allow communication between hundreds of different reactions within the cell. NADH transfers electrons; consider it a donor of **high-energy electrons**. ATP, on the other hand, transfers a terminal phosphoryl group; think of it as a **high-energy phosphate** compound.

The connection between electron flow and ATP formation is a profound one in biochemistry. In the global energy cycle (**FIGURE 1.3**), radiation from the sun induces the formation of high-energy electrons by driving the formation of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), a close analog of NADH. NADPH then donates electrons to convert atmospheric carbon dioxide (CO₂) to sugars and other molecules. Subsequently, animals consume those sugars, converting their electrons to NADH, which is used to form ATP. In the process, sugars and other molecules form CO₂, which is once again utilized by plants.

1.4 Cell Theory

The *cell*—the smallest unit of life—is a key organizing principle in biology. Single-celled organisms, such as bacteria, yeasts, and protozoans (e.g., the paramecium), comprise the largest number of species. Our major focus, however, is on multicellular organisms, primarily human.

The features of a typical mammalian cell are illustrated in **FIGURE 1.4**, which shows internal organelles and their arrangement within

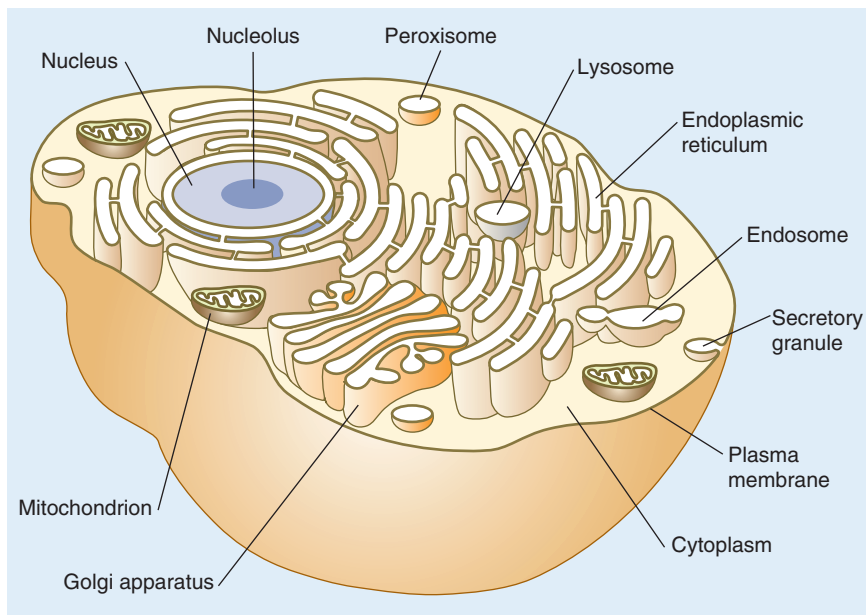


FIGURE 1.4 The Cell. A representative drawing of the cell shows the compartments that are created by membranes. The cell itself is separated from its exterior by a plasma membrane. Several interior membranes, such as those for the mitochondria, the lysosome, and the endoplasmic reticulum, define separate reaction spaces within the cell.

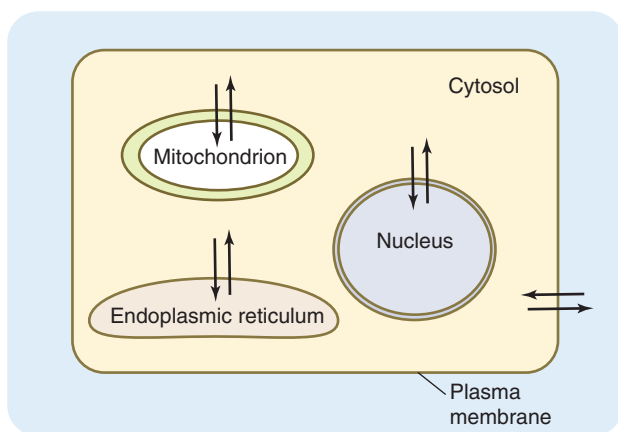


FIGURE 1.5 The Cell as a Concept. The cell can be represented as separate reaction spaces, set apart by semipermeable membranes. Specific exchanges across the relatively few membrane-delimited spaces are indicated by the arrows drawn across the membranes in both directions.

the cell. A much simplified functional representation is depicted in **FIGURE 1.5**, where the properties relevant to biochemical analysis can be readily seen. Consider, for example, the outer *plasma membrane* that encloses the cell, serving as its boundary with the outside world. The plasma membrane, like all cell membranes, is **semipermeable**. Some molecules readily cross the membrane, such as O_2 , H_2O , and CO_2 , whereas many others can traverse the membrane only if there is a carrier protein that selectively allows them to cross. From a chemical viewpoint, the membranes create separate water spaces to isolate chemical reactions. Only those molecules that can communicate across these spaces—those which can diffuse, or those which have a specific transporter—can participate in the reactions. The major water space within the cell is called the **cytosol**. Other membrane-delimited organelles shown in Figure 1.5 that define separate water spaces for reaction are the **mitochondria**, the **endoplasmic reticulum**, and the **nuclear membrane**. In addition to defining reactive spaces, membranes are the sites of lipid biosynthesis.

1.5 The Species Hierarchy and Evolution

Evolution is a fundamental principle of biology. It is also commonly misunderstood, perhaps due to the popularization of the phrase “survival of the fittest.” It is ironic that this phrase is so strongly embedded in the popular lexicon, because it does not exist in *On the Origin of the Species*, Darwin’s landmark work on evolution.

Evolution was first suggested from the extremes of plant and animal species that exist on isolated islands, notably the Galápagos of present day Ecuador. The idea was that species arose from other species, and those that could adapt best to their environment were able to survive because they lived to reproduce. Eventually, adaptive characteristics emerged. It is now commonplace to apply evolutionary principles

to biochemistry, mapping the formation of numerous enzymes and DNA sequences from early origins to divergent present forms.

In order to catalog the complexity of the immense number of known species, currently believed to number in the tens of millions, scientists have traditionally turned to the classification hierarchy. The classical organization is kingdom, phylum, class, order, family, genus, and species. While there are currently considered to be five kingdoms, we will focus on just three: animals, plants, and prokaryotes. Our emphasis is on mammalian species, although plant, bacterial, and occasionally yeast examples will be included. Most discoveries in biochemistry have come from only a very small sampling of the biological universe, largely for practical purposes, but there are many indications that a unity of pathways and mechanisms exists between different organisms. Some differences between organisms will be considered to get a sense of how biological variation is expressed.

1.6 Biological Systems

There is another hierarchy that identifies the viewpoint of the investigator toward biological systems. **FIGURE 1.6** shows a ranking with organisms at the top level and subatomic particles at the bottom. There are even higher levels of organization than organisms, such as populations, which have medical as well as scientific importance (as in studies of the transmission of infections between organisms). However, levels beyond that of organism are usually of interest in other fields, such as the social sciences.

The rankings in Figure 1.6 also can be used to exemplify two extremes in the analysis of biochemistry. A view close to the top, taking in as wide a swath as possible, is called **holistic**. Those who favor the holistic view argue that it leads to answers that are physiologically relevant. A view close to the bottom, examining specific chemicals or molecular interactions, is called **reductionist**. Those who favor the reductionist view argue that it alone allows firm conclusions to be drawn because the number of variables is much smaller than in the holistic approach. Both are essential, and we will move between them in the text. We begin with a reductionist approach, examining the chemical properties of water, the molecule most essential for the existence of life.

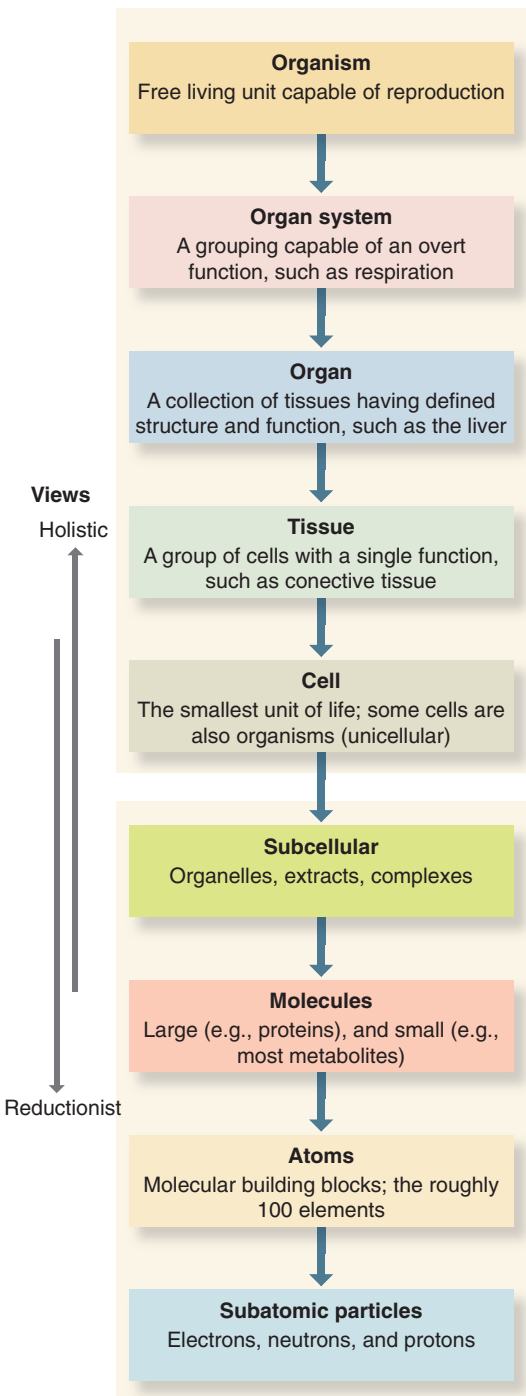


FIGURE 1.6 Hierarchies of Biological Study. Different levels of investigation are possible in the experimental study of biology. Physiologists typically study the higher levels, such as organisms, organ systems, and organs, whereas physicists typically study subatomic particles. Biochemists usually examine the intermediate levels. If the study is closer to the top of the hierarchy (i.e., if the approach is holistic), it is considered to be *physiologically relevant*, but there is greater uncertainty about the findings. If the study is closer to the bottom (i.e., if the approach is reductionist), it is considered to be more exact, but less relevant. Both approaches, while in conflict, require each other.

Key Terms

collision theory	high-energy electrons	saturation
cytosol	high-energy molecules	semipermeable
endoplasmic reticulum	high-energy phosphate	species hierarchies
energy	holistic	spontaneous generation
enthalpy	internal energy	steady state
entropy	mitochondria	vitalism
equilibrium	nuclear membrane	vitalist
free energy	rate constant	work
heat	reductionist	

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