CHAPTER 2

The Plasma Membrane, Membrane Transport, and the Resting Membrane Potential

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CHAPTER OUTLINE

KEY CONCEPTS

1. The two major components of the plasma membrane of a cell are proteins and lipids, present in about equal proportions.
2. Membrane proteins are responsible for most of the functions of the plasma membrane, including the transport of water and solutes across the membrane and providing specific binding sites for extracellular signaling molecules such as hormones.
3. Carrier-mediated transport systems allow the rapid transport of polar molecules, reach a maximum rate at high substrate concentration, exhibit structural specificity, and are competitively inhibited by molecules of similar structure.
4. Voltage-gated channels are opened by a change in the membrane potential, and ligand-gated channels are opened by the binding of a specific agonist.
5. The Na^+/K^+-ATPase pump is an example of primary active transport, and Na^+-coupled glucose transport is an example of secondary active transport.
6. The polarized organization of epithelial cells produces a directional movement of solutes and water across the epithelium.
7. Many cells regulate their volume when exposed to osmotic stress by activating transport systems that allow the exit or entry of solute so that water will follow.
8. The Goldman equation gives the value of the membrane potential when all the permeable ions are accounted for.
9. In most cells, the resting membrane potential is close to the Nernst potential for K^+.

The intracellular fluid of living cells, the **cytosol**, has a composition very different from that of the extracellular fluid. For example, the concentrations of potassium and phosphate ions are higher inside cells than outside, whereas sodium, calcium, and chloride ion concentrations are much lower inside cells than outside. These differences are necessary for the proper functioning of many intracellular enzymes; for instance, the synthesis of proteins by the ribosomes requires a relatively high potassium concentration. The cell membrane or **plasma membrane** creates and maintains these differences by establishing a permeability barrier around the cytosol. The ions and cell proteins needed for normal cell function are prevented from leaking out; those not needed by the cell are unable to enter the cell freely. The cell membrane also keeps metabolic intermediates near where they will be needed for further synthesis or processing and retains metabolically expensive proteins inside the cell.

The plasma membrane is necessarily selectively permeable. Cells must receive nutrients in order to function, and they must dispose of metabolic waste products. To function in coordination with the rest of the organism, cells receive and send information in the form of hormones and neurotransmitters. The plasma membrane has mechanisms that allow specific molecules to cross the barrier around the cell. A selective barrier surrounds not only the cell but also every intracellular organelle that requires an internal milieu different from that of the cytosol. The cell nucleus, mitochondria, endoplasmic reticulum, Golgi apparatus, and lysosomes are delimited by membranes similar in composi-

tion to the plasma membrane. This chapter describes the specific types of membrane transport mechanisms for ions and other solutes, their relative contributions to the resting membrane potential, and how their activities are coordinated to achieve directional transport from one side of a cell layer to the other.

THE STRUCTURE OF THE PLASMA MEMBRANE

The first theory of membrane structure proposed that cells are surrounded by a double layer of lipid molecules, a **lipid bilayer**. This theory was based on the known tendency of lipid molecules to form lipid bilayers with low permeability to water-soluble molecules. However, the lipid bilayer theory did not explain the selective movement of certain water-soluble compounds, such as glucose and amino acids, across the plasma membrane. In 1972, Singer and Nicolson proposed the **fluid mosaic model** of the plasma membrane (Fig. 2.1). With minor modifications, this model is still accepted as the correct picture of the structure of the plasma membrane.

The Plasma Membrane Has Proteins Inserted in the Lipid Bilayer

Proteins and lipids are the two major components of the plasma membrane, present in about equal proportions by weight. The various lipids are arranged in a lipid bilayer, and two different types of proteins are associated with this bilayer. **Integral proteins** (or intrinsic proteins) are embedded in the lipid bilayer; many span it completely, being accessible from the inside and outside of the membrane. The polypeptide chain of these proteins may cross the lipid bilayer once or may make multiple passes across it. The membrane-spanning segments usually contain amino acids with nonpolar side chains and are arranged in an ordered α-helical conformation. **Peripheral proteins** (or extrinsic proteins) do not penetrate the lipid bilayer. They are in contact with the outer side of only one of the lipid layers—either the layer facing the cytoplasm or the layer facing the extracellular fluid (see Fig. 2.1). Many membrane proteins have carbohydrate molecules, in the form of specific sugars, attached to the parts of the proteins that are exposed to the extracellular fluid. These molecules are known as **glycoproteins**. Some of the integral membrane proteins can move in the plane of the membrane, like small boats floating in the "sea" formed by the bilayer arrangement of the lipids. Other membrane proteins are anchored to the cytoskeleton inside the cell or to proteins of the extracellular matrix.

The proteins in the plasma membrane play a variety of roles. Many peripheral membrane proteins are enzymes, and many membrane-spanning integral proteins are carriers or channels for the movement of water-soluble molecules and ions into and out of the cell. Another important role of membrane proteins is structural; for example, certain membrane proteins in the erythrocyte help maintain the biconcave shape of the cell. Finally, some membrane proteins serve as highly specific recognition sites or receptors on the outside of the cell membrane to which extracellular molecules, such as hormones, can bind. If the receptor is a membrane-spanning protein, it provides a mechanism for converting an extracellular signal into an intracellular response.

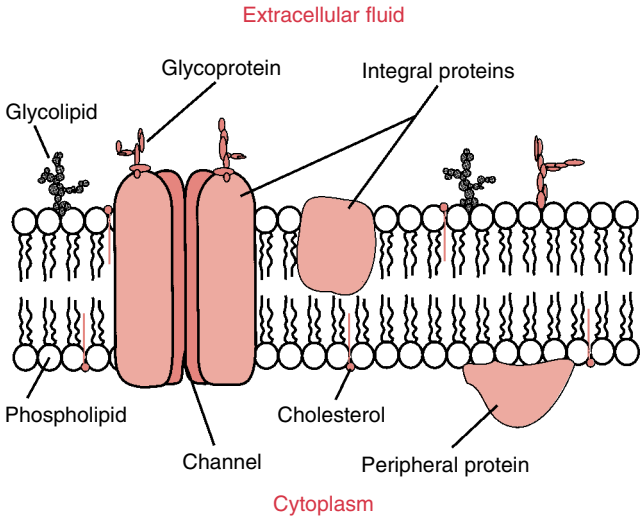

FIGURE 2.1 **The fluid mosaic model of the plasma membrane.** Lipids are arranged in a bilayer. Integral proteins are embedded in the bilayer and often span it. Some membrane-spanning proteins form channels. Peripheral proteins do not penetrate the bilayer.

There Are Different Types of Membrane Lipids

Lipids found in cell membranes can be classified into two broad groups: those that contain fatty acids as part of the lipid molecule and those that do not. Phospholipids are an example of the first group, and cholesterol is the most important example of the second group.

Phospholipids. The fatty acids present in **phospholipids** are molecules with a long hydrocarbon chain and a carboxyl terminal group. The hydrocarbon chain can be saturated (no double bonds between the carbon atoms) or unsaturated (one or more double bonds present). The composition of fatty acids gives them some peculiar characteristics. The long hydrocarbon chain tends to avoid contact with water and is described as **hydrophobic**. The carboxyl group at the other end is compatible with water and is termed **hydrophilic**. Fatty acids are said to be **amphipathic** because both hydrophobic and hydrophilic regions are present in the same molecule.

Phospholipids are the most abundant complex lipids found in cell membranes. They are amphipathic molecules formed by two fatty acids (normally, one saturated and one unsaturated) and one phosphoric acid group substituted on the backbone of a glycerol or sphingosine molecule. This arrangement produces a hydrophobic area formed by the two fatty acids and a polar hydrophilic head. When phospholipids are arranged in a bilayer, the polar heads are on the outside and the hydrophobic fatty acids on the inside. It is difficult for water-soluble molecules and ions to pass directly through the hydrophobic interior of the lipid bilayer.

The phospholipids, with a backbone of sphingosine (a long amino alcohol), are usually called sphingolipids and are present in all plasma membranes in small amounts. They are especially abundant in brain and nerve cells.

Glycolipids are lipid molecules that contain sugars and sugar derivatives (instead of phosphoric acid) in the polar head. They are located mainly in the outer half of the lipid bilayer, with the sugar molecules facing the extracellular fluid.

Cholesterol. **Cholesterol** is an important component of mammalian plasma membranes. The proportion of cholesterol in plasma membranes varies from 10% to 50% of total lipids. Cholesterol has a rigid structure that stabilizes the cell membrane and reduces the natural mobility of the complex lipids in the plane of the membrane. Increasing amounts of cholesterol make it more difficult for lipids and proteins to move in the membrane. Some cell functions, such as the response of immune system cells to the presence of an antigen, depend on the ability of membrane proteins to move in the plane of the membrane to bind the antigen. A decrease in membrane fluidity resulting from an increase in cholesterol will impair these functions.

MECHANISMS OF SOLUTE TRANSPORT

All cells need to import oxygen, sugars, amino acids, and some small ions and to export carbon dioxide, metabolic wastes, and secretions. At the same time, specialized cells require mechanisms to transport molecules such as enzymes, hormones, and neurotransmitters. The movement of large molecules is carried out by endocytosis and exocytosis, the transfer of substances into or out of the cell, respectively, by vesicle formation and vesicle fusion with the plasma membrane. Cells also have mechanisms for the rapid movement of ions and solute molecules across the plasma membrane. These mechanisms are of two general types: **passive movement,** which requires no direct expenditure of metabolic energy, and **active movement,** which uses metabolic energy to drive solute transport.

Macromolecules Cross the Plasma Membrane by Vesicle Fusion

Phagocytosis and Endocytosis. **Phagocytosis** is the ingestion of large particles or microorganisms, usually occurring only in specialized cells such as macrophages (Fig. 2.2). An important function of macrophages in humans is to remove invading bacteria. The phagocytic vesicle (1 to 2 μm in diameter) is almost as large as the phagocytic cell itself. Phagocytosis requires a specific stimulus. It occurs only after the extracellular particle has bound to the extracellular surface. The particle is then enveloped by expansion of the cell membrane around it.

Endocytosis is a general term for the process in which a region of the plasma membrane is pinched off to form an endocytic vesicle inside the cell. During vesicle formation, some fluid, dissolved solutes, and particulate material from the extracellular medium are trapped inside the vesicle and internalized by the cell. Endocytosis produces much smaller endocytic vesicles (0.1 to 0.2 μm in diameter) than phagocytosis. It occurs in almost all cells and is termed a constitutive process because it occurs continually and specific stimuli are not required. In further contrast to phagocytosis, endocytosis originates with the formation of depressions in the cell membrane. The depressions pinch off

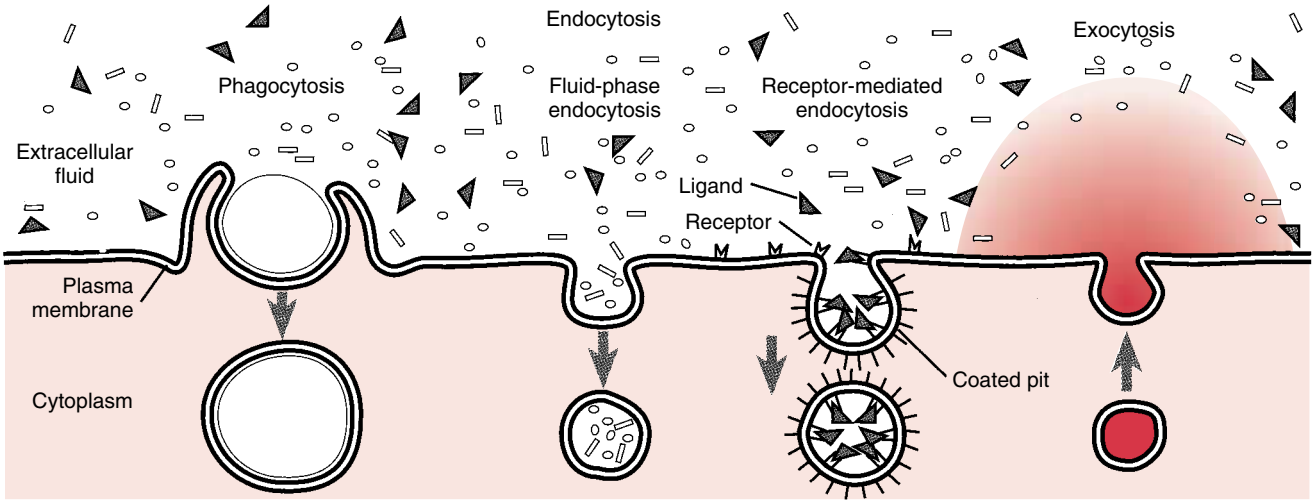

FIGURE 2.2 The transport of macromolecules across the plasma membrane by the formation of vesicles. Particulate matter in the extracellular fluid is engulfed and internalized by phagocytosis. During fluid-phase endocytosis, extracellular fluid and dissolved macromolecules enter the cell in endocytic vesicles that pinch off at depressions in the plasma membrane. Receptor-mediated endocytosis uses membrane receptors at coated pits to bind and internalize specific solutes (ligands). Exocytosis is the release of macromolecules destined for export from the cell. These are packed inside secretory vesicles that fuse with the plasma membrane and release their contents outside the cell. (Modified from Dautry-Varsat A, Lodish HF. How receptors bring proteins and particles into cells. Sci Am 1984;250(5):52–58.)

within a few minutes after they form and give rise to endocytic vesicles inside the cell.

Two main types of endocytosis can be distinguished (see Fig. 2.2). **Fluid-phase endocytosis** is the nonspecific uptake of the extracellular fluid and all its dissolved solutes. The material is trapped inside the endocytic vesicle as it is pinched off inside the cell. The amount of extracellular material internalized by this process is directly proportional to its concentration in the extracellular solution. **Receptor-mediated endocytosis** is a more efficient process that uses receptors on the cell surface to bind specific molecules. These receptors accumulate at specific depressions known as **coated pits,** so named because the cytosolic surface of the membrane at this site is covered with a coat of several proteins. The coated pits pinch off continually to form endocytic vesicles, providing the cell with a mechanism for rapid internalization of a large amount of a specific molecule without the need to endocytose large volumes of extracellular fluid. The receptors also aid the cellular uptake of molecules present at low concentrations outside the cell. Receptor-mediated endocytosis is the mechanism by which cells take up a variety of important molecules, including hormones; growth factors; and serum transport proteins, such as **transferrin** (an iron carrier). Foreign substances, such as diphtheria toxin and certain viruses, also enter cells by this pathway.

Exocytosis. Many cells synthesize important macromolecules that are destined for **exocytosis** or export from the cell. These molecules are synthesized in the endoplasmic reticulum, modified in the Golgi apparatus, and packed inside transport vesicles. The vesicles move to the cell surface, fuse with the cell membrane, and release their contents outside the cell (see Fig. 2.2).

There are two exocytic pathways—constitutive and regulated. Some proteins are secreted continuously by the cells that make them. Secretion of mucus by **goblet cells** in the small intestine is a specific example. In this case, exocytosis follows the **constitutive pathway**, which is present in all cells. In other cells, macromolecules are stored inside the cell in secretory vesicles. These vesicles fuse with the cell membrane and release their contents only when a specific extracellular stimulus arrives at the cell membrane. This pathway, known as the **regulated pathway**, is responsible for the rapid "on-demand" secretion of many specific hormones, neurotransmitters, and digestive enzymes.

The Passive Movement of Solutes Tends to Equilibrate Concentrations

Simple Diffusion. Any solute will tend to uniformly occupy the entire space available to it. This movement, known as **diffusion**, is due to the spontaneous Brownian (random) movement that all molecules experience and that explains many everyday observations. Sugar diffuses in coffee, lemon diffuses in tea, and a drop of ink placed in a glass of water will diffuse and slowly color all the water. The net result of diffusion is the movement of substances according to their difference in concentrations, from regions of high concentration to regions of low concentration. Diffusion is an effective way for substances to move short distances.

The speed with which the diffusion of a solute in water occurs depends on the difference of concentration, the size of the molecules, and the possible interactions of the diffusible substance with water. These different factors appear in Fick's law, which describes the diffusion of any solute in water. In its simplest formulation, Fick's law can be written as:

$$J = DA\,(C_1 - C_2)/\Delta X \qquad (1)$$

where J is the flow of solute from region 1 to region 2 in the solution, D is the diffusion coefficient of the solute and takes into consideration such factors as solute molecular size and interactions of the solute with water, A is the cross-sectional area through which the flow of solute is measured, C is the concentration of the solute at regions 1 and 2, and ΔX is the distance between regions 1 and 2. J is expressed in units of amount of substance per unit area per unit time, for example, mol/cm^2 per hour, and is also referred to as the solute **flux**.

Diffusive Membrane Transport. Solutes can enter or leave a cell by diffusing passively across the plasma membrane. The principal force driving the diffusion of an uncharged solute is the difference of concentration between the inside and the outside of the cell (Fig. 2.3). In the case of an electrically charged solute, such as an ion, diffusion is also driven by the membrane potential, which is the electrical gradient across the membrane. The membrane potential of most living cells is negative inside the cell relative to the outside.

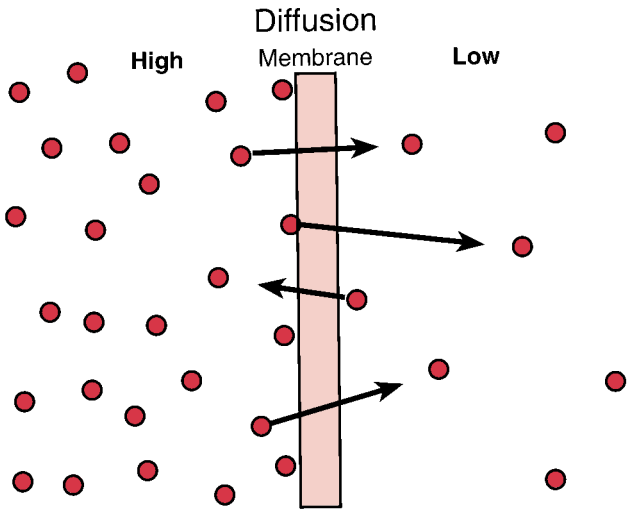

FIGURE 2.3 **The diffusion of gases and lipid-soluble molecules through the lipid bilayer.** In this example, the diffusion of a solute across a plasma membrane is driven by the difference in concentration on the two sides of the membrane. The solute molecules move randomly by Brownian movement. Initially, random movement from left to right across the membrane is more frequent than movement in the opposite direction because there are more molecules on the left side. This results in a net movement of solute from left to right across the membrane until the concentration of solute is the same on both sides. At this point, equilibrium (no net movement) is reached because solute movement from left to right is balanced by equal movement from right to left.

Diffusion across a membrane has no preferential direction; it can occur from the outside of the cell toward the inside or from the inside of the cell toward the outside. For any substance, it is possible to measure the **permeability coefficient** (P), which gives the speed of the diffusion across a unit area of plasma membrane for a defined driving force. Fick's law for the diffusion of an uncharged solute across a membrane can be written as:

$$J = PA\,(C_1 - C_2) \qquad (2)$$

which is similar to equation 1. P includes the membrane thickness, diffusion coefficient of the solute within the membrane, and solubility of the solute in the membrane. Dissolved gases, such as oxygen and carbon dioxide, have high permeability coefficients and diffuse across the cell membrane rapidly. Since diffusion across the plasma membrane usually implies that the diffusing solute enters the lipid bilayer to cross it, the solute's solubility in a lipid solvent (e.g., olive oil or chloroform) compared with its solubility in water is important in determining its permeability coefficient.

A substance's solubility in oil compared with its solubility in water is its **partition coefficient**. Lipophilic substances that mix well with the lipids in the plasma membrane have high partition coefficients and, as a result, high permeability coefficients; they tend to cross the plasma membrane easily. Hydrophilic substances, such as ions and sugars, do not interact well with the lipid component of the membrane, have low partition coefficients and low permeability coefficients, and diffuse across the membrane more slowly.

For solutes that diffuse across the lipid part of the plasma membrane, the relationship between the rate of movement and the difference in concentration between the two sides of the membrane is linear (Fig. 2.4). The higher the difference in concentration ($C_1 - C_2$), the greater the amount of substance crossing the membrane per unit time.

Facilitated Diffusion via Carrier Proteins. For many solutes of physiological importance, such as sugars and amino acids, the relationship between transport rate and concentration difference follows a curve that reaches a plateau (Fig. 2.5). Furthermore, the rate of transport of these hydrophilic substances across the cell membrane is much faster than expected for simple diffusion through a lipid bilayer. Membrane transport with these characteristics is often called **carrier-mediated transport** because an integral membrane protein, the carrier, binds the transported solute on one side of the membrane and releases it at the other side. Although the details of this transport mechanism are unknown, it is hypothesized that the binding of the solute causes a conformational change in the carrier protein, which results in translocation of the solute (Fig. 2.6). Because there are limited numbers of these carriers in any cell membrane, increasing the concentration of the solute initially uses the existing "spare" carriers to transport the solute at a higher rate than by simple diffusion. As the concentration of the solute increases further and more solute molecules bind to carriers, the transport system eventually reaches **saturation**, when all the carriers are involved in translocating molecules of solute. At this point, additional increases in solute concentration do not increase the rate of solute transport (see Fig. 2.5).

The types of carrier-mediated transport mechanisms considered here can transport a solute along its concentration gradient only, as in simple diffusion. Net movement

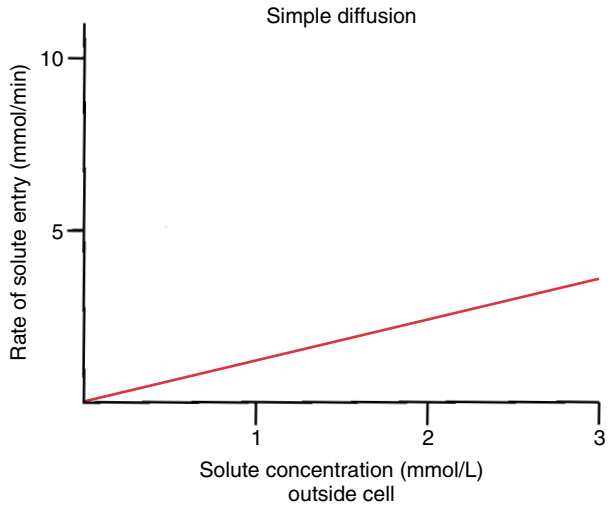

FIGURE 2.4 **A graph of solute transport across a plasma membrane by simple diffusion.** The rate of solute entry increases linearly with extracellular concentration of the solute. Assuming no change in intracellular concentration, increasing the extracellular concentration increases the gradient that drives solute entry.

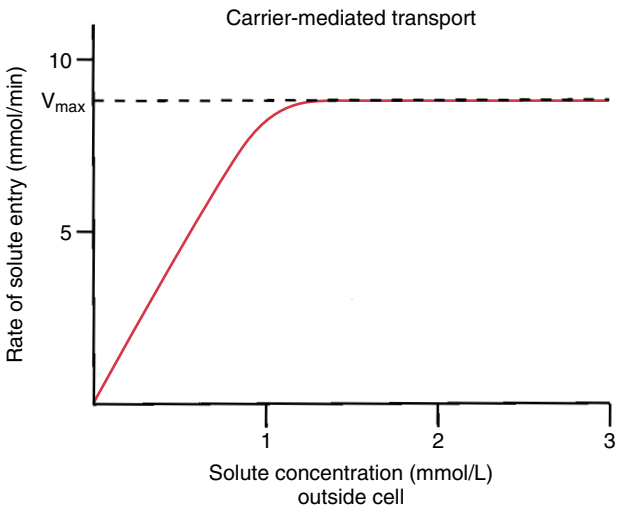

FIGURE 2.5 **A graph of solute transport across a plasma membrane by carrier-mediated transport.** The rate of transport is much faster than that of simple diffusion (see Fig. 2.4) and increases linearly as the extracellular solute concentration increases. The increase in transport is limited, however, by the availability of carriers. Once all are occupied by solute, further increases in extracellular concentration have no effect on the rate of transport. A maximum rate of transport (V_{max}) is achieved that cannot be exceeded.

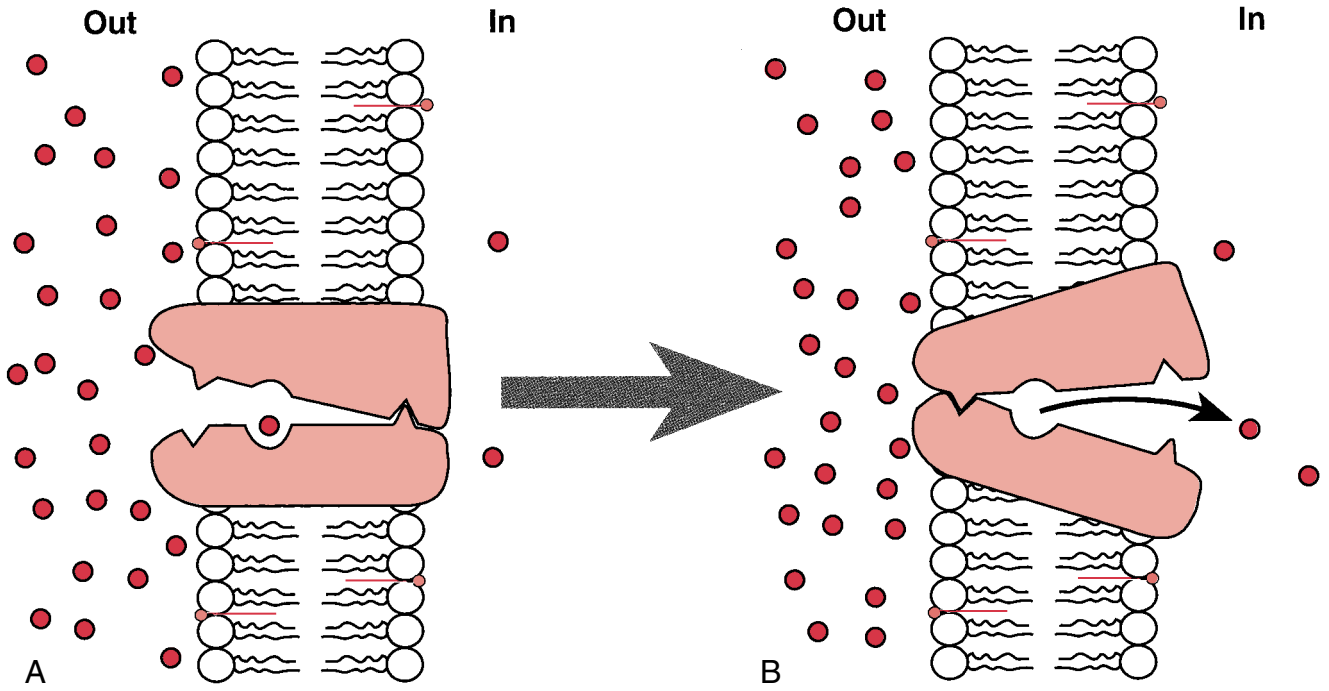

FIGURE 2.6 The role of a carrier protein in facilitated diffusion of solute molecules across a plasma membrane. In this example, solute transport into the cell is driven by the high solute concentration outside compared to inside. **A,** Binding of extracellular solute to the carrier, a membrane-spanning integral protein, may trigger a change in protein conformation that exposes the bound solute to the interior of the cell. **B,** Bound solute readily dissociates from the carrier because of the low intracellular concentration of solute. The release of solute may allow the carrier to revert to its original conformation (A) to begin the cycle again.

stops when the concentration of the solute has the same value on both sides of the membrane. At this point, with reference to equation 2, $C_1 = C_2$ and the value of J is 0. The transport systems function until the solute concentrations have **equilibrated**. However, equilibrium is attained much faster than with simple diffusion.

Equilibrating carrier-mediated transport systems have several characteristics:

- They allow the transport of polar (hydrophilic) molecules at rates much higher than expected from the partition coefficient of these molecules.
- They eventually reach saturation at high substrate concentration.
- They have structural specificity, meaning each carrier system recognizes and binds specific chemical structures (a carrier for D-glucose will not bind or transport L-glucose).
- They show competitive inhibition by molecules with similar chemical structure. For example, carrier-mediated transport of D-glucose occurs at a slower rate when molecules of D-galactose also are present. This is because galactose, structurally similar to glucose, competes with glucose for the available glucose carrier proteins.

A specific example of this type of carrier-mediated transport is the movement of glucose from the blood to the interior of cells. Most mammalian cells use blood glucose as a major source of cellular energy, and glucose is transported into cells down its concentration gradient. The transport process in many cells, such as erythrocytes and the cells of fat, liver, and muscle tissues, involves a plasma membrane protein called GLUT 1 (glucose transporter 1). The erythrocyte GLUT 1 has an affinity for D-glucose that is about 2,000-fold greater than the affinity for L-glucose. It is an integral membrane protein that contains 12 membrane-spanning α-helical segments.

Equilibrating carrier-mediated transport, like simple diffusion, does not have directional preferences. It functions equally well in bringing its specific solutes into or out of the cell, depending on the concentration gradient. Net movement by equilibrating carrier-mediated transport ceases once the concentrations inside and outside the cell become equal.

The anion exchange protein (AE1), the predominant integral protein in the mammalian erythrocyte membrane, provides a good example of the reversibility of transporter action. AE1 is folded into at least 12 transmembrane α-helices and normally permits the one-for-one exchange of Cl^- and HCO_3^- ions across the plasma membrane. The direction of ion movement is dependent only on the concentration gradients of the transported ions. AE1 has an important role in transporting CO_2 from the tissues to the lungs. The erythrocytes in systemic capillaries pick up CO_2 from tissues and convert it to HCO_3^-, which exits the cells via AE1. When the erythrocytes enter pulmonary capillaries, the AE1 allows plasma HCO_3^- to enter erythrocytes, where it is converted back to CO_2 for expiration by the lungs (see Chapter 21).

Facilitated Diffusion Through Ion Channels. Small ions, such as Na^+, K^+, Cl^-, and Ca^{2+}, also cross the plasma membrane faster than would be expected based on their partition coefficients in the lipid bilayer. An ion's electrical charge makes it difficult for the ion to move across the lipid bilayer. The rapid movement of ions across the membrane, however, is an aspect of many cell functions. The nerve action potential, the contraction of muscle, the pacemaker function of the heart, and many other physiological events are possible because of the ability of small ions to enter or leave the cell rapidly. This movement occurs through selective ion channels.

Ion channels are integral proteins spanning the width of the plasma membrane and are normally composed of several polypeptide subunits. Certain specific stimuli cause the protein subunits to open a **gate**, creating an aqueous channel through which the ions can move (Fig. 2.7). In this way, ions do not need to enter the lipid bilayer to cross the membrane; they are always in an aqueous medium. When the channels are open, the ions move rapidly from one side of the membrane to the other by facilitated diffusion. Specific interactions between the ions and the sides of the channel produce an extremely rapid rate of ion movement; in fact, ion channels permit a much faster rate of solute transport (about 10^8 ions/sec) than carrier-mediated systems.

Ion channels are often selective. For example, some channels are selective for Na^+, for K^+, for Ca^{2+}, for Cl^-, and for other anions and cations. It is generally assumed that some kind of ionic selectivity filter must be built into the structure of the channel (see Fig. 2.7). No clear relation between the amino acid composition of the channel protein and ion selectivity of the channel has been established.

A great deal of information about the characteristic behavior of channels for different ions has been revealed by the **patch clamp technique**. The small electrical current caused by ion movement when a channel is open can be detected with this technique, which is so sensitive that the opening and closing of a single ion channel can be observed (Fig. 2.8). In general, ion channels exist either fully open or completely closed, and they open and close very rapidly. The frequency with which a channel opens is variable, and the time the channel remains open (usually a few milliseconds) is also variable. The overall rate of ion transport across a membrane can be controlled by changing the frequency of a channel opening or by changing the time a channel remains open.

Most ion channels usually open in response to a specific stimulus. Ion channels can be classified according to their gating mechanisms, the signals that make them open or close. There are voltage-gated channels and ligand-gated channels. Some ion channels are always open and these are referred to as nongated channels (see Chapter 3).

Voltage-gated ion channels open when the membrane potential changes beyond a certain threshold value. Channels of this type are involved in the conduction of action potentials along nerve axons and they include sodium and potassium channels (see Chapter 3). Voltage-gated ion channels are found in many cell types. It is thought that some charged amino acids located in a membrane-spanning α-helical segment of the channel protein are sensitive to the transmembrane potential. Changes in the membrane potential cause these amino acids to move and induce a conformational change of the protein that opens the way for the ions.

Ligand-gated (or, **chemically gated**) **ion channels** cannot open unless they first bind to a specific agonist. The opening of the gate is produced by a conformational change in the protein induced by the ligand binding. The ligand can be a neurotransmitter arriving from the extracellular medium. It also can be an intracellular second messenger, produced in response to some cell activity or hormone action, that reaches the ion channel from the inside of the cell. The nicotinic acetylcholine receptor channel found in the postsynaptic neuromuscular junction (see Chapters 3 and 9) is a ligand-gated ion channel that is opened by an extracellular ligand (acetylcholine). Examples of ion channels gated by intracel-

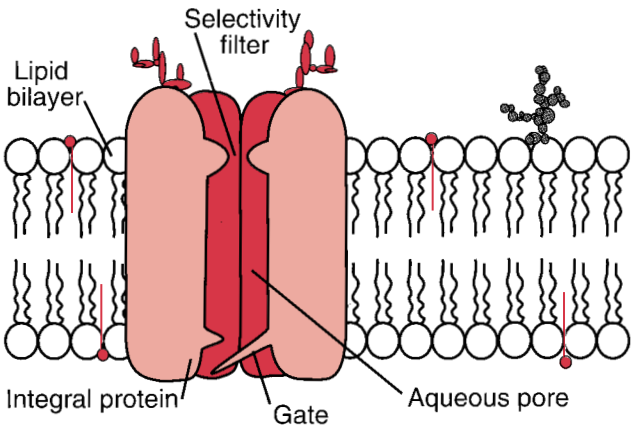

FIGURE 2.7 **An ion channel.** Ion channels are formed between the polypeptide subunits of integral proteins that span the plasma membrane, providing an aqueous pore through which ions can cross the membrane. Different types of gating mechanisms are used to open and close channels. Ion channels are often selective for a specific ion.

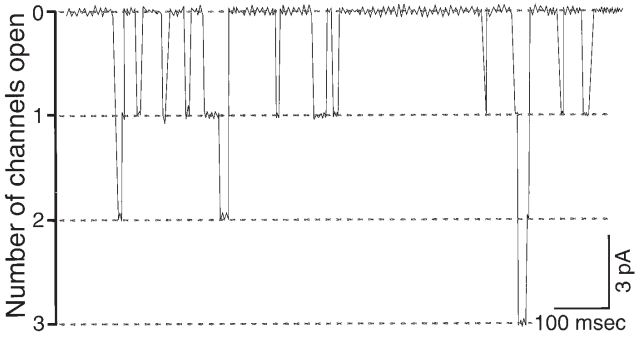

FIGURE 2.8 **A patch clamp recording from a frog muscle fiber.** Ions flow through the channel when it opens, generating a current. The current in this experiment is about 3 pA and is detected as a downward deflection in the recording. When more than one channel opens, the current and the downward deflection increase in direct proportion to the number of open channels. This record shows that up to three channels are open at any instant. (Modified from Kandel ER, Schwartz JH, Jessell TM. Principles of Neural Science. 3rd Ed. New York: Elsevier, 1991.)

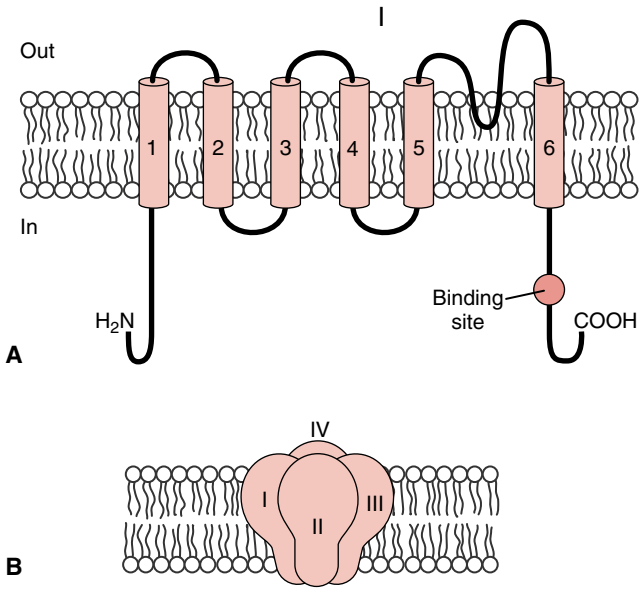

FIGURE 2.9 **Structure of a cyclic nucleotide-gated ion channel.** A, The secondary structure of a single subunit has six membrane-spanning regions and a binding site for cyclic nucleotides on the cytosolic side of the membrane. B, Four identical subunits (I–IV) assemble together to form a functional channel that provides a hydrophilic pathway across the plasma membrane.

lular messengers also abound in nature. This type of gating mechanism allows the channel to open or close in response to events that occur at other locations in the cell. For example, a sodium channel gated by intracellular cyclic GMP is involved in the process of vision (see Chapter 4). This channel is located in the rod cells of the retina and it opens in the presence of cyclic GMP. The generalized structure of one subunit of an ion channel gated by cyclic nucleotides is shown in Figure 2.9. There are six membrane-spanning regions and a cyclic nucleotide-binding site is exposed to the cytosol. The functional protein is a tetramer of four identical subunits. Other cell membranes have potassium channels that open when the intracellular concentration of calcium ions increases. Several known channels respond to inositol 1,4,5-trisphosphate, the activated part of G proteins, or ATP. The gating of the epithelial chloride channel by ATP is described in the Clinical Focus Box 2.1 in this chapter.

Solutes Are Moved Against Gradients by Active Transport Systems

The passive transport mechanisms discussed all tend to bring the cell into equilibrium with the extracellular fluid. Cells must oppose these equilibrating systems and preserve intracellular concentrations of solutes, particularly ions, that are compatible with life.

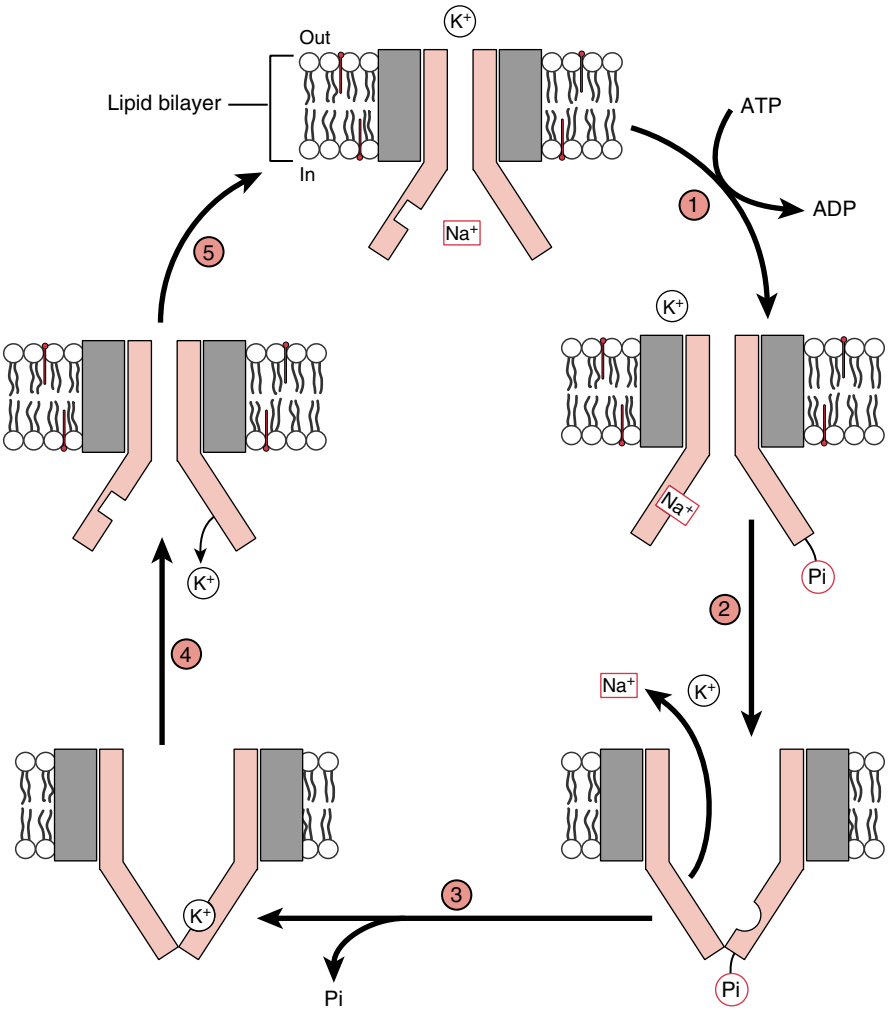

FIGURE 2.10 **The possible sequence of events during one cycle of the sodium-potassium pump.** The functional form may be a tetramer of two large catalytic subunits and two smaller subunits of unknown function. Binding of intracellular Na^+ and phosphorylation by ATP inside the cell may induce a conformational change that transfers Na^+ to the outside of the cell (steps 1 and 2). Subsequent binding of extracellular K^+ and dephosphorylation return the protein to its original form and transfer K^+ into the cell (steps 3, 4, and 5). There are thought to be three Na^+ binding sites and two K^+ binding sites. During one cycle, three Na^+ are exchanged for two K^+, and one ATP molecule is hydrolyzed.

CLINICAL FOCUS BOX 2.1

Cystic Fibrosis

Cystic fibrosis is one of the most common lethal genetic diseases of Caucasians. In northern Europe and the United States, for example, about 1 child in 2,500 is born with the disease. It was first recognized clinically in the 1930s, when it appeared to be a gastrointestinal problem because patients usually died from malnutrition during the first year of life. Survival has improved as management has improved; afflicted newborns now have a life expectancy of about 40 years. Cystic fibrosis affects several organ systems, with the severity varying enormously among individuals. Clinical features can include deficient secretion of digestive enzymes by the pancreas; infertility in males; increased concentration of chloride ions in sweat; intestinal and liver disease; and airway disease, leading to progressive lung dysfunction. Involvement of the lungs determines survival: 95% of cystic fibrosis patients die from respiratory failure.

The basic defect in cystic fibrosis is a failure of chloride transport across epithelial plasma membranes, particularly in the epithelial cells that line the airways. Much of the information about defective chloride transport was obtained by studying individual chloride channels using the patch clamp technique. One hypothesis is that all the pathophysiology of cystic fibrosis is a direct result of chloride transport failure. In the lungs, for example, reduced secretion of chloride ion is usually accompanied by a reduced secretion of sodium and bicarbonate ions. These changes retard the secretion of water, so the mucus secretions that line airways become thick and sticky and the smaller airways become blocked. The thick mucus also traps bacteria, which may lead to bacterial infection. Once established, bacterial infection is difficult to eradicate from the lungs of a patient with cystic fibrosis.

It was predicted that the flawed gene in patients with cystic fibrosis would normally encode either a chloride channel protein or a membrane protein that regulates chloride channels. The gene was identified in 1989 and encodes a protein of 1,480 amino acids, the **cystic fibrosis transmembrane conductance regulator** (CFTR). Evidence indicates that CFTR contains both a chloride channel and a channel regulator. Although it functions as an ion channel, it has structural similarities to adenosine triphosphate (ATP)-driven ion pumps that are integral membrane proteins. The CFTR protein is anchored in the plasma membrane by 12 membrane-spanning segments that also form a channel. A large regulatory domain is exposed to the cytosol and contains several sites that can be phosphorylated by various protein kinases, such as cyclic adenosine monophosphate (AMP)-dependent protein kinase. Two nucleotide-binding domains (NBD) control channel activity through interactions with nucleotides, such as ATP, present in the cell cytosol. A two-step process controls the gating of CFTR: (1) phosphorylation of specific sites within the regulatory domain, and (2) binding and hydrolysis of ATP at the NBD. After initial phosphorylation, gating between the closed and open states is controlled by ATP hydrolysis. It is believed that the channel is opened by ATP hydrolysis at one NBD and closed by subsequent ATP hydrolysis at the other NBD.

A common mutation in CFTR, found in 70% of cystic fibrosis patients, results in the loss of the amino acid phenylalanine from one of the NBD. This mutation produces severe symptoms because it results in defective targeting of newly synthesized CFTR proteins to the plasma membrane. The number of functional CFTR proteins at the correct location is decreased to an inadequate level.

Increased understanding of the pathophysiology of airway disease in cystic fibrosis has given rise to new therapies, and a definitive solution may be close at hand. Two approaches are undergoing clinical trials. One approach is to design pharmacological agents that will either regulate (open or close) defective CFTR chloride channels or bypass CFTR and stimulate other membrane chloride channels in the same cells. The other approach is the use of gene therapy to insert a normal gene for CFTR into affected airway epithelial cells. This has the advantage of restoring both the known and unknown functions of the gene. The field of gene therapy is in its infancy, and although there have been no "cures" for cystic fibrosis, much has been learned about the problems presented by the inefficient and short-lived transfer of genes *in vivo*. The next phase of gene therapy will focus on improving the technology for gene delivery. Gene therapy may become a reality for many lung diseases during this century.

Primary Active Transport. Integral membrane proteins that directly use metabolic energy to transport ions against a gradient of concentration or electrical potential are known as **ion pumps**. The direct use of metabolic energy to carry out transport defines a **primary active transport mechanism**. The source of metabolic energy is ATP synthesized by mitochondria, and the different ion pumps hydrolyze ATP to ADP and use the energy stored in the third phosphate bond to carry out transport. Because of this ability to hydrolyze ATP, ion pumps also are called **ATPases**.

The most abundant ion pump in higher organisms is the **sodium-potassium pump** or Na^+/K^+**-ATPase**. It is found in the plasma membrane of practically every eukaryotic cell and is responsible for maintaining the low sodium and high potassium concentrations in the cytoplasm. The sodium-potassium pump is an integral membrane protein consisting of two subunits, one large and one small. Sodium ions are transported out of the cell and potassium ions are brought in. It is known as a **P-type ATPase** because the protein is phosphorylated during the transport cycle (Fig. 2.10). The pump counterbalances the tendency of sodium ions to enter the cell passively and the tendency of potassium ions to leave passively. It maintains a high intracellular potassium concentration necessary for protein synthesis. It also plays a role in the resting membrane potential by maintaining ion gradients. The sodium-potassium pump can be inhibited either by metabolic poisons that stop the synthesis and supply of ATP or by specific pump blockers, such as the cardiac glycoside **digitalis**.

Calcium pumps, Ca^{2+}**-ATPases**, are found in the plasma membrane, in the membrane of the endoplasmic reticulum, and, in muscle cells, in the sarcoplasmic reticu-

lum membrane. They are also P-type ATPases. They pump calcium ions from the cytosol of the cell either into the extracellular space or into the lumen of these organelles. The organelles store calcium and, as a result, help maintain a low cytosolic concentration of this ion (see Chapter 1).

The **H^+/K^+-ATPase** is another example of a P-type ATPase. It is present in the luminal membrane of the parietal cells in oxyntic (acid-secreting) glands of the stomach. By pumping protons into the lumen of the stomach in exchange for potassium ions, this pump maintains the low pH in the stomach that is necessary for proper digestion (see Chapter 28). It is also found in the colon and in the collecting ducts of the kidney. Its role in the kidney is to secrete H^+ ions into the urine and to reabsorb K^+ ions (see Chapter 25).

Proton pumps, H^+-ATPases, are found in the membranes of the lysosomes and the Golgi apparatus. They pump protons from the cytosol into these organelles, keeping the inside of the organelles more acidic (at a lower pH) than the rest of the cell. These pumps, classified as **V-type ATPases** because they were first discovered in intracellular vacuolar structures, have now been detected in plasma membranes. For example, the proton pump in the luminal plasma membrane of kidney cells is characterized as a V-type ATPase. By secreting protons, it plays an important role in acidifying the tubular urine.

Mitochondria have **F-type ATPases** located in the inner mitochondrial membrane. This type of proton pump normally functions in reverse. Instead of using the energy stored in ATP molecules to pump protons, its principal function is to synthesize ATP by using the energy stored in a gradient of protons. The proton gradient is generated by the respiratory chain.

Secondary Active Transport. The net effect of ion pumps is maintenance of the various environments needed for the proper functioning of organelles, cells, and organs. Metabolic energy is expended by the pumps to create and maintain the differences in ion concentrations. Besides the importance of local ion concentrations for cell function, differences in concentrations represent stored energy. An ion releases potential energy when it moves down an electrochemical gradient, just as a body releases energy when falling to a lower level. This energy can be used to perform work. Cells have developed several carrier mechanisms to transport one solute against its concentration gradient by using the energy stored in the favorable gradient of another solute. In mammals, most of these mechanisms use sodium as the driver solute and use the energy of the sodium gradient to carry out the "uphill" transport of another important solute (Fig. 2.11). Because the sodium gradient is low. A conformational change in the carrier protein may expose the binding sites to the cytosol, where Na^+ readily dissociates because of the low intracellular Na^+ concentration. The release of Na^+ decreases the affinity of the carrier for solute and forces the release of the solute inside the cell, where solute concentration is already high.

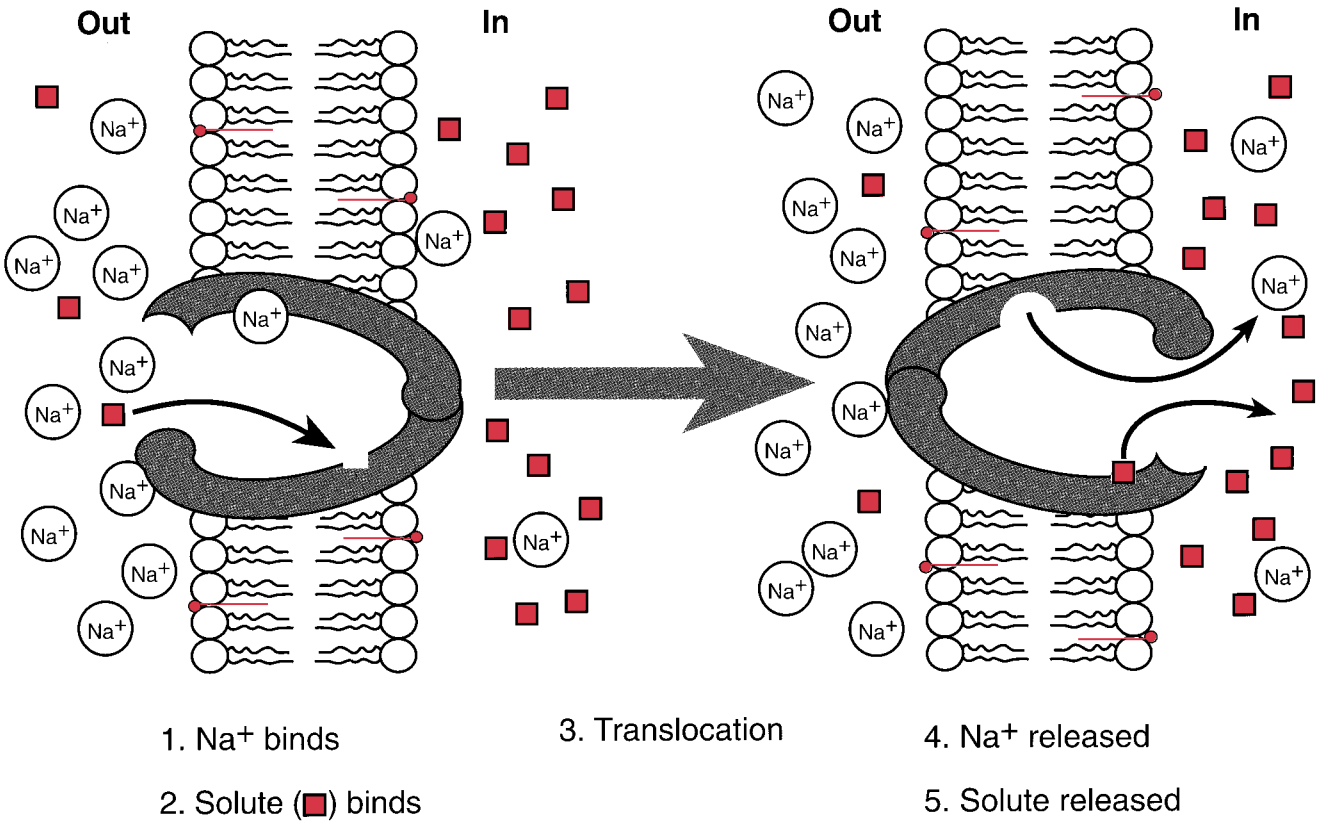

FIGURE 2.11 **A possible mechanism of secondary active transport.** A solute is moved against its concentration gradient by coupling it to Na^+ moving down a favorable gradient. Binding of extracellular Na^+ to the carrier protein may increase the affinity of binding sites for solute, so that solute also can bind to the carrier, even though its extracellular concentration is low. A conformational change in the carrier protein may expose the binding sites to the cytosol, where Na^+ readily dissociates because of the low intracellular Na^+ concentration. The release of Na^+ decreases the affinity of the carrier for solute and forces the release of the solute inside the cell, where solute concentration is already high.

dient is maintained by the action of the sodium-potassium pump, the function of these transport systems also depends on the function of the pump. Although they do not directly use metabolic energy for transport, these systems ultimately depend on the proper supply of metabolic energy to the sodium-potassium pump. They are called **secondary active transport mechanisms**. Disabling the pump with metabolic inhibitors or pharmacological blockers causes these transport systems to stop when the sodium gradient has been dissipated.

Similar to passive carrier-mediated systems, secondary active transport systems are integral membrane proteins; they have specificity for the solute they transport and show saturation kinetics and competitive inhibition. They differ, however, in two respects. First, they cannot function in the absence of the driver ion, the ion that moves along its electrochemical gradient and supplies energy. Second, they transport the solute against its own concentration or electrochemical gradient. Functionally, the different secondary active transport systems can be classified into two groups: **symport** (cotransport) systems, in which the solute being transported moves in the same direction as the sodium ion; and **antiport** (exchange) systems, in which sodium moves in one direction and the solute moves in the opposite direction (Fig. 2.12).

Examples of symport mechanisms are the sodium-coupled sugar transport system and the several sodium-coupled amino acid transport systems found in the small intestine and the renal tubule. The symport systems allow efficient absorption of nutrients even when the nutrients are present at very low concentrations. The Na^+-glucose cotransporter

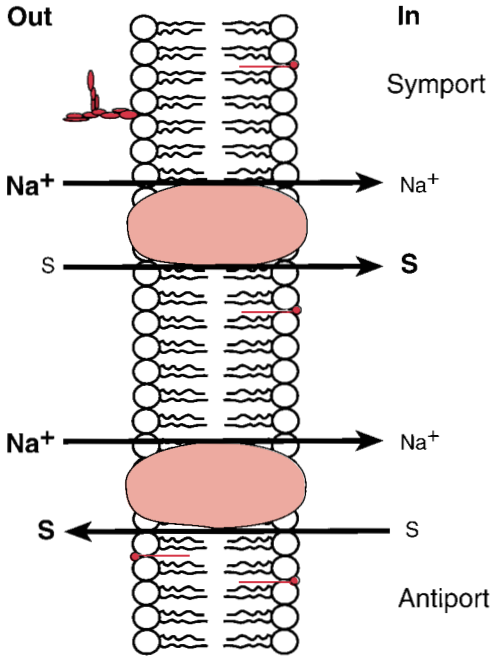

FIGURE 2.12 **Secondary active transport systems.** In a symport system (top), the transported solute (S) is moved in the same direction as the Na^+ ion. In an antiport system (bottom), the solute is moved in the opposite direction to Na^+. Large and small type indicate high and low concentrations, respectively, of Na^+ ions and solute.

in the human intestine has been cloned and sequenced. It is called sodium-dependent glucose transporter (SGLT). The protein contains 664 amino acids, and the polypeptide chain is thought to contain 14 membrane-spanning segments (Fig. 2.13). Another example of a symport system is the family of sodium-coupled phosphate transporters (termed NaPi, types I and II) in the intestine and renal proximal tubule. These transporters have 6 to 8 membrane-spanning segments and contain 460 to 690 amino acids. Sodium-coupled chloride transporters in the kidney are targets for inhibition by specific diuretics. The Na^+-Cl^- cotransporter in the distal tubule, known as NCC, is inhibited by thiazide diuretics, and the Na^+-K^+-$2Cl^-$ cotransporter in the ascending limb of the loop of Henle, referred to as NKCC, is inhibited by bumetanide.

The most important examples of antiporters are the Na^+/H^+ exchange and Na^+/Ca^{2+} exchange systems, found mainly in the plasma membrane of many cells. The first uses the sodium gradient to remove protons from the cell, controlling the intracellular pH and counterbalancing the production of protons in metabolic reactions. It is an **electroneutral system** because there is no net movement of charge. One Na^+ enters the cell for each H^+ that leaves. The second antiporter removes calcium from the cell and, together with the different calcium pumps, helps maintain a low cytosolic calcium concentration. It is an **electrogenic system** because there is a net movement of charge. Three Na^+ enter the cell and one Ca^{2+} leaves during each cycle.

The structures of the symport and antiport protein transporters that have been characterized (see Fig. 2.13) share a common property with ion channels (see Fig. 2.9) and equilibrating carriers, namely the presence of multiple membrane-spanning segments within the polypeptide chain. This supports the concept that, regardless of the mechanism, the membrane-spanning regions of a transport protein form a hydrophilic pathway for rapid transport of ions and solutes across the hydrophobic interior of the membrane lipid bilayer.

The Movement of Solutes Across Epithelial Cell Layers.

Epithelial cells occur in layers or sheets that allow the directional movement of solutes not only across the plasma membrane but also from one side of the cell layer to the other. Such regulated movement is achieved because the plasma membranes of epithelial cells have two distinct regions with different morphology and different transport systems. These regions are the **apical membrane**, facing the lumen, and the **basolateral membrane**, facing the blood supply (Fig. 2.14). The specialized or polarized organization of the cells is maintained by the presence of **tight junctions** at the areas of contact between adjacent cells. Tight junctions prevent proteins on the apical membrane from migrating to the basolateral membrane those on the basolateral membrane from migrating to the apical membrane. Thus, the entry and exit steps for solutes can be localized to opposite sides of the cell. This is the key to **transcellular transport** across epithelial cells.

An example is the absorption of glucose in the small intestine. Glucose enters the intestinal epithelial cells by active transport using the electrogenic Na^+-glucose cotransporter system (SGLT) in the apical membrane. This

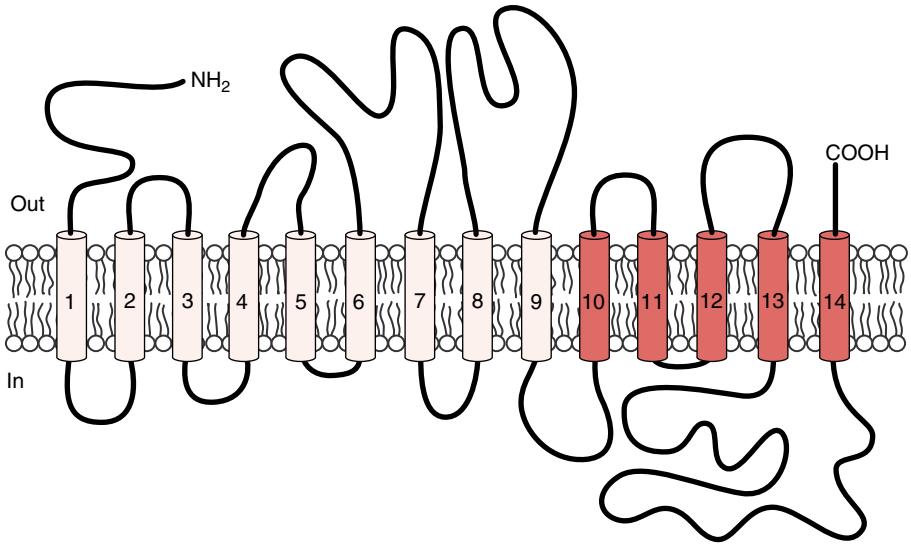

FIGURE 2.13 A model of the secondary structure of the Na^+-glucose cotransporter protein (SGLT) in the human intestine. The polypeptide chain of 664 amino acids passes back and forth across the membrane 14 times. Each membrane-spanning segment consists of 21 amino acids arranged in an α-helical conformation. Both the NH_2 and the COOH ends are located on the extracellular side of the plasma membrane. In the functional protein, it is likely that the membrane-spanning segments are clustered together to provide a hydrophilic pathway across the plasma membrane. The N-terminal portion of the protein, including helices 1 to 9, is required to couple Na^+ binding to glucose transport. The five helices (10 to 14) at the C-terminus may form the transport pathway for glucose. (Modified from Panayotova-Heiermann M, Eskandari S, Turk E, et al. Five transmembrane helices form the sugar pathway through the Na^+-glucose cotransporter. J Biol Chem 1997;272:20324–20327.)

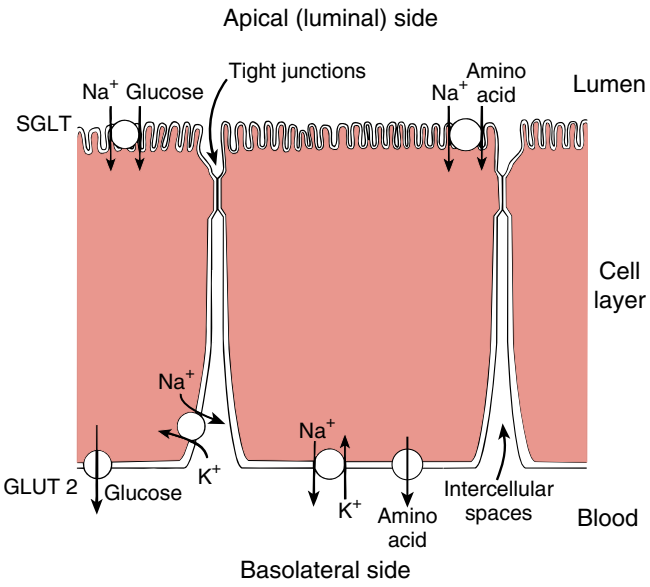

FIGURE 2.14 The localization of transport systems to different regions of the plasma membrane in epithelial cells of the small intestine. A polarized cell is produced, in which entry and exit of solutes, such as glucose, amino acids, and Na^+, occur at opposite sides of the cell. Active entry of glucose and amino acids is restricted to the apical membrane and exit requires equilibrating carriers located only in the basolateral membrane. For example, glucose enters on SGLT and exits on GLUT 2. Na^+ that enters via the apical symporters is pumped out by the Na^+/K^+-ATPase on the basolateral membrane. The result is a net movement of solutes from the luminal side of the cell to the basolateral side, ensuring efficient absorption of glucose, amino acids, and Na^+ from the intestinal lumen.

increases the intracellular glucose concentration above the blood glucose concentration, and the glucose molecules move passively out of the cell and into the blood via an equilibrating carrier mechanism (GLUT 2) in the basolateral membrane (see Fig. 2.14). The intestinal GLUT 2, like the erythrocyte GLUT 1, is a sodium-independent transporter that moves glucose down its concentration gradient. Unlike GLUT 1, the GLUT 2 transporter can accept other sugars, such as galactose and fructose, that are also absorbed in the intestine. The sodium ions that enter the cell with the glucose molecules on SGLT are pumped out by the Na^+/K^+-ATPase that is located in the basolateral membrane only. The polarized organization of the epithelial cells and the integrated functions of the plasma membrane transporters form the basis by which cells accomplish transcellular movement of both glucose and sodium ions.

THE MOVEMENT OF WATER ACROSS THE PLASMA MEMBRANE

Since the lipid part of the plasma membrane is very hydrophobic, the movement of water across it is too slow to explain the speed at which water can move in and out of the cells. The partition coefficient of water into lipids is very low; therefore, the permeability of the lipid bilayer for water is also very low. Specific membrane proteins that function as **water channels** explain the rapid movement of water across the plasma membrane. These water channels are small (molecular weight about 30 kDa) integral membrane proteins known as **aquaporins.** Ten different forms have been discovered so far in mammals. At least six forms are expressed in cells in the kidney and seven forms in the gas-

trointestinal tract, tissues where water movement across plasma membranes is particularly rapid.

In the kidney, aquaporin-2 (AQP2) is abundant in the collecting duct and is the target of the hormone **arginine vasopressin,** also known as antidiuretic hormone. This hormone increases water transport in the collecting duct by stimulating the insertion of AQP2 proteins into the apical plasma membrane. Several studies have shown that AQP2 has a critical role in inherited and acquired disorders of water reabsorption by the kidney. For example, **diabetes insipidus** is a condition in which the kidney loses its ability to reabsorb water properly, resulting in excessive loss of water and excretion of a large volume of very dilute urine (polyuria). Although inherited forms of diabetes insipidus are relatively rare, it can develop in patients receiving chronic lithium therapy for psychiatric disorders, giving rise to the term **lithium-induced polyuria**. Both of these conditions are associated with a decrease in the number of AQP2 proteins in the collecting ducts of the kidney.

The Movement of Water Across the Plasma Membrane Is Driven by Differences in Osmotic Pressure

The spontaneous movement of water across a membrane driven by a gradient of water concentration is the process known as **osmosis**. The water moves from an area of high concentration of water to an area of low concentration. Since concentration is defined by the number of particles per unit of volume, a solution with a high concentration of solutes has a low concentration of water, and vice versa. Osmosis can, therefore, be viewed as the movement of water from a solution of high water concentration (low concentration of solute) toward a solution with a lower concentration of water (high solute concentration). Osmosis is a passive transport mechanism that tends to equalize the total solute concentrations of the solutions on both sides of every membrane.

If a cell that is normally in osmotic equilibrium is transferred to a more dilute solution, water will enter the cell, the cell volume will increase, and the solute concentration of the cytoplasm will be reduced. If the cell is transferred to a more concentrated solution, water will leave the cell, the cell volume will decrease, and the solute concentration of the cytoplasm will increase. As we will see below, many cells have regulatory mechanisms that keep cell volume within a certain range. Other cells, such as mammalian erythrocytes, do not have volume regulatory mechanisms and large volume changes occur when the solute concentration of the extracellular fluid is changed.

The driving force for the movement of water across the plasma membrane is the difference in water concentration between the two sides of the membrane. For historical reasons, this driving force is not called the chemical gradient of water but the difference in osmotic pressure. The **osmotic pressure** of a solution is defined as the pressure necessary to stop the net movement of water across a selectively permeable membrane that separates the solution from pure water. When a membrane separates two solutions of different osmotic pressure, water will move from the solution with low osmotic pressure (high water concentration) to the solution of high osmotic pressure (low water concentration). In this context, the term *selectively permeable* means that the membrane is permeable to water but not solutes. In reality, most biological membranes contain membrane transport proteins that permit solute movement.

The osmotic pressure of a solution depends on the number of particles dissolved in it, the total concentration of all solutes. Many solutes, such as salts, acids, and bases, dissociate in water, so the number of particles is greater than the molar concentration. For example, NaCl dissociates in water to give Na^+ and Cl^-, so one molecule of NaCl will produce two osmotically active particles. In the case of $CaCl_2$, there are three particles per molecule. The equation giving the osmotic pressure of a solution is:

$$\pi = n\,R\,T\,C \qquad (3)$$

where π is the osmotic pressure of the solution, n is the number of particles produced by the dissociation of one molecule of solute (2 for NaCl, 3 for $CaCl_2$), R is the universal gas constant (0.0821 L·atm/mol·K), T is the absolute temperature, and C is the concentration of the solute in mol/L. Osmotic pressure can be expressed in **atmospheres (atm)**. Solutions with the same osmotic pressure are called **isosmotic**. A solution is **hyperosmotic** with respect to another solution if it has a higher osmotic pressure and **hypoosmotic** if it has a lower osmotic pressure.

Equation 3, called the **van't Hoff equation**, is valid only when applied to very dilute solutions, in which the particles of solutes are so far away from each other that no interactions occur between them. Generally, this is not the case at physiological concentrations. Interactions between dissolved particles, mainly between ions, cause the solution to behave as if the concentration of particles is less than the theoretical value (nC). A correction coefficient, called the **osmotic coefficient** (ϕ) of the solute, needs to be introduced in the equation. Therefore, the osmotic pressure of a solution can be written more accurately as:

$$\pi = n\,R\,T\,\phi\,C \qquad (4)$$

The osmotic coefficient varies with the specific solute and its concentration. It has values between 0 and 1. For example, the osmotic coefficient of NaCl is 1.00 in an infinitely dilute solution but changes to 0.93 at the physiological concentration of 0.15 mol/L.

At any given T, since R is constant, equation 4 shows that the osmotic pressure of a solution is directly proportional to the term $n\phi C$. This term is known as the **osmolality** or **osmotic concentration** of a solution and is expressed in osm/kg H_2O. Most physiological solutions, such as blood plasma, contain many different solutes, and each contributes to the total osmolality of the solution. The osmolality of a solution containing a complex mixture of solutes is usually measured by freezing point depression. The freezing point of an aqueous solution of solutes is lower than that of pure water and depends on the total number of solute particles. Compared with pure water, which freezes at 0°C, a solution with an osmolality of 1 osm/kg H_2O will freeze at −1.86°C. The ease with which osmolality can be measured has led to the wide use of this parameter for comparing the osmotic pressure of different solutions. The osmotic pressures of physiological solutions

are not trivial. Consider blood plasma, for example, which usually has an osmolality of 0.28 osm/kg H_2O, determined by freezing point depression. Equation 4 shows that the osmotic pressure of plasma at 37°C is 7.1 atm, about 7 times greater than atmospheric pressure.

Many Cells Can Regulate Their Volume

Cell volume changes can occur in response to changes in the osmolality of extracellular fluid in both normal and pathophysiological situations. Accumulation of solutes also can produce volume changes by increasing the intracellular osmolality. Many cells can correct these volume changes.

Volume regulation is particularly important in the brain, for example, where cell swelling can have serious consequences because expansion is strictly limited by the rigid skull.

Osmolality and Tonicity. A solution's osmolality is determined by the total concentration of all the solutes present. In contrast, the solution's **tonicity** is determined by the concentrations of only those solutes that do not enter ("penetrate") the cell. Tonicity determines cell volume, as illustrated in the following examples. Na^+ behaves as a nonpenetrating solute because it is pumped out of cells by the Na^+/K^+-ATPase at the same rate that it enters. A solution of NaCl at 0.2 osm/kg H_2O is hypoosmotic compared to cell cytosol at 0.3 osm/kg H_2O. The NaCl solution is also **hypotonic** because cells will accumulate water and swell when placed in this solution. A solution containing a mixture of NaCl (0.3 osm/kg H_2O) and urea (0.1 osm/kg H_2O) has a total osmolality of 0.4 osm/kg H_2O and will be hyperosmotic compared to cell cytosol. The solution is **isotonic**, however, because it produces no permanent change in cell volume. The reason is that cells shrink initially as a result of loss of water but urea is a penetrating solute that rapidly enters the cells. Urea entry increases the intracellular osmolality so water also enters and increases the volume. Entry of water ceases when the urea concentration is the same inside and outside the cells. At this point, the total osmolality both inside and outside the cells will be 0.4 osm/kg H_2O and the cell volume will be restored to normal.

Volume Regulation. When cell volume increases because of extracellular hypotonicity, the response of many cells is rapid activation of transport mechanisms that tend to decrease the cell volume (Fig. 2.15A). Different cells use different **regulatory volume decrease** (**RVD**) **mechanisms** to move solutes out of the cell and decrease the number of particles in the cytosol, causing water to leave the cell. Since cells have high intracellular concentrations of potassium, many RVD mechanisms involve an increased efflux of K^+, either by stimulating the opening of potassium channels or by activating symport mechanisms for KCl. Other cells activate the efflux of some amino acids, such as taurine or proline. The net result is a decrease in intracellular solute content and a reduction of cell volume close to its original value (see Fig. 2.15A).

When placed in a **hypertonic** solution, cells rapidly lose water and their volume decreases. In many cells, a decreased volume triggers **regulatory volume increase** (**RVI**) **mechanisms**, which increase the number of intracellular particles, bringing water back into the cells. Because Na^+ is the main extracellular ion, many RVI mechanisms involve an influx of sodium into the cell. Na^+-Cl^- symport, Na^+-K^+-$2Cl^-$ symport, and Na^+/H^+ antiport are some of the mechanisms activated to increase the intracellular concentration of Na^+ and increase the cell volume toward its original value (Fig. 2.15B).

Mechanisms based on an increased Na^+ influx are effective for only a short time because, eventually, the sodium pump will increase its activity and reduce intracellular Na^+

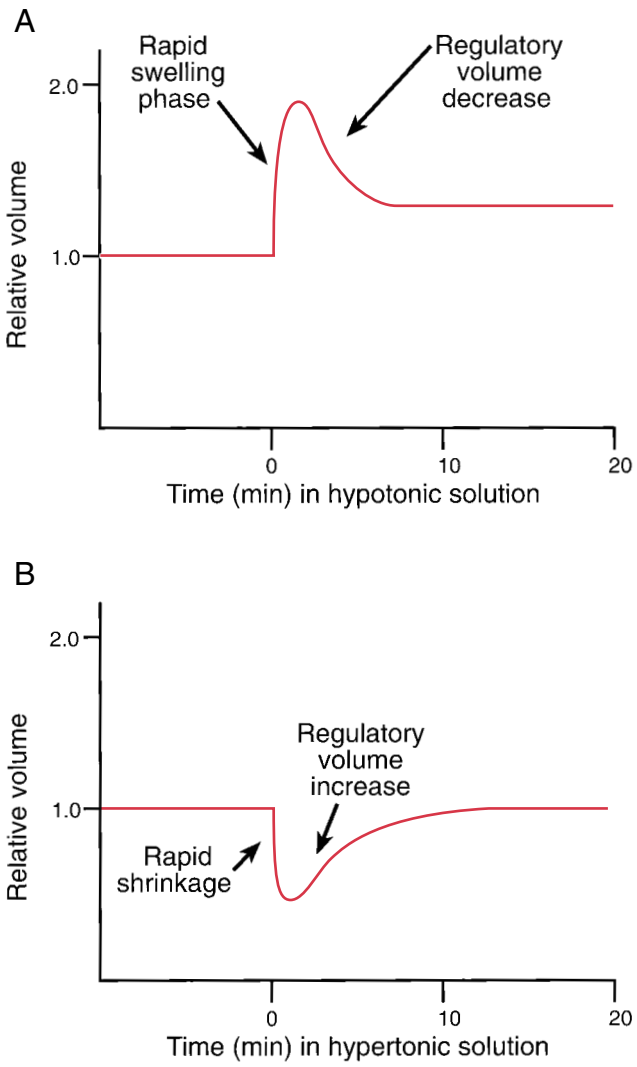

FIGURE 2.15 **The effect of tonicity changes on cell volume.** Cell volume changes when a cell is placed in either a hypotonic or a hypertonic solution. **A,** In a hypotonic solution, the reversal of the initial increase in cell volume is known as a regulatory volume decrease. Transport systems for solute exit are activated, and water follows movement of solute out of the cell. **B,** In a hypertonic solution, the reversal of the initial decrease in cell volume is a regulatory volume increase. Transport systems for solute entry are activated, and water follows solute into the cell.

to its normal value. Cells that regularly encounter hypertonic extracellular fluids have developed additional mechanisms for maintaining normal volume. These cells can synthesize specific organic solutes, enabling them to increase intracellular osmolality for a long time and avoiding altering the concentrations of ions they must maintain within a narrow range of values. The organic solutes are usually small molecules that do not interfere with normal cell function when they accumulate inside the cell. For example, cells of the medulla of the mammalian kidney can increase the level of the enzyme aldose reductase when subjected to elevated extracellular osmolality. This enzyme converts glucose to an osmotically active solute, sorbitol. Brain cells can synthesize and store inositol. Synthesis of sorbitol and inositol represents different answers to the problem of increasing the total intracellular osmolality, allowing normal cell volume to be maintained in the presence of hypertonic extracellular fluid.

Oral Rehydration Therapy

Oral administration of rehydration solutions has dramatically reduced the mortality resulting from cholera and other diseases that involve excessive losses of water and solutes from the gastrointestinal tract. The main ingredients of rehydration solutions are glucose, NaCl, and water. The glucose and Na^+ ions are reabsorbed by SGLT and other transporters in the epithelial cells lining the lumen of the small intestine (see Fig. 2.14). Deposition of these solutes on the basolateral side of the epithelial cells increases the osmolality in that region compared with the intestinal lumen and drives the osmotic absorption of water. Absorption of glucose increases the absorption of NaCl and water and helps to compensate for excessive diarrheal losses of salt and water.

THE RESTING MEMBRANE POTENTIAL

The different passive and active transport systems are coordinated in a living cell to maintain intracellular ions and other solutes at concentrations compatible with life. Consequently, the cell does not equilibrate with the extracellular fluid, but rather exists in a **steady state** with the extracellular solution. For example, intracellular Na^+ concentration (10 mmol/L in a muscle cell) is much lower than extracellular Na^+ concentration (140 mmol/L), so Na^+ enters the cell by passive transport through nongated Na^+ channels. The rate of Na^+ entry is matched, however, by the rate of active transport of Na^+ out of the cell via the sodium-potassium pump (Fig. 2.16). The net result is that intracellular Na^+ is maintained constant and at a low level, even though Na^+ continually enters and leaves the cell. The reverse is true for K^+, which is maintained at a high concentration inside the cell relative to the outside. The passive exit of K^+ through nongated K^+ channels is matched by active entry via the pump (see Fig. 2.16). Maintenance of this steady state with ion concentrations inside the cell different from those outside the cell is the basis for the difference in electrical potential across the plasma membrane or the **resting membrane potential**.

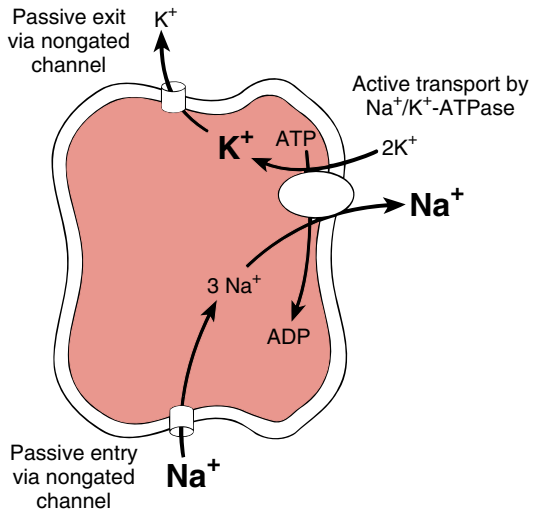

FIGURE 2.16 **The concept of a steady state.** Na^+ enters a cell through nongated Na^+ channels, moving passively down the electrochemical gradient. The rate of Na^+ entry is matched by the rate of active transport of Na^+ out of the cell via the Na^+/K^+-ATPase. The intracellular concentration of Na^+ remains low and constant. Similarly, the rate of passive K^+ exit through nongated K^+ channels is matched by the rate of active transport of K^+ into the cell via the pump. The intracellular K^+ concentration remains high and constant. During each cycle of the ATPase, two K^+ are exchanged for three Na^+ and one molecule of ATP is hydrolyzed to ADP. Large type and small type indicate high and low ion concentrations, respectively.

Ion Movement Is Driven by the Electrochemical Potential

If there are no differences in temperature or hydrostatic pressure between the two sides of a plasma membrane, two forces drive the movement of ions and other solutes across the membrane. One force results from the difference in the concentration of a substance between the inside and the outside of the cell and the tendency of every substance to move from areas of high concentration to areas of low concentration. The other force results from the difference in electrical potential between the two sides of the membrane, and it applies only to ions and other electrically charged solutes. When a difference in electrical potential exists, positive ions tend to move toward the negative side, while negative ions tend to move toward the positive side.

The sum of these two driving forces is called the gradient (or difference) of **electrochemical potential** across the membrane for a specific solute. It measures the tendency of that solute to cross the membrane. The expression of this force is given by:

$$\Delta\mu = RT \ln \frac{C_i}{C_o} + zF (E_i - E_o) \qquad (5)$$

where μ represents the electrochemical potential ($\Delta\mu$ is the difference in electrochemical potential between two sides of the membrane); C_i and C_o are the concentrations of the solute inside and outside the cell, respectively; E_i is the electrical potential inside the cell measured with respect to the electrical potential outside the cell (E_o); R is the universal gas constant (2 cal/mol·K); T is the absolute tem-

perature (K); z is the valence of the ion; and F is the Faraday constant (23 cal/mV·mol). By inserting these units in equation 5 and simplifying, the electrochemical potential will be expressed in cal/mol, which are units of energy. If the solute is not an ion and has no electrical charge, then z = 0 and the last term of the equation becomes zero. In this case, the electrochemical potential is defined only by the different concentrations of the uncharged solute, called the **chemical potential**. The driving force for solute transport becomes solely the difference in chemical potential.

Net Ion Movement Is Zero at the Equilibrium Potential

Net movement of an ion into or out of a cell continues as long as the driving force exists. Net movement stops and equilibrium is reached only when the driving force of electrochemical potential across the membrane becomes zero. The condition of equilibrium for any permeable ion will be $\Delta\mu = 0$. Substituting this condition into equation 5, we obtain:

$$0 = RT \ln \frac{C_i}{C_o} + zF (E_i - E_o)$$

$$E_i - E_o = -\frac{RT}{zF} \ln \frac{C_i}{C_o} \qquad (6)$$

$$E_i - E_o = \frac{RT}{zF} \ln \frac{C_o}{C_i}$$

Equation 6, known as the **Nernst equation**, gives the value of the electrical potential difference ($E_i - E_o$) necessary for a specific ion to be at equilibrium. This value is known as the **Nernst equilibrium potential** for that particular ion and it is expressed in millivolts (mV), units of voltage. At the equilibrium potential, the tendency of an ion to move in one direction because of the difference in concentrations is exactly balanced by the tendency to move in the opposite direction because of the difference in electrical potential. At this point, the ion will be in equilibrium and there will be no net movement. By converting to $\log_{10}$ and assuming a physiological temperature of 37°C and a value of +1 for z (for Na^+ or K^+), the Nernst equation can be expressed as:

$$E_i - E_o = 61 \log_{10} \frac{C_o}{C_i} \qquad (7)$$

Since Na^+ and K^+ (and other ions) are present at different concentrations inside and outside a cell, it follows from equation 7 that the equilibrium potential will be different for each ion.

The Resting Membrane Potential Is Determined by the Passive Movement of Several Ions

The resting membrane potential is the electrical potential difference across the plasma membrane of a normal living cell in its unstimulated state. It can be measured directly by the insertion of a microelectrode into the cell with a reference electrode in the extracellular fluid. The resting membrane potential is determined by those ions that can cross the membrane and are prevented from attaining equilibrium by active transport systems. Potassium, sodium, and chloride ions can cross the membranes of every living cell, and each of these ions contributes to the resting membrane potential. By contrast, the permeability of the membrane of most cells to divalent ions is so low that it can be ignored in this context.

The **Goldman equation** gives the value of the membrane potential (in mV) when all the permeable ions are accounted for:

$$E_i - E_o = \frac{RT}{F} \ln \frac{P_K[K^+]_o + P_{Na}[Na^+]_o + P_{Cl}[Cl^-]_i}{P_K[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_o} \qquad (8)$$

where P_K, P_{Na}, and P_{Cl} represent the permeability of the membrane to potassium, sodium, and chloride ions, respectively; and brackets indicate the concentration of the ion inside (i) and outside (o) the cell. If a certain cell is not permeable to one of these ions, the contribution of the impermeable ion to the membrane potential will be zero. If a specific cell is permeable to an ion other than the three considered in equation 8, that ion's contribution to the membrane potential must be included in the equation.

It can be seen from equation 8 that the contribution of any ion to the membrane potential is determined by the membrane's permeability to that particular ion. The higher the permeability of the membrane to one ion relative to the others, the more that ion will contribute to the membrane potential. The plasma membranes of most living cells are much more permeable to potassium ions than to any other ion. Making the assumption that P_{Na} and P_{Cl} are zero relative to P_K, equation 8 can be simplified to:

$$E_i - E_o = \frac{RT}{F} \ln \frac{P_K[K^+]_o}{P_K[K^+]_i}$$

$$E_i - E_o = \frac{RT}{F} \ln \frac{[K^+]_o}{[K^+]_i} \qquad (9)$$

which is the Nernst equation for the equilibrium potential for K^+ (see equation 6). This illustrates two important points:

- In most cells, the resting membrane potential is close to the equilibrium potential for K^+.
- The resting membrane potential of most cells is dominated by K^+ because the plasma membrane is more permeable to this ion compared to the others.

As a typical example, the K^+ concentrations outside and inside a muscle cell are 3.5 mmol/L and 155 mmol/L, respectively. Substituting these values in equation 7 gives an equilibrium potential for K^+ of −100 mV, negative inside the cell relative to the outside. The resting membrane potential in a muscle cell is −90 mV (negative inside). This value is close to, although not the same as, the equilibrium potential for K^+.

The reason the resting membrane potential in the muscle cell is less negative than the equilibrium potential for K^+ is as follows. Under physiological conditions, there is passive entry of Na^+ ions. This entry of positively charged ions has a small but significant effect on the negative potential inside the cell. Assuming intracellular Na^+ to be 10 mmol/L and extracellular Na^+ to be 140 mmol/L, the Nernst equation gives a value of +70 mV for the Na^+ equilibrium potential (positive inside the cell). This is far from

the resting membrane potential of −90 mV. Na^+ makes only a small contribution to the resting membrane potential because membrane permeability to Na^+ is very low compared to that of K^+.

The contribution of Cl^- ions need not be considered because the resting membrane potential in the muscle cell is the same as the equilibrium potential for Cl^-. Therefore, there is no net movement of chloride ions.

In most cells, as shown above using a muscle cell as an example, the equilibrium potentials of K^+ and Na^+ are different from the resting membrane potential, which indicates that neither K^+ ions nor Na^+ ions are at equilibrium. Consequently, these ions continue to cross the plasma membrane via specific nongated channels, and these passive ion movements are *directly* responsible for the resting membrane potential.

The Na^+/K^+-ATPase is important *indirectly* for maintaining the resting membrane potential because it sets up the gradients of K^+ and Na^+ that drive passive K^+ exit and Na^+ entry. During each cycle of the pump, two K^+ ions are moved into the cell in exchange for three Na^+, which are moved out (see Fig. 2.16). Because of the unequal exchange mechanism, the pump's activity contributes slightly to the negative potential inside the cell.

REVIEW QUESTIONS

DIRECTIONS: Each of the numbered items or incomplete statements in this section is followed by answers or by completions of the statement. Select the ONE lettered answer or completion that is BEST in each case.

1. Which one of the following is a common property of all phospholipid molecules?
   (A) Hydrophilic
   (B) Steroid structure
   (C) Water-soluble
   (D) Amphipathic
   (E) Hydrophobic
2. Select the true statement about membrane phospholipids.
   (A) A phospholipid contains cholesterol
   (B) Phospholipids move rapidly in the plane of the bilayer
   (C) Specific phospholipids are always present in equal proportions in the two halves of the bilayer
   (D) Phospholipids form ion channels through the membrane
   (E) Na^+-glucose symport is mediated by phospholipids
3. Several segments of the polypeptide chain of integral membrane proteins usually span the lipid bilayer. These segments frequently
   (A) Adopt an α-helical configuration
   (B) Contain many hydrophilic amino acids
   (C) Form covalent bonds with cholesterol
   (D) Contain unusually strong peptide bonds
   (E) Form covalent bonds with phospholipids
4. The electrical potential difference necessary for a single ion to be at equilibrium across a membrane is best described by the
   (A) Goldman equation
   (B) van't Hoff equation
   (C) Fick's law
   (D) Nernst equation
   (E) Permeability coefficient
5. The ion present in highest concentration inside most cells is
   (A) Sodium
   (B) Potassium
   (C) Calcium
   (D) Chloride
   (E) Phosphate
6. Solute movement by active transport can be distinguished from solute transport by equilibrating carrier-mediated transport because active transport
   (A) Is saturable at high solute concentration
   (B) Is inhibited by other molecules with structures similar to that of the solute
   (C) Moves the solute against its electrochemical gradient
   (D) Allows movement of polar molecules
   (E) Is mediated by specific membrane proteins
7. A sodium channel that opens in response to an increase in intracellular cyclic GMP is an example of
   (A) A ligand-gated ion channel
   (B) An ion pump
   (C) Sodium-coupled solute transport
   (D) A peripheral membrane protein
   (E) Receptor-mediated endocytosis
8. During regulatory volume decrease, many cells will increase
   (A) Their volume
   (B) Influx of Na^+
   (C) Efflux of K^+
   (D) Synthesis of sorbitol
   (E) Influx of water
9. At equilibrium the concentrations of Cl^- inside and outside a cell are 8 mmol/L and 120 mmol/L, respectively. The equilibrium potential for Cl^- at 37°C is calculated to be
   (A) +4.07 mV
   (B) –4.07 mV
   (C) +71.7 mV
   (D) –71.7 mV
   (E) +91.5 mV
   (F) –91.5 mV
10. What is the osmotic pressure (in atm) of an aqueous solution of 100 mmol/L $CaCl_2$ at 27 °C? (Assume the osmotic coefficient is 0.86 and the gas constant is 0.0821 L·atm/mol·K).
    (A) 738 atm
    (B) 635 atm
    (C) 211 atm
    (D) 7.38 atm
    (E) 6.35 atm
    (F) 2.11 atm

SUGGESTED READING

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