CHAPTER 5

TRANSPORT OF SOLUTES AND WATER

Peter S. Aronson, Walter F. Boron, and Emile L. Boulpaep

The cells of the human body live in a carefully regulated fluid environment. The fluid inside the cells, the **intracellular fluid** (**ICF**), occupies what is called the intracellular compartment, and the fluid outside the cells, the **extracellular fluid** (**ECF**), occupies the extracellular compartment. The barriers that separate these two compartments are the **cell membranes**. For life to be sustained, the body must rigorously maintain the volume and composition of the intracellular and extracellular compartments. To a large extent, such regulation is the result of transport across the cell membrane. In this chapter, we discuss how cell membranes regulate the distribution of ions and water in the intracellular and extracellular compartments.

THE INTRACELLULAR AND EXTRACELLULAR FLUIDS

Total body water is the sum of the intracellular and extracellular fluid volumes

Total body water is ~60% of total body weight in a young adult human male, approximately 50% of total body weight in a young adult human female (Table 5-1), and 65% to 75% of total body weight in an infant. Total body water accounts for a lower percentage of weight in females because they typically have more adipose tissue, and fat cells have a lower water content than muscle does. Even if gender and age are taken into consideration, the fraction of total body weight contributed by water is not constant for all individuals under all conditions. For example, variability in the amount of adipose tissue can influence the fraction. Because water represents such a large fraction of body weight, acute changes in total body water can be detected simply by monitoring body weight.

The anatomy of the body fluid compartments is illustrated in Figure 5-1. The prototypic 70-kg male has ~42 liters of total body water (60% of 70 kg). Of these 42 liters, ~60% (25 liters) is intracellular and ~40% (17 liters) is extracellular. *Extracellular fluid* is composed of blood plasma, interstitial fluid, and transcellular fluid.

Plasma Volume Of the ~17 liters of ECF, only ~20% (~3 liters) is contained within the cardiac chambers and blood vessels, that is, within the intravascular compartment. The *total* volume of this intravascular compartment is the **blood volume**, ~5.5 liters. The extracellular 3 liters of the blood volume is the plasma volume. The balance, ~2.5 liters, consists of the cellular elements of blood: erythrocytes, leukocytes, and platelets. The fraction of blood volume that is occupied by these cells is called the **hematocrit**. The hematocrit is determined by centrifuging blood that is treated with an anticoagulant and measuring the fraction of the total volume that is occupied by the packed cells.

Interstitial Fluid About 75% (~13 liters) of the ECF is outside the intravascular compartment, where it bathes the nonblood cells of the body. Within this interstitial fluid are two smaller compartments that communicate only slowly with the bulk of the interstitial fluid: dense connective tissue, such as cartilage and tendons, and bone matrix.

The barriers that separate the intravascular and interstitial compartments are the walls of **capillaries**. Water and solutes can move between the interstitium and blood plasma by crossing capillary walls and between the interstitium and cytoplasm by crossing cell membranes.

Transcellular Fluid Finally, ~5% (~1 liter) of ECF is trapped within spaces that are completely surrounded by epithelial cells. This transcellular fluid includes the synovial fluid within joints and the cerebrospinal fluid surrounding the brain and spinal cord. Transcellular fluid does *not* include fluids that are, strictly speaking, outside the body, such as the contents of the gastrointestinal tract or urinary bladder.

Intracellular fluid is rich in K^+, whereas the extracellular fluid is rich in Na^+ and Cl^-

Not only do the various body fluid compartments have very different volumes, they also have radically different compositions, as summarized in Figure 5-1. Table 5-2 is a more comprehensive listing of these values. *Intra*cellular *fluid* is high in K^+ and low in Na^+ and Cl^-; *extra*cellular *fluids* (interstitial and plasma) are high in Na^+ and Cl^- and low in K^+.

Table 5-1 Approximate Water Distribution in Adult Humans*

	Men	Typical Volume (liters)	Women	Typical Volume (liters)
Total body water (TBW)	60% of body weight	42	50% of body weight	35
Intracellular fluid (ICF)	60% of TBW	25	60% of TBW	21
Extracellular fluid (ECF)	40% of TBW	17	40% of TBW	14
Interstitial fluid	75% of ECF	13	75% of ECF	10
Plasma volume (PV)	20% of ECF	3	20% of ECF	3
Blood volume (BV)	PV/(1 – Hct)	5.5	PV/(1 – Hct)	5
Transcellular fluid	5% of ECF	1	5% of ECF	1

*Assuming a body weight of 70 kg for both sexes and a hematocrit (Hct) of 45% for men and 40% for women.

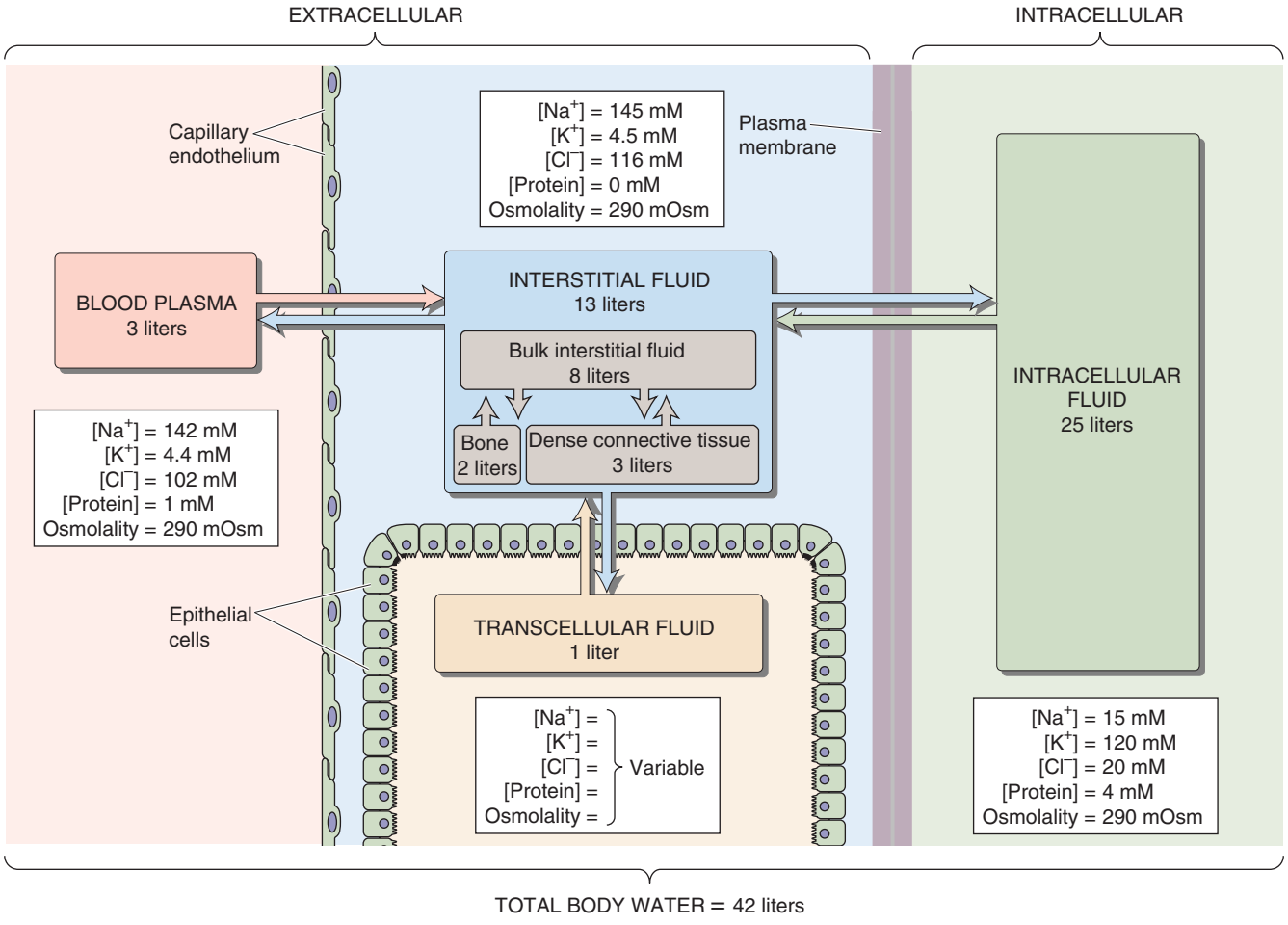


Figure 5-1 The fluid compartments of a prototypic adult human weighing 70 kg. Total body water is divided into four major compartments: intracellular fluid *(green),* interstitial fluid *(blue),* blood plasma *(red),* and transcellular water such as synovial fluid *(tan).* Color codes for each of these compartments are maintained throughout this book.

The cell maintains a relatively high K^+ concentration ($[K^+]_i$) and low Na^+ concentration ($[Na^+]_i$), not by making its membrane totally impermeable to these ions but by using the Na-K pump to actively extrude Na^+ from the cell and to actively transport K^+ into the cell.

The composition of transcellular fluids differs greatly both from each other and from plasma because they are secreted by different epithelia. The two major constituents of ECF, the plasma and interstitial fluid, have similar composition as far as small solutes are concerned. For most cells, it is the composition of the interstitial fluid enveloping the cells that is the relevant parameter. The major difference between plasma and interstitial fluid is the absence of plasma proteins from the interstitium. These plasma proteins, which

Table 5-2 Approximate Solute Composition of Key Fluid Compartments

Solute	Plasma	Protein-Free Plasma	Interstitium	Cell
Na^+ (mM)	142	153	145	15
K^+ (mM)	4.4	4.7	4.5	120
Ca^{2+} (mM)	1.2 (ionized) 2.4 (total)*	1.3 (ionized)	1.2 (ionized)	0.0001 (ionized)
Mg^{2+} (mM)	0.6 (ionized) 0.9 (total)*	0.6 (ionized)	0.55 (ionized)	1 (ionized) 18 (total)
Cl^- (mM)	102	110	116	20
HCO_3^- (mM)	22†	24	25	16
$H_2PO_4^-$ and HPO_4^{2-} (mM)	0.7 (ionized) 1.4 (total)‡	0.75 (ionized)	0.8 (ionized)	0.7 (free)
Proteins	7 g/dL 1 mmol/L 14 mEq/L	—	1 g/dL	30 g/dL
Glucose (mM)	5.5	5.9	5.9	Very low
pH	7.4	7.4	7.4	~7.2
Osmolality (mosmole/kg H_2O)	291	290	290	290

*Total includes amounts ionized, complexed to small solutes, and protein bound.
†Arterial value. The value in mixed-venous blood would be ~24 mM.
‡As discussed in Chapter 52, levels of total plasma inorganic phosphate are not tightly regulated and vary between 0.8 and 1.5 mM.

cannot equilibrate across the walls of most capillaries, are responsible for the usually slight difference in small-solute concentrations between plasma and interstitial fluid. Plasma proteins affect solute distribution because of the volume they occupy and the electrical charge they carry.

Volume Occupied by Plasma Proteins The proteins and, to a much lesser extent, the lipids in plasma ordinarily occupy ~7% of the total plasma volume. Clinical laboratories report the plasma composition of ions (e.g., Na^+, K^+) in units of milliequivalents (meq) per liter of plasma solution. However, for cells bathed by interstitial fluid, more meaningful units would be **milliequivalents per liter of protein-free plasma solution** because it is only the protein-free portion of plasma—and not the proteins dissolved in this water—that can equilibrate across the capillary wall. For example, we can obtain $[Na^+]$ in protein-free plasma (which clinicians call plasma water) by dividing the laboratory value for plasma $[Na^+]$ by the plasma water content (usually 93%):

$$[Na^+]_{\text{plasma water}} = \frac{142\,\text{meq/L plasma}}{0.93} = 153\,\text{meq/L plasma water} \quad (5\text{-}1)$$

Similarly, for Cl^-,

$$[Cl^-]_{\text{plasma water}} = \frac{102\,\text{meq/L plasma}}{0.93} = 110\,\text{meq/L plasma water} \quad (5\text{-}2)$$

Table 5-2 lists solute concentrations in terms of both liters of *plasma* and liters of *plasma water*. If the plasma water fraction is less than 93% because of hyperproteinemia (high levels of protein in blood) or hyperlipemia (high levels of lipid in blood), the values that the clinical laboratory reports for electrolytes may appear abnormal even though the physiologically important concentration (solute concentration per liter of plasma water) is normal. For example, if a patient's plasma proteins and lipids occupy 20% of plasma volume and consequently plasma water is only 80% of plasma, a correction factor of 0.80 (rather than 0.93) should be used in Equation 5-1. If the clinical laboratory were to report a very low plasma $[Na^+]$ of 122 meq/L plasma, the patient's $[Na^+]$ relevant to interstitial fluid would be 122/0.80 = 153 meq/L plasma water, which is quite normal.

Effect of Protein Charge For noncharged solutes such as glucose, the correction for protein and lipid volume is the only correction needed to predict interstitial concentrations from plasma concentrations. Because plasma proteins carry a net negative charge and because the capillary wall confines

them to the plasma, they tend to retain cations in plasma. Thus, the **cation** concentration of the protein-free solution of the interstitium is lower by ~5%. Conversely, because these negatively charged plasma proteins repel anions, the **anion** concentration of the protein-free solution of the interstitium is higher by ~5%. We consider the basis for these 5% corrections in the discussion of the Gibbs-Donnan equilibrium.

Thus, for a monovalent cation such as Na^+, the interstitial concentration is 95% of the $[Na^+]$ of the protein-free plasma water, the value from Equation 5-1:

$$[Na^+]_{interstitium} = 153\,meq/L\ plasma\ water \times 0.95 = 145\,meq/L\ interstitial\ fluid \quad (5\text{-}3)$$

For a monovalent anion such as Cl^-, the interstitial concentration is 105% of the $[Cl^-]$ of the protein-free water of plasma, a value already obtained in Equation 5-2:

$$[Cl^-]_{interstitium} = 110\,meq/L\ plasma\ water \times 1.05 = 116\,meq/L\ interstitial\ fluid \quad (5\text{-}4)$$

Thus, for cations (e.g., Na^+), the two corrections (0.95/0.93) nearly cancel each other. On the other hand, for anions (e.g., Cl^-), the two corrections (1.05/0.93) are cumulative and yield a total correction of ~13%.

All body fluids have approximately the same osmolality, and each fluid has equal numbers of positive and negative charges

Osmolality Despite the differences in solute composition among the intracellular, interstitial, and plasma compartments, they all have approximately the same osmolality. Osmolality describes the total concentration of all particles that are free in a solution. Thus, glucose contributes one particle, whereas fully dissociated NaCl contributes two. Particles bound to macromolecules do not contribute at all to osmolality. In all body fluid compartments, humans have an osmolality—expressed as the number of osmotically active particles per kilogram of water—of ~290 mosmol/kg H_2O (290 mOsm).

Plasma proteins contribute ~14 meq/L (Table 5-2). However, because these proteins usually have many negative charges per molecule, not many particles (~1 mM) are necessary to account for these milliequivalents. Moreover, even though the protein concentration—measured in terms of grams per liter—may be high, the high molecular weight of the average protein means that the protein concentration—measured in terms of moles per liter—is very low. Thus, proteins actually contribute only slightly to the total number of osmotically active particles (~1 mOsm).

Summing the *total* concentrations of all the solutes in the cells and interstitial fluid (including metabolites not listed in Table 5-2), we would see that the *total* solute concentration of the intracellular compartment is higher than that of the interstitium. Because the flow of water across cell membranes is governed by differences in osmolality across the membrane, and because the net flow is normally zero, intracellular and extracellular osmolality must be the same. How, then, do we make sense of this discrepancy? For some ions, a considerable fraction of their total intracellular store is bound to cellular proteins or complexed to other small solutes. In addition, some of the proteins are themselves attached to other materials that are out of solution. In computing osmolality, we count each particle once, whether it is a free ion, a complex of two ions, or several ions bound to a protein. For example, most of the intracellular Mg^{2+} and phosphate and virtually all the Ca^{2+} are either complexed or bound. Some of the electrolytes in blood plasma are also bound to plasma proteins; however, the bound fraction is generally much lower than the fraction in the cytosol.

Electroneutrality All solutions must respect the principle of bulk electroneutrality: the number of positive charges in the overall solution must be the same as the number of negative charges. If we add up the major cations and anions in the cytosol (Table 5-2), we see that the sum of $[Na^+]_i$ and $[K^+]_i$ greatly exceeds the sum of $[Cl^-]_i$ and $[HCO_3^-]_i$. The excess positive charge reflected by this difference is balanced by the negative charge on intracellular macromolecules (e.g., proteins) as well as smaller anions such as organic phosphates.

There is a similar difference between major cations and anions in blood plasma, where it is often referred to as the **anion gap**. The clinical definition of anion gap is

$$\text{Anion gap}_{plasma} = [Na^+]_{plasma} - ([Cl^-]_{plasma} + [HCO_3^-]_{plasma}) \quad (5\text{-}5)$$

Note that plasma $[K^+]$ is ignored. The anion gap, usually 9 to 14 meq/L, is the difference between ignored anions and ignored cations. Among the ignored anions are anionic proteins as well as small anionic metabolites. Levels of anionic metabolites, such as acetoacetate and β-hydroxybutyrate, can become extremely high, for example, in type 1 diabetic patients with very low levels of insulin (see Chapter 51). Thus, the anion gap increases under these conditions.

The differences in ionic composition between the ICF and ECF compartments are extremely important for normal functioning of the body. For example, because the K^+ gradient across cell membranes is a major determinant of electrical excitability, clinical disorders of extracellular $[K^+]$ can cause life-threatening disturbances in the heart rhythm. Disorders of extracellular $[Na^+]$ cause abnormal extracellular osmolality, with water being shifted into or out of brain cells; if uncorrected, such disorders lead to seizures, coma, or death.

These examples of clinical disorders emphasize the absolute necessity of understanding the processes that control the volume and composition of the body fluid compartments. These processes are the ones that move water and solutes between the compartments and between the body and the outside world.

SOLUTE TRANSPORT ACROSS CELL MEMBRANES

In passive, noncoupled transport across a permeable membrane, a solute moves down its electrochemical gradient

We are all familiar with the way that water can flow from one side of a dike to another, provided the water levels between

the two sides of the dike are different and the water has an open pathway (a breach in the dike) to move from one side to the other. In much the same way, a substance can passively move across a membrane that separates two compartments when there is both a favorable driving force and an open pathway through which the driving force can exert its effect.

When a pathway exists for transfer of a substance across a membrane, the membrane is said to be **permeable** to that substance. The **driving force** that determines the passive transport of solutes across a membrane is the **electrochemical gradient** or electrochemical potential energy difference acting on the solute between the two compartments. This **electrochemical potential energy difference** includes a contribution from the concentration gradient of the solute—the chemical potential energy difference—and, for charged solutes (e.g., Na^+, Cl^-), a contribution from any difference in voltage that exists between the two compartments—the electrical potential energy difference.

This concept of how force and pathway determine passive movement of solutes is most easily illustrated by the example of passive, noncoupled transport. **Noncoupled transport** of a substance X means that movement of X across the membrane is not directly coupled to the movement of any other solute or to any chemical reaction (e.g., the hydrolysis of ATP). What, then, are the driving forces for the net movement of X? Clearly, if the concentration of X is higher in the outside compartment ($[X]_o$) than in the inside compartment ($[X]_i$), and assuming no voltage difference, the concentration gradient will act as the driving force to bring about the net movement of X across the membrane from outside to inside (Fig. 5-2). If [X] is the same on both sides but there is a voltage difference across the membrane—that is, the electrical potential energy on the outside (Ψ_o) is not the same as on the inside (Ψ_i)—this voltage difference will also drive the net movement of X, provided X is charged. The concentration gradient for X and the voltage difference across the membrane are the two determinants of the electrochemical potential energy difference for X between the two compartments. Because the movement of X by such a noncoupled mechanism is not directly coupled to the movement of other solutes or to any chemical reactions, the electrochemical gradient for X is the only driving force that contributes to the transport of X. Thus, the transport of X by a noncoupled, passive mechanism must always proceed "downhill," in the direction down the electrochemical potential energy difference for X.

Regardless of *how* X moves passively through the membrane—whether X moves through lipid or through a membrane protein—the direction of the overall driving force acting on X determines the direction of net transport. In the example in Figure 5-2, the overall driving force favors net transport from outside to inside (influx). However, X may still move from inside to outside (efflux). Movement of X across the membrane in one direction or the other is known as **unidirectional flux**. The algebraic sum of the two unidirectional fluxes is the **net flux**, or the net transport rate. Net transport occurs only when the unidirectional fluxes are unequal. In Figure 5-2, the overall driving force makes unidirectional influx greater than unidirectional efflux, resulting in net influx.

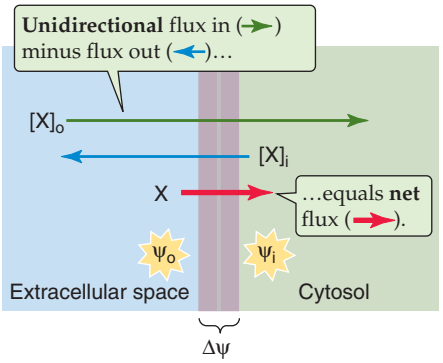


Figure 5-2 Uncoupled transport of a solute across a cell membrane. The net passive movement of a solute (X) depends on both the difference in concentration between the inside of the cell ($[X]_i$) and the outside of the cell ($[X]_o$) and the difference in voltage between the inside of the cell (Ψ_i) and the outside of the cell (Ψ_o).

When no net driving force is acting on X, we say that X is at **equilibrium** across the membrane and there is no net transport of X across the membrane. However, even when X is in equilibrium, there may be and usually are equal and opposite movements of X across the membrane. Net transport takes place only when the net driving force acting on X is displaced from the equilibrium point, and transport proceeds in the direction that would bring X back to equilibrium.

Equilibrium is actually a special case of a **steady state**. In a steady state, by definition, the conditions related to X do not change with time. Thus, a transport system is in a steady state when both the driving forces acting on it and the rate of transport are constant with time. Equilibrium is the particular steady state in which there is no net driving force and thus no net transport.

How can a steady state persist when X is not in equilibrium? Returning to the dike analogy, the downhill flow of water can be constant only if some device, such as a pump, keeps the water levels constant on both sides of the dike. A cell can maintain a nonequilibrium steady state for X only when some device, such as a mechanism for actively transporting X, can compensate for the passive movement of X and prevent the intracellular and extracellular concentrations of X from changing with time. This combination of a pump and a leak maintains both the concentrations of X and the passive flux of X.

At equilibrium, the chemical and electrical potential energy differences across the membrane are equal but opposite

As noted in the preceding section, the driving force for the passive, uncoupled transport of a solute is the electrochemical potential energy difference for that solute across the membrane that separates the inside (i) from the outside (o). We define the electrochemical potential energy difference as

$$\underbrace{\Delta\tilde{\mu}_X}_{\substack{\text{Electrochemical}\\ \text{potential energy}\\ \text{difference}}} = \underbrace{RT\ln\frac{[X]_i}{[X]_o}}_{\substack{\text{Chemical}\\ \text{potential energy}\\ \text{difference}}} + \underbrace{z_X F(\Psi_i - \Psi_o)}_{\substack{\text{Electrical}\\ \text{potential energy}\\ \text{difference}}} \qquad (5\text{-}6)$$

where z_X is the valence of X, T is absolute temperature, R is the gas constant, and F is Faraday's constant. The first term on the right-hand side of Equation 5-6, the difference in chemical potential energy, describes the energy change (joules/mole) as X moves across the membrane if we disregard the charge—if any—on X. The second term, the difference in electrical potential energy, describes the energy change as a mole of charged particles (each with a valence of z_X) moves across the membrane. The difference $(\Psi_i - \Psi_o)$ is the **voltage difference across the membrane** ($\mathbf{V_m}$), also known as the **membrane potential**.

By definition, X is at equilibrium when the electrochemical potential energy difference for X across the membrane is zero:

$$\Delta\tilde{\mu}_X = 0 \qquad (5\text{-}7)$$

Thus, $\Delta\tilde{\mu}_x$ is the *net driving force* (*units:* joules/mole). When $\Delta\tilde{\mu}_x$ is *not* zero, X is *not* in equilibrium and will obviously tend either to enter the cell or to leave the cell, provided a pathway exists for X to cross the membrane.

It is worthwhile to consider two special cases of the equilibrium state (Equation 5-7). First, when either the chemical or the electrical term in Equation 5-6 is zero, the other must also be zero. For example, when X is uncharged ($z_X = 0$), as in the case of glucose, equilibrium can occur only when [X] is equal on the two sides of the membrane. Alternatively, when X is charged, as in the case of Na^+, but the voltage difference (i.e., V_m) is zero, equilibrium likewise can occur only when [X] is equal on the two sides of the membrane. Second, when neither the chemical nor the electrical term in Equation 5-6 is zero, equilibrium can occur only when the two terms are equal but of opposite sign. Thus, if we set $\Delta\tilde{\mu}_x$ in Equation 5-6 to zero, as necessary for a state of equilibrium,

$$\begin{aligned} 0 &= RT\ln\frac{[X]_i}{[X]_o} + z_X F V_m \\ -\frac{RT}{z_X F}\ln\frac{[X]_i}{[X]_o} &= V_m \\ V_m = E_X &= -\frac{RT}{z_X F}\ln\frac{[X]_i}{[X]_o} \end{aligned} \qquad (5\text{-}8)$$

This relationship is the **Nernst equation**, which describes the conditions when an ion is in equilibrium across a membrane. Given values for $[X]_i$ and $[X]_o$, X can be in equilibrium only when the voltage difference across the membrane equals the **equilibrium potential** (E_X), also known as the Nernst potential. Stated somewhat differently, E_X is the value that the membrane voltage *would have to have* for X to be in equilibrium. If we express the logarithm to the base 10, then for the special case in which the temperature is 29.5°C:

$$V_m = E_X = -\frac{(60\,\text{mV})}{z_X}\log_{10}\frac{[X]_i}{[X]_o} \qquad (5\text{-}9)$$

At normal body temperature (37°C), the coefficient is ~61.5 mV instead of 60 mV. At 20°C, it is ~58.1 mV.

To illustrate the use of Equation 5-9, we compute E_X for a monovalent cation, such as K^+. If $[K^+]_i$ is 100 mM and $[K^+]_o$ is 10 mM, a 10-fold concentration gradient, then

$$E_K = -\frac{(60\,\text{mV})}{1}\log_{10}\frac{100}{10} = -60\,\text{mV} \qquad (5\text{-}10)$$

Thus, a 10-fold gradient of a monovalent ion such as K^+ is equivalent, as a driving force, to a voltage difference of 60 mV. For a divalent ion such as Ca^{2+}, a 10-fold concentration gradient can be balanced as a driving force by a voltage difference of 60 mV/2, or only 30 mV.

($V_m - E_X$) is the net electrochemical driving force acting on an ion

When dealing with an ion (X), it is more convenient to think about the net driving force in voltage (*units:* mV) rather than electrochemical potential energy difference (*units:* joules/mole). If we divide all terms in Equation 5-6 by the product of valence and Faraday's constant ($z_X F$), we obtain

$$\underbrace{\frac{\Delta\mu_X}{z_X F}}_{\substack{\text{Net}\\ \text{driving}\\ \text{force}}} = \underbrace{\frac{RT}{z_X F}\ln\frac{[X]_i}{[X]_o}}_{-E_X} + \underbrace{(\Psi_i - \Psi_o)}_{V_m} \qquad (5\text{-}11)$$

Because the energy terms previously expressed as *joules* per mole were divided by *coulombs* per mole (i.e., $z_X F$)—all three energy terms enclosed in braces are now in units of joules per coulomb or *volts*. The term on the left is the net electrochemical driving force acting on ion X. The first term on the right, as defined in Equation 5-8, is the negative of the Nernst equilibrium potential ($-E_X$). The second term on the right is the membrane voltage (V_m). Thus, a convenient equation expressing the net driving force is

$$\text{Net driving force in volts} = (V_m - E_X) \qquad (5\text{-}12)$$

In Table 5-3, we use this equation—along with the values in Table 5-2 for extracellular (i.e., interstitial) and intracellular concentrations and a typical V_m of −60 mV—to compute the net driving force of Na^+, K^+, Ca^{2+}, Cl^-, HCO_3^-, and H^+. When the net driving force is negative, cations will enter the cell and anions will exit. Stated differently, when V_m is more negative than E_X (i.e., the cell is *too negative* for X to be in equilibrium), a cation will tend to enter the cell and an anion will tend to exit.

In simple diffusion, the flux of an uncharged substance through membrane lipid is directly proportional to its concentration difference

The difference in electrochemical potential energy of a solute X across the membrane is a useful parameter because it

Table 5-3 Net Electrochemical Driving Forces Acting on Ions in a Typical Cell*

Extracellular Concentration $[X]_o$	Intracellular Concentration $[X]_i$	Membrane Voltage V_m	Equilibrium Potential (mV) $E_X = -(RT/zF)$ ln $([X]_i/[X]_o)$	Electrochemical Driving Force $(V_m - E_X)$
Na^+ 145 mM	15 mM	–60 mV	+61 mV	–121 mV
K^+ 4.5 mM	120 mM	–60 mV	–88 mV	+28 mV
Ca^{2+} 1.2 mM	10^{-7} M	–60 mV	+125 mV	–185 mV
Cl^- 116 mM	20 mM	–60 mV	–47 mV	–13 mV
HCO_3^- 25 mM	16 mM	–60 mV	–12 mV	–48 mV
H^+ 40 nM pH 7.4	63 nM 7.2	–60 mV	–12 mV	–48 mV

*Calculated at 37°C using -RT/zF = -26.71 mV.

allows us to predict whether X is in equilibrium across the cell membrane (i.e., Is $\Delta\tilde{\mu}_x = 0$?) or, if not, whether X would tend to passively move into the cell or out of the cell. As long as the movement of X is not coupled to the movement of another substance or to some biochemical reaction, the only factor that determines the direction of net transport is the driving force $\Delta\tilde{\mu}_x = 0$. The ability to predict the movement of X is independent of any detailed knowledge of the actual transport pathway mediating its passive transport. In other words, we can understand the overall *energetics* of X transport without knowing anything about the transport mechanism itself, other than knowing that it is passive.

So far, we have discussed only the *direction* of net transport, not the *rate*. How will the rate of X transport vary if we vary the driving force $\Delta\tilde{\mu}_x$? Unlike the issue of direction, determining the rate—that is, the **kinetics**—of transport requires knowing the peculiarities of the actual mechanism that mediates passive X transport.

Most transport systems are so complicated that a straightforward relationship between transport rate and $\Delta\tilde{\mu}_x$ may not exist. Here we examine the simplest case, which is **simple diffusion**. How fast does an uncharged, hydrophobic solute move through a lipid bilayer? Gases (e.g., CO_2), a few endogenous compounds (e.g., steroid hormones), and many drugs (e.g., anesthetics) are both uncharged and hydrophobic. Imagine that such a solute is present on both sides of the membrane but at a higher concentration on the outside (Fig. 5-2). Because X has no electrical charge and because $[X]_o$ is greater than $[X]_i$, the net movement of X will be *into* the cell. How *fast* X moves is described by its **flux** (J_X), namely, the number of moles of X crossing a unit area of membrane (typically 1 cm^2) per unit time (typically 1 second). Thus J_X has the units moles/($cm^2 \cdot s$). The better that X can dissolve in the membrane lipid (i.e., the higher the lipid-water **partition coefficient** of X), the more easily X will be able to traverse the membrane-lipid barrier. The flux of X will also be greater if X moves more readily once it is in the membrane (i.e., a higher **diffusion coefficient**) and if the distance that it must traverse is short (i.e., a smaller **membrane thickness**). We can combine these three factors into a single parameter called the **permeability coefficient** of X (P_X). Finally, the flux of X will be greater as the difference in [X] between the two sides of the membrane increases (a large **gradient**).

These concepts governing the simple diffusion of an electrically neutral substance were quantified by Adolf Fick in the 1800s and applied by others to the special case of a cell membrane. They are embodied in the following equation, which is a simplified version of **Fick's law**:

$$J_X = P_X([X]_o - [X]_i) \qquad \textbf{(5-13)}$$

As already illustrated in Figure 5-2, we can separate the *net* flux of X into a *unidirectional influx* ($J_X^{o\to i}$) and a *unidirectional efflux* ($J_X^{i\to o}$). The net flux of X into the cell is simply the difference between the unidirectional fluxes:

$$\begin{aligned} \text{Unidirectional influx: } J_X^{o\to i} &= P_X[X]_o \\ \text{Unidirectional efflux: } J_X^{i\to o} &= P_X[X]_i \\ \text{Net flux: } J_X &= P_X([X]_o - [X]_i) \end{aligned} \qquad \textbf{(5-14)}$$

Thus, unidirectional influx is proportional to the outside concentration, unidirectional efflux is proportional to the inside concentration, and net flux is proportional to the concentration *difference* (not the *ratio* $[X]_o/[X]_i$, but the *difference* $[X]_o - [X]_i$). In all cases, the proportionality constant is P_X.

A description of the *kinetic* behavior of a transport system (Equation 5-14)—that is, how fast things move—cannot violate the laws of *energetics,* or thermodynamics (Equation 5-6)—that is, the direction in which things move to restore equilibrium. For example, the laws of thermodynamics (Equation 5-6) predict that when the concentration gradient for a neutral substance is zero (i.e., when $[X]_o/[X]_i = 1$), the system is in equilibrium and therefore the net flux must be zero. The law of simple diffusion (Equation 5-14), which is a kinetic description, also predicts that when the concentra-

tion gradient for a neutral substance is zero (i.e., $[X]_o - [X]_i = 0$), the flux is zero.

Some substances cross the membrane passively through intrinsic membrane proteins that can form pores, channels, or carriers

Because most ions and hydrophilic solutes of biological interest partition poorly into the lipid bilayer, simple passive diffusion of these solutes through the lipid portion of the membrane is negligible. Noncoupled transport across the plasma membrane generally requires specialized pathways that allow particular substances to cross the lipid bilayer. In all known cases, such pathways are formed from **integral membrane proteins**. Three types of protein pathways through the membrane are recognized:

1. The membrane protein forms a **pore** that is always open (Fig. 5-3A). Physiological examples are the porins in the outer membranes of mitochondria, cytotoxic pore-forming proteins such as the perforin released by lymphocytes, and perhaps the aquaporin water channels. A physical equivalent is a straight, open tube. If you look though this tube, you always see light coming through from the opposite side.
2. The membrane protein forms a **channel** that is alternately open and closed because it is equipped with a movable barrier or gate (Fig. 5-3B). Physiological examples include virtually all ion channels, such as the ones that allow Na^+, Cl^-, K^+, and Ca^{2+} to cross the membrane. The process of opening and closing of the barrier is referred to as **gating**. Thus, a channel is a gated pore, and a pore is a nongated channel. A physical equivalent is a tube with a shutter near one end. As you look through this tube, you see the light flickering as the shutter opens and closes.
3. The membrane protein forms a **carrier** surrounding a conduit that never offers a continuous transmembrane path because it is equipped with at least two gates that are never open at the same time (Fig. 5-3C). Between the two gates is a compartment that can contain one or more binding sites for the solute. If the two gates are both closed, one (or more) of the transiting particles is trapped, or **occluded**, in that compartment. Physiological examples include carriers that move single solutes through the membrane by a process known as facilitated diffusion, which is discussed in the next section. A physical equivalent is a tube with shutters at both ends. As you look through this tube, you never see any light passing through because both shutters are never open simultaneously.

Water-filled pores can allow molecules, some as large as 45 kDa, to cross membranes passively

Some membrane proteins form **pores** that provide an aqueous transmembrane conduit that is always open (Fig. 5-3A). Among the large-size pores are the **porins** (Fig. 5-4) found in the outer membranes of gram-negative bacteria and mitochondria. Mitochondrial porin allows solutes as large as 5 kDa to diffuse passively from the cytosol into the mitochondria's intermembrane space.

One mechanism by which cytotoxic T lymphocytes kill their target cells is by releasing monomers of a pore-forming protein known as **perforin**. Perforin monomers polymerize within the target cell membrane and assemble like staves of a barrel to form large, doughnut-like channels with an internal diameter of 16 nm. The passive flow of ions, water, and other small molecules through these pores kills the target cell. A similar pore plays a crucial role in the defense against bacterial infections. The binding of antibodies to an invading bacterium ("classic" pathway), or simply the presence of native polysaccharides on bacteria ("alternative" pathway), triggers a cascade of reactions known as the **complement cascade**. This cascade culminates in the formation of a doughnut-like structure with an internal diameter of 10 nm. This pore is made up of monomers of C9, the final component of the complement cascade.

The **nuclear pore complex (NPC)**, which regulates traffic into and out of the nucleus (see Chapter 2), is remarkably large. The NPC is made up of at least 30 different proteins and has a molecular mass of 10^8 Da and an outer diameter of ~100 nm. It can transport huge molecules (approaching 10^6 Da) in a complicated process that involves ATP hydrolysis. In addition to this active component of transport, the NPC also has a passive component. Contained within the massive NPC is a simple aqueous pore with an internal diameter of ~9 nm that allows molecules smaller than 45 kDa to move between the cytoplasm and nucleus but almost completely restricts the movement of globular proteins that are larger than ~60 kDa.

The plasma membranes of many types of cells have proteins that form channels just large enough to allow water molecules to pass through. The first water channel to be studied was **aquaporin** 1 (AQP1), a 28-kDa protein. AQP1 belongs to a larger family of aquaporins that has representatives in organisms as diverse as bacteria, plants, and animals. In mammals, the various aquaporin isoforms have different tissue distributions, different mechanisms of regulation, and varying abilities to transport small neutral molecules other than water. In the lipid bilayer, AQP1 (Fig. 5-5) exists as tetramers. Each monomer consists of six membrane-spanning helices as well as two shorter helices that dip into the plane of the membrane. These structures form a permeation pathway for the single-file diffusion of water. For his discovery of the aquaporins, Peter Agre shared the 2003 Nobel Prize in Chemistry.

Gated channels, which alternately open and close, allow ions to cross the membrane passively

Gated **ion channels**, like the aquaporins just discussed, consist of one or more polypeptide subunits with α-helical membrane-spanning segments. These channels have several functional components (Fig. 5-3B). The first is a **gate** that determines whether the channel is open or closed, each state reflecting a different conformation of the membrane protein. Second, the channel generally has one or more **sensors** that can respond to one of several different types of signals: (1) changes in membrane voltage, (2) second-messenger systems

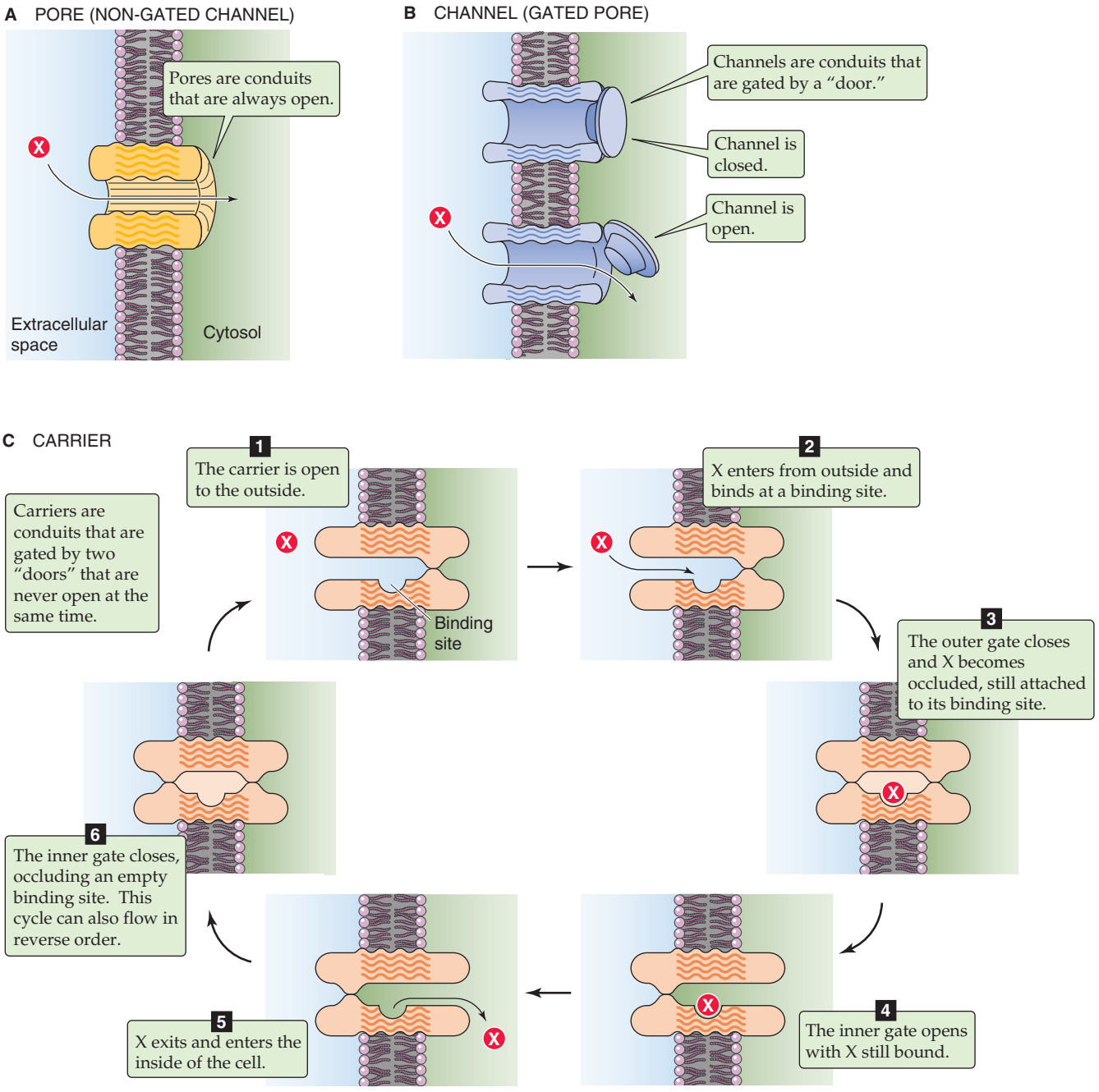


Figure 5-3 Three types of passive, noncoupled transport through integral membrane proteins.

that act at the cytoplasmic face of the membrane protein, or (3) ligands, such as neurohumoral agonists, that bind to the extracellular face of the membrane protein. These signals regulate transitions between the open and closed states. A third functional component is a **selectivity filter**, which determines the *classes* of ions (e.g., anions or cations) or the *particular* ions (e.g., Na^+, K^+, Ca^{2+}) that have access to the channel pore. The fourth component is the actual **open-channel pore** (Fig. 5-3B). Each time that a channel assumes the open conformation, it provides a continuous pathway between the two sides of the membrane so that ions can flow through it passively by diffusion until the channel closes again. During each channel opening, many ions flow through the channel pore, usually a sufficient number to be detected as a small current by sensitive patch-clamping techniques (see Chapter 6).

Na^+ Channels Because **the electrochemical driving force for Na^+** ($V_m - E_{Na}$) is always strongly *negative* (Table 5-3), a large, inwardly directed net driving force or gradient favors the passive movement of Na^+ into virtually every cell of the body. Therefore, an open Na^+ channel will act as a conduit

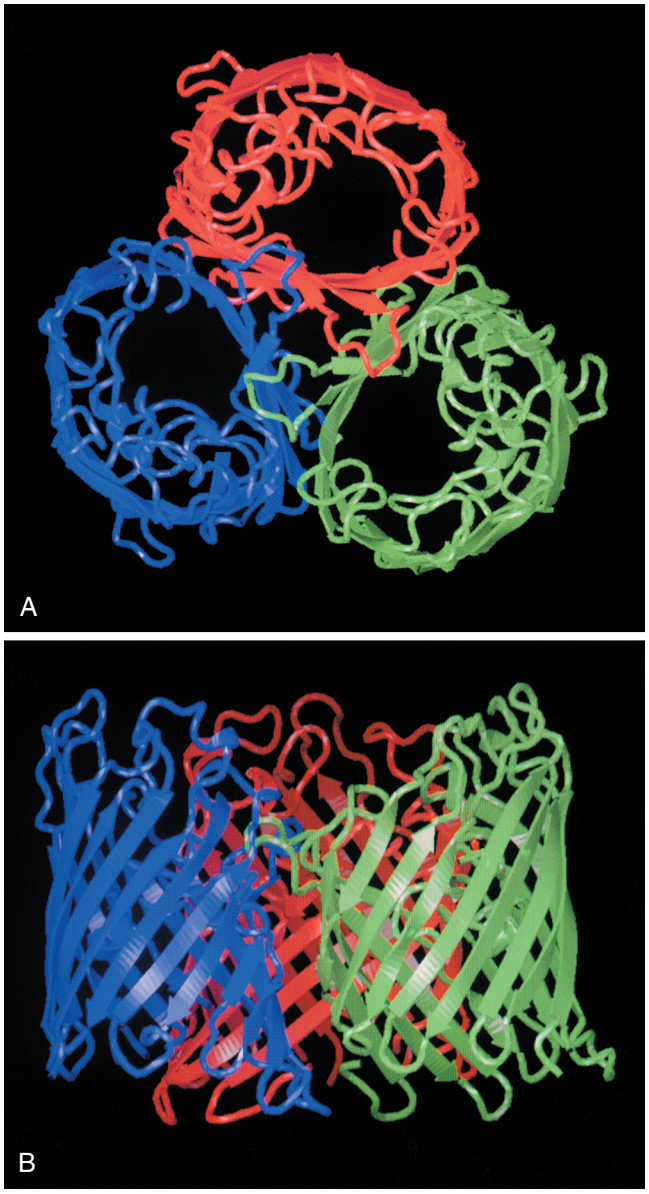


Figure 5-4 Structure of the PhoE porin of *Escherichia coli.* **A,** Top view of a porin trimer that shows the backbones of the polypeptide chains. Each of the three identical monomers, which are shown in different colors, contains 330 amino acids. The center of each monomer is a pore. **B,** Side view of a porin trimer. The extracellular surface is shown at the top. Each monomer consists of a β barrel with 16 antiparallel β sheets (i.e., adjacent polypeptide strands are oriented in opposite directions) surrounding a large cavity that at its narrowest point has an oval cross section (internal diameter, 0.7 × 1.1 nm). The images are based on high-resolution electron microscopy, at a resolution of 3.5 Å (0.35 nm). *(Reproduced from Jap BK, Walian PJ: Structure and functional mechanisms of porins. Physiol Rev 1996; 76:1073-1088.)*

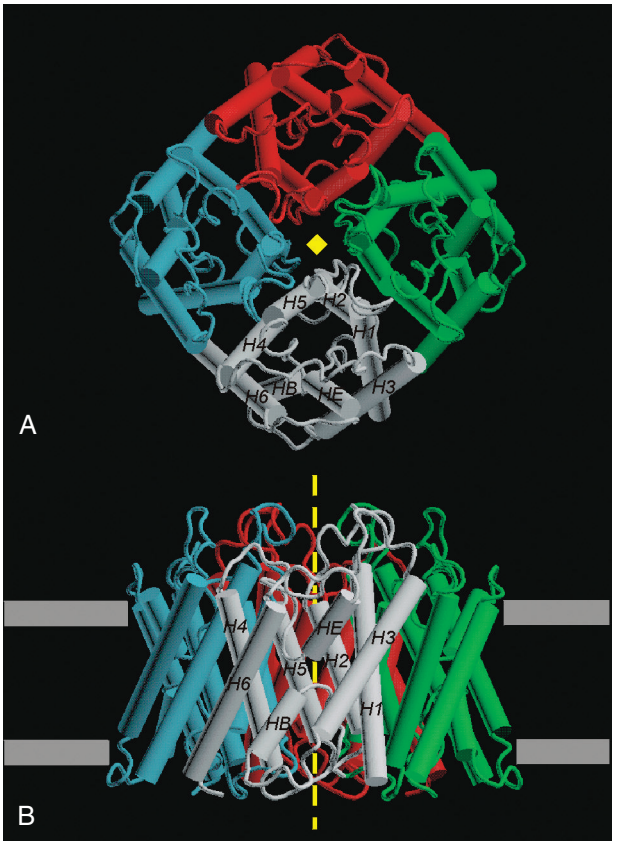


Figure 5-5 Structure of the human AQP1 water channel. **A,** Top view of an aquaporin tetramer. Each of the four identical monomers is made up of 269 amino acids and has a pore at its center. **B,** Side view of aquaporin. The extracellular surface is shown at the top. The images are based on high-resolution electron microscopy at a resolution of 3.8 Å (0.38 nm). *(Reproduced from Murata K, Mitsuoka K, Hirai T, et al: Structural determinants of water permeation through aquaporin-1. Nature 407:599-605, 2000. © 2000 Macmillan Magazines Ltd.)*

for the passive entry of Na^+. One physiological use for channel-mediated Na^+ entry is the transmission of information. Thus, voltage-gated Na^+ channels are responsible for generating the action potential (e.g., "nerve impulse") in many excitable cells. Another physiological use of Na^+ channels can be found in epithelial cells such as those in certain segments of the renal tubule and intestine. In this case, ENaC Na^+ channels are largely restricted to the apical surface of the cell, where they allow Na^+ to enter the epithelial cell from the renal tubule lumen or intestinal lumen. This passive influx is a key step in the movement of Na^+ across the entire epithelium, from lumen to blood.

K^+ Channels The **electrochemical driving force for K^+** ($V_m - E_K$) is usually fairly close to zero or somewhat *positive* (Table 5-3), so K^+ is either at equilibrium or tends to move *out* of the cell. In virtually all cells, K^+ channels play a major role in generating a resting membrane voltage that is inside-negative. Other kinds of K^+ channels play a key role in excitable cells, where these channels help terminate action potentials.

Ca^{2+} Channels The **electrochemical driving force for Ca^{2+}** ($V_m - E_{Ca}$) is always strongly *negative* (Table 5-3), so Ca^{2+} tends to move *into* the cell. When Ca^{2+} channels are open, Ca^{2+} rapidly enters the cell down a steep electrochemical gradient. This inward movement of Ca^{2+} plays a vital role in transmembrane signaling for both excitable and

nonexcitable cells as well as in generating action potentials in some excitable cells.

Proton Channels The plasma membranes of many cell types contain Hv1 H^+ channels. Under normal conditions, the H^+ driving force generally tends to move H^+ *into* cells if Hv1 channels are open. However, Hv1 channels tend to be *closed* under normal conditions and activate only when the membrane depolarizes or the cytoplasm acidifies—that is, when the driving force favors the *outward* movement of H^+. Hv1 channels may therefore help mediate H^+ extrusion from the cell during states of strong membrane depolarization (e.g., during an action potential) or severe intracellular acidification.

Anion Channels Most cells contain one or more types of anion-selective channels through which the passive, noncoupled transport of Cl^-—and, to a lesser extent, HCO_3^-—can take place. The electrochemical driving force for Cl^- ($V_m - E_{Cl}$) in most cells is modestly *negative* (Table 5-3), so Cl^- tends to move *out of* these cells. In certain epithelial cells with Cl^- channels on their basolateral membranes, the passive movement of Cl^- through these channels plays a role in the transepithelial movement of Cl^- from lumen to blood.

Some carriers facilitate the passive diffusion of small solutes such as glucose

Carrier-mediated transport systems transfer a broad range of ions and organic solutes across the plasma membrane. Each carrier protein has a specific affinity for binding one or a small number of solutes and transporting them across the bilayer. The simplest passive carrier-mediated transporter is one that mediates *facilitated diffusion*. Later, we will introduce *cotransporters* (which carry two or more solutes in the same direction) and *exchangers* (which move them in opposite directions).

All carriers that do not either hydrolyze ATP or couple to an electron transport chain are members of the **solute carrier (SLC) superfamily**, which is organized according to the homology of the deduced amino acid sequences (Table 5-4). Each of the 43 SLC families contains 1 to 27 variants, which share a relatively high amino acid sequence identity (20% to 25%) among the isoforms. Members of an SLC family may differ in molecular mechanism (facilitated diffusion versus exchange), kinetic properties (e.g., solute specificity and affinity), regulation (e.g., phosphorylation), sites of membrane targeting (e.g., plasma membrane versus intracellular organelles), tissues in which they are expressed (e.g., kidney versus brain), or developmental stage at which they are expressed.

Carrier-mediated transport systems behave according to a general kinetic scheme for facilitated diffusion that is outlined in Figure 5-3C. This model illustrates how, in a cycle of six steps, a carrier could passively move a solute X into the cell.

This mechanism can mediate only the downhill, or passive, transport of X. Therefore, it mediates a type of diffusion called **facilitated diffusion**. When [X] is equal on the two sides of the membrane, no *net* transport will take place, although equal and opposite *unidirectional* fluxes of X may still occur.

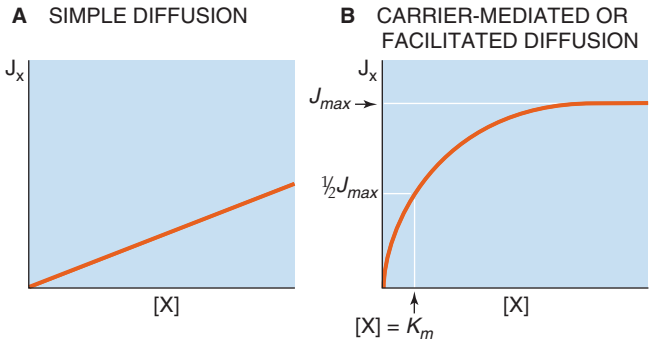


Figure 5-6 Dependence of transport rates on solute concentration. **A,** The net flux of the solute X through the cell membrane is J_X. **B,** The maximal flux of X (J_{max}) occurs when the carriers are saturated. The flux is half of its maximal value (1/2 J_{max}) when the concentration of X is equal to the K_m.

In a cell membrane, a fixed number of carriers is available to transport X. Furthermore, each carrier has a limited speed with which it can cycle through the steps illustrated in Figure 5-3C. Thus, if the extracellular X concentration is gradually increased, for example, the influx of X will eventually reach a maximal value once all the carriers have become loaded with X. This situation is very different from the one that exists with simple diffusion, that is, the movement of a solute through the lipid phase of the membrane. Influx by simple diffusion increases linearly with increases in $[X]_o$, with no maximal rate of transport. As an example, if X is initially absent on *both* sides of the membrane and we gradually increase [X] on *one* side, the net flux of X (J_X) is described by a straight line that passes through the origin (Fig. 5-6A). However, with carrier-mediated transport, J_X reaches a maximum (J_{max}) when [X] is high enough to occupy all the carriers in the membrane (Fig. 5-6B). Thus, the relationship describing carrier-mediated transport follows the same Michaelis-Menten kinetics as enzymes do:

$$V = \frac{[S]\,V_{max}}{K_m + [S]} \qquad (5\text{-}15)$$

This equation describes how the velocity of an enzymatic reaction (V) depends on the substrate concentration ([S]), the Michaelis constant (K_m), and the maximal velocity (V_{max}). The comparable equation for carrier-mediated transport is identical, except that fluxes replace reaction velocities:

$$J_X = \frac{[X]\,J_{max}}{K_m + [X]} \qquad (5\text{-}16)$$

Thus, K_m is the solute concentration at which J_X is half of the maximal flux (J_{max}). The lower the K_m, the higher the apparent **affinity** of the transporter for the solute.

Historically, the name *carrier* suggested that carrier-mediated transport occurs as the solute binds to a miniature ferryboat that shuttles back and forth across the membrane.

Table 5-4 Some Families in the SLC Superfamily of Solute Carriers

Family	Description	Examples
SLC1 (7)*	Glutamate transporters	EAAT1
SLC2 (14)	Facilitated transport of hexoses	GLUT1, GLUT4
SLC3 (2)	Heavy subunits of heterodimeric amino acid transporters (with SLC7)	
SLC4 (10)	HCO_3^- exchangers and cotransporters	AE1 (Cl-HCO_3 exchanger) NBCe1 (electrogenic Na/HCO_3 cotransporter) NBCn1 (electroneutral Na/HCO_3 cotransporter) NDCBE (Na^+-driven Cl-HCO_3 exchanger)
SLC5 (8)	Na^+/glucose cotransporters	SGLT1 to 5 (glucose)
SLC6 (16)	Na^+- and Cl^--coupled cotransport of "neurotransmitters"	B^0AT1 (Na^+-coupled amino acid) GAT1-3, GBT1 (Na^+- and Cl^--coupled GABA) ATB^{0+} (Na^+- and Cl^--coupled amino acids)
SLC7 (14)	Transporter subunits of heterodimeric amino acid transporters (with SLC3)	
SLC8 (3)	Na-Ca exchangers	NCX1 to 3
SLC9 (9)	Na-H exchangers	NHE1 to 8
SLC10 (6)	Na/bile salt cotransporters	
SLC11 (2)	H^+-driven metal ion cotransporters	DMT1
SLC12 (9)	Cation-coupled Cl^- cotransporters	NKCC1, NKCC2 (Na/K/Cl cotransporter) NCC (Na/Cl cotransporter) KCC1 (K/Cl cotransporter)
SLC13 (5)	Na^+-coupled sulfate and carboxylate cotransporters	NaDC1 (mono-, di-, and tricarboxylates) NaSi (sulfate)
SLC14 (2)	Facilitated transport of urea	UT
SLC15 (4)	H^+-driven oligopeptide cotransporters	PepT1
SLC16 (14)	Monocarboxylate transporters	MCT1 (H^+-coupled monocarboxylate cotransporter) TAT1 (facilitated diffusion of aromatic amino acids)
SLC17 (8)	Type I Na/phosphate cotransporters and vesicular Glu transporters	NaPi-I
SLC18 (3)	Vesicular amine transporters	
SLC19 (3)	Folate/thiamine transporters	
SLC20 (2)	Type III Na/phosphate cotransporters	NaPi-III
SLC21 (11)	Organic anion transporters	OATP PGT

*Number of genes in parentheses.

Table 5-4 The SLC Superfamily of Solute Carriers—cont'd

Family	Description	Examples
SLC22 (18)*	Organic cations, anions, zwitterions	OCT1 to 3 (facilitated diffusion of organic *cations*) OAT1 to 5 (exchange or facilitated diffusion of organic *anions*) URAT (urate exchanger)
SLC23 (4)	Na/ascorbic acid transporters	
SLC26 (10)	Multifunctional anion exchangers	DRA (Cl-HCO_3 exchanger) Pendrin (exchanges HCO_3^-, Cl^-, or I^-) CFEX (exchanges Cl^-, HCO_3^-, oxalate, formate)
SLC28 (3)	Na/nucleoside transporters	
SLC34 (3)	Type II Na/phosphate cotransporters	NaPi-IIa, NaPi-IIc
SLC36 (4)	H^+-coupled amino acid cotransporters	PAT1
SLC38 (6)	Na^+-driven neutral amino acid transporters (system A and N)	SNAT3 (cotransports amino acids with Na^+ in exchange for H^+) System N (cotransports amino acids with Na^+ in exchange for H^+) SNAT1, 2, 4 (system A cotransports amino acids with Na^+)
SLC39 (14)	Metal ion transporters	ZIP1 (uptake of Zn^{2+})
SLC40 (1)	Basolateral Fe transporter (facilitated diffusion)	Ferroportin (MTP1, Fe^{2+})
SLC42 (3)	NH_3 channels	RhAG

*Number of genes in parentheses.

Small polypeptides that act as shuttling carriers exist in nature, as exemplified by the antibiotic valinomycin. Such "ion carriers," or **ionophores**, bind to an ion on one side of the membrane, diffuse across the lipid phase of the membrane, and release the ion on the opposite side of the membrane. Valinomycin is a K^+ ionophore that certain bacteria produce to achieve a selective advantage over their neighbors. However, none of the known carrier-mediated transport pathways in animal cell membranes are ferries.

An example of a membrane protein that mediates facilitated diffusion is the **glucose transporter GLUT1** (Fig. 5-7), a member of the SLC2 family (Table 5-4). The GLUTs have 12 membrane-spanning segments as well as multiple hydrophilic polypeptide loops facing either the ECF or ICF. It could not possibly act as a ferryboat shuttling back and forth across the membrane. Instead, some of the membrane-spanning segments of carrier-mediated transport proteins most likely form a permeation pathway through the lipid bilayer, as illustrated by the amphipathic membrane-spanning segments 7, 8, and 11 in Figure 5-7. These membrane-spanning segments, as well as other portions of the protein, probably also act as the gates and solute-binding sites that allow transport to proceed in the manner outlined in Figure 5-3C.

The SLC2 family includes 12 hexose transporters (GLUTs). Whereas GLUT1 is constitutively expressed on the cell surface, GLUT4 in the basal state is predominantly present in the membranes of intracellular vesicles, which represent a storage pool for the transporters. Because a solute such as glucose permeates the lipid bilayer so poorly, its uptake by the cell depends strictly on the activity of a carrier-mediated transport system for glucose. Insulin increases the rate of carrier-mediated glucose transport into certain cells by recruiting the GLUT4 isoform to the plasma membrane from the storage pool (see Chapter 51).

Two other examples of transporters that mediate facilitated diffusion are the **urea transporter (UT)** family, which are members of the SLC14 family (Table 5-4), and the **organic cation transporter (OCT)** family, which are members of the SLC22 family. Because OCT moves an electrical charge (i.e., carries current), it is said to be **electrogenic.**

Table 5-5 Comparison of Properties of Pores, Channels, and Carriers

	Pores	Channels	Carriers
Example	Water channel (AQP1)	Shaker K^+ channel	Glucose transporter (GLUT1)
Conduit through membrane	Always open	Intermittently open	Never open
Unitary event	None (continuously open)	Opening	Cycle of conformational changes
Particles translocated per "event"	—	6×10^4*	1-5
Particles translocated per second	up to 2×10^9	10^6 to 10^8 when open	200-50,000

*Assuming a 100-pS channel, a driving force of 100 mV, and an opening time of 1 ms.

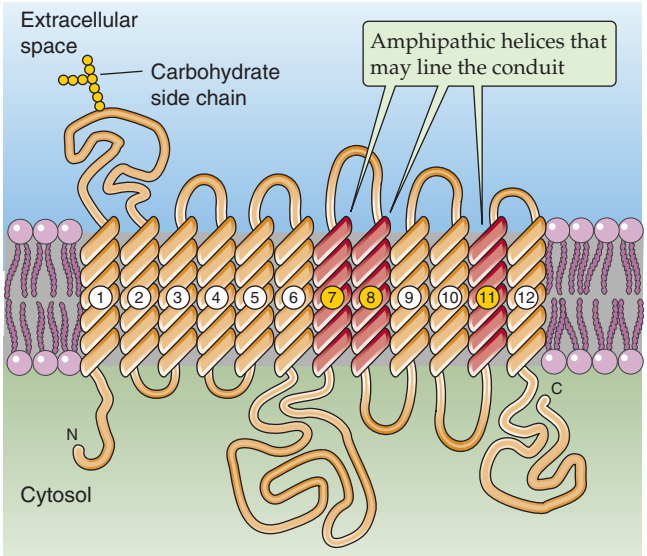


Figure 5-7 Structure of the GLUT family of glucose transporters. The 12 membrane-spanning segments are connected to each other by intracellular and extracellular loops.

The physical structure of pores, channels, and carriers is quite similar

Pores, ion channels, and carriers all have multiple transmembrane segments surrounding a solute permeation pathway. Moreover, some channels also contain binding sites within their permeation pathways, so transport is saturable with respect to ion concentration. However, pores, channels, and carriers are fundamentally distinct kinetically (Table 5-5). *Pores,* such as the porins, are thought to be continuously open and allow vast numbers of particles to cross the membrane. No evidence suggests that pores have conformational states. *Channels* undergo conformational transitions between closed and open states. When they are open, they are open to both intracellular and extracellular solutions simultaneously. Thus, while the channel is open, it allows multiple ions, perhaps millions, to cross the membrane per open event. Because the length of time that a particular channel remains open varies from one open event to the next, the number of ions flowing through that channel per open event is not fixed. *Carriers* have a permeation pathway that is virtually never open simultaneously to both intracellular and extracellular solutions. Whereas the fundamental event for a channel is opening, the fundamental event for a carrier is a complete cycle of conformational changes. Because the binding sites in a carrier are limited, each cycle of a carrier can transport only one or a *small, fixed* number of solute particles. Thus, the number of particles per second that can move across the membrane is generally several orders of magnitude lower for a single carrier than for a single channel.

We have seen how carriers can mediate facilitated diffusion of glucose, which is a passive or downhill process. However, carriers can also mediate **coupled** modes of transport. The remainder of this section is devoted to these carriers, which act as pumps, cotransporters, and exchangers.

The Na-K pump, the most important primary active transporter in animal cells, uses the energy of ATP to extrude Na^+ and to take up K^+

Active transport is a process that can transfer a solute uphill across a membrane—that is, against its electrochemical potential energy difference. In **primary active transport**, the driving force needed to cause net transfer of a solute against its electrochemical gradient comes from the favorable energy change that is associated with an exergonic chemical reaction, such as ATP hydrolysis. In **secondary active transport**, the driving force is provided by coupling the *uphill* movement of that solute to the *downhill* movement of one or more other solutes for which a favorable electrochemical potential energy difference exists. A physical example is to use a motor-driven winch to lift a large weight into the air (primary active transport) and then to transfer this large weight to a seesaw, on the other end of which is a lighter child. The potential energy stored in the elevated weight will then lift the child (secondary active transport). For transporters, it is commonly the favorable inwardly directed Na^+ electrochemical gradient, which itself is set up by a *primary* active transporter, that drives the *secondary* active transport of another solute. In this and the next section, we discuss

primary active transporters, which are also referred to as **pumps**. The pumps discussed here are all energized by ATP hydrolysis and hence are **ATPases**.

As a prototypic example of a primary active transporter, consider the nearly ubiquitous **Na-K pump** (or Na,K-ATPase, **NKA**). This substance was the first enzyme recognized to be an ion pump, a discovery for which Jens Skou shared the 1997 Nobel Prize in Chemistry. The Na-K pump is located in the plasma membrane and has both α and β subunits (Fig. 5-8A). The α subunit, which has 10 transmembrane segments, is the catalytic subunit that mediates active transport. The β subunit, which has one transmembrane segment, is essential for proper assembly and membrane targeting of the Na-K pump. Four α isoforms and two β isoforms have been described. These isoforms have different tissue and developmental patterns of expression as well as different kinetic properties.

With each forward cycle, the pump couples the extrusion of three Na^+ ions and the uptake of two K^+ ions to the intracellular hydrolysis of one ATP molecule. By themselves, the transport steps of the Na-K pump are energetically uphill; that is, if the pump were not an ATPase, the transporter would run in reverse, with Na^+ leaking into the cell and K^+ leaking out. Indeed, under extreme experimental conditions, the Na-K pump can be reversed and forced to synthesize ATP! However, under physiological conditions, hydrolysis of one ATP molecule releases so much free energy—relative to the aggregate free energy needed to fuel the uphill movement of three Na^+ and two K^+ ions—that the pump is poised far from its equilibrium and brings about the net active exchange of Na^+ for K^+ in the desired directions.

Although animal cells may have other pumps in their plasma membranes, the Na-K pump is the only primary active transport process for Na^+. The Na-K pump is also the most important primary active transport mechanism for K^+. In cells throughout the body, the Na-K pump is responsible for maintaining a low $[Na^+]_i$ and a high $[K^+]_i$ relative to ECF. In most epithelial cells, the Na-K pump is restricted to the basolateral side of the cell.

The Na-K pump exists in two major conformational states: E_1, in which the binding sites for the ions face the inside of the cell; and E_2, in which the binding sites face the outside. The Na-K pump is a member of a large superfamily of pumps known as **E_1-E_2 ATPases** or **P-type ATPases**. It is the ordered cycling between these two states that underlies the action of the pump. Figure 5-8B is a simplified model showing the eight stages of this catalytic cycle of the α subunit:

Stage 1: **ATP-bound E_1 • ATP state.** The cycle starts with the ATP-bound E_1 conformation, just after the pump has released its bound K^+ to the ICF. The Na^+-binding sites face the ICF and have high affinities for Na^+.

Stage 2: **Na^+-bound E_1 • ATP • 3Na^+ state.** Three intracellular Na^+ ions bind.

Stage 3: **Occluded E_1-P • (3Na^+) state.** The ATP previously bound to the pump phosphorylates the pump at an aspartate residue. Simultaneously, ADP leaves. This phosphorylation triggers a minor conformational change in which the E_1 form of the pump now occludes the three bound Na^+ ions within the permeation pathway. In this state, the Na^+ binding sites are inaccessible to both the ICF and ECF.

Stage 4: **Deoccluded E_2-P • 3Na^+ state.** A major conformational change shifts the pump from the E_1 to the E_2 conformation and has two effects. First, the pump becomes deoccluded, so that the Na^+-binding sites now communicate with the *extracellular* solution. Second, the Na^+ affinities of these binding sites decrease.

Stage 5: **Empty E_2-P state.** The three bound Na^+ ions dissociate into the external solution, and the protein undergoes a minor conformational change to the empty **E_2-P** form, which has high affinity for binding of extracellular K^+. However, the pore still communicates with the extracellular solution.

Stage 6: **K^+-bound E_2-P • 2K^+ state.** Two K^+ ions bind to the pump.

Stage 7: **Occluded E_2 • (2K^+) state.** Hydrolysis of the acylphosphate bond, which links the phosphate group to the aspartate residue, releases the inorganic phosphate into the intracellular solution and causes a minor conformational change. In this E_2 • ($2K^+$) state, the pump occludes the two bound K^+ ions within the permeation pathway so that the K^+-binding sites are inaccessible to both the ECF and ICF.

Stage 8: **Deoccluded E_1 • ATP • 2K^+ state.** Binding of intracellular ATP causes a major conformational change that shifts the pump from the E_2 back to the E_1 state. This conformational change has two effects. First, the pump becomes deoccluded, so that the K^+-binding sites now communicate with the *intracellular* solution. Second, the K^+ affinities of these binding sites decrease.

Stage 1: **ATP-bound E_1 • ATP state.** Dissociation of the two bound K^+ ions into the intracellular solution returns the pump to its original E_1 • ATP state, ready to begin another cycle.

Because each cycle of hydrolysis of one ATP molecule is coupled to the extrusion of three Na^+ ions from the cell and the uptake of two K^+ ions, the *stoichiometry* of the pump is three Na^+ to two K^+, and each cycle of the pump is associated with the net extrusion of one positive charge from the cell. Thus, the Na-K pump is *electrogenic*.

Just as glucose flux through the GLUT1 transporter is a saturable function of [glucose], the rate of active transport by the Na-K pump is a saturable function of $[Na^+]_i$ and $[K^+]_o$. The reason is that the number of pumps is finite and each must bind three Na^+ ions and two K^+ ions. The transport rate is also a saturable function of $[ATP]_i$ and therefore depends on the metabolic state of the cell. In cells with high Na-K pump rates, such as renal proximal tubules, a third or more of cellular energy metabolism is devoted to supplying ATP to the Na-K pump.

A hallmark of the Na-K pump is that it is blocked by a class of compounds known as **cardiac glycosides**, examples of which are ouabain and digoxin; digoxin is widely used for a variety of cardiac conditions. These compounds have a high affinity for the extracellular side of the E_2-P state of the pump, which also has a high affinity for extracellular K^+. Thus, the binding of extracellular K^+ competitively antagonizes the binding of cardiac glycosides. An important clinical correlate is that hypokalemia (a low $[K^+]$ in blood plasma) potentiates digitalis toxicity in patients.

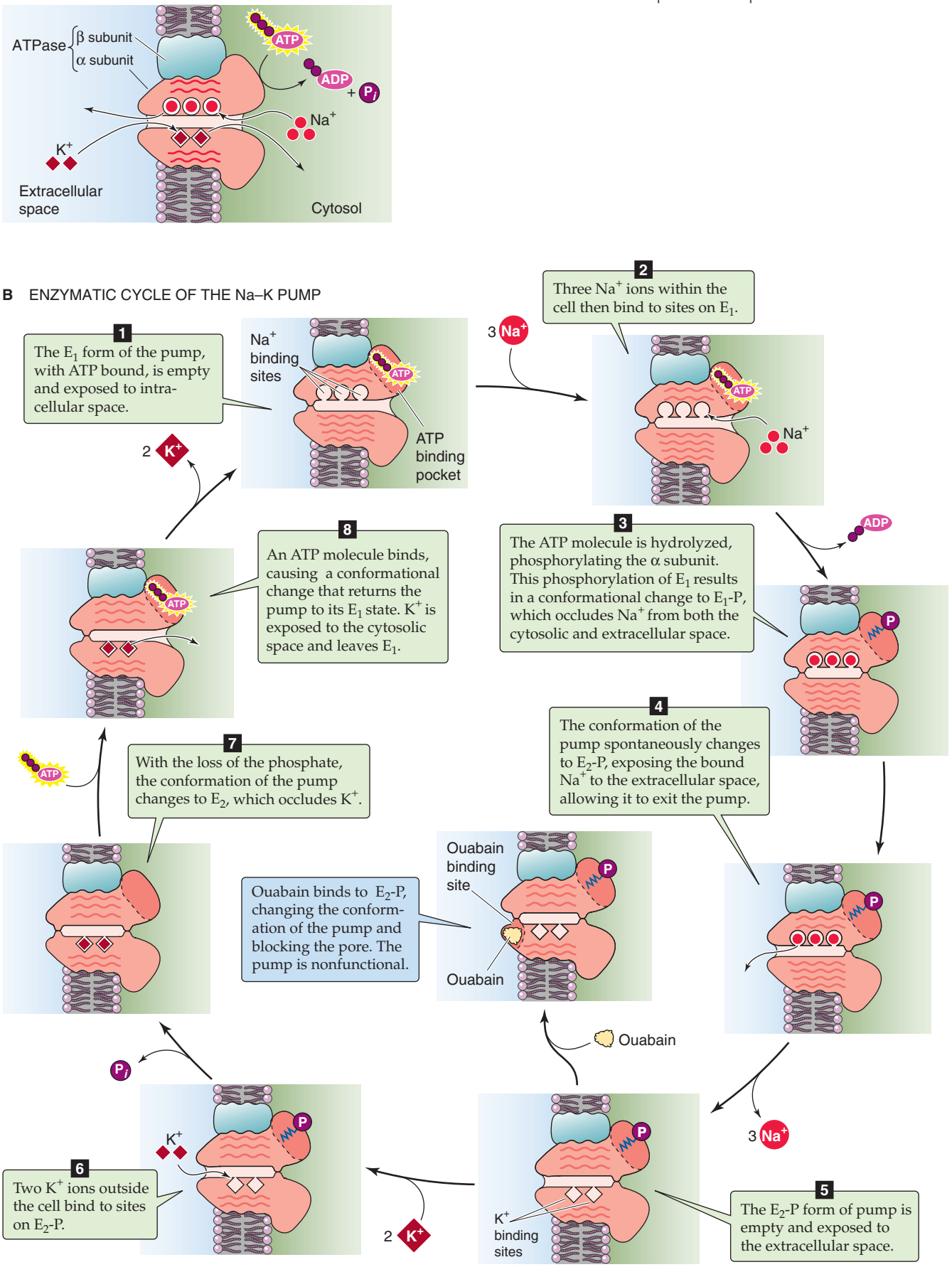


Figure 5-8 Model of the sodium pump. **A,** Schematic representation of the α and β subunits of the pump. **B,** The protein cycles through at least eight identifiable stages as it moves 3 Na^+ ions out of the cell and 2 K^+ ions into the cell.

Besides the Na-K pump, other P-type ATPases include the H-K and Ca^{2+} pumps

The family of P-type ATPases—all of which share significant sequence similarity with the α subunit of the Na-K pump—includes several subfamilies.

The H-K Pump Other than the Na-K pump, relatively few primary active transporters are located on the plasma membranes of animal cells. In the parietal cells of the gastric gland, an H-K pump (**HKA**) extrudes H^+ across the apical membrane into the gland lumen. Similar pumps are present in the kidney and intestines. The H-K pump mediates the active extrusion of H^+ and the uptake of K^+, all fueled by ATP hydrolysis, probably in the ratio of two H^+ ions, two K^+ ions, and one ATP molecule. Like the Na-K pump, the H-K pump is composed of α and β subunits, each with multiple isoforms. The α subunit of the H-K pump also undergoes phosphorylation through E_1 and E_2 intermediates during its catalytic cycle (Fig. 5-8B) and, like the α subunit of the Na-K pump, is a member of the P_{2C} subfamily of P-type ATPases. The Na-K and H-K pumps are the only two P-type ATPases with known β subunits, all of which share significant sequence similarity.

Ca^{2+} Pumps Most if not all cells have a primary active transporter at the plasma membrane that extrudes Ca^{2+} from the cell. These pumps are abbreviated **PMCA** (for plasma membrane Ca^{2+}-ATPase), and at least four PMCA isoforms appear in the P_{2B} subfamily of P-type ATPases. These pumps exchange one H^+ for one Ca^{2+} for each molecule of ATP that is hydrolyzed.

Ca^{2+} pumps (or Ca^{2+}-ATPases) also exist on the membrane surrounding such intracellular organelles as the sarcoplasmic reticulum in muscle cells and the endoplasmic reticulum in other cells, where they play a role in the active sequestration of Ca^{2+} into intracellular stores. The **SERCAs** (for sarcoplasmic and endoplasmic reticulum calcium ATPase) appear to transport two H^+ and two Ca^{2+} ions for each molecule of ATP hydrolyzed. The three known SERCAs, which are in the P_{2A} subfamily of P-type ATPases, are expressed in different muscle types (see Table 9-1).

Other Pumps Among the other P-type ATPases is the copper pump ATP7B. This member of the P_{1B} subfamily of P-type ATPases is mutated in Wilson disease (see the box on this topic in Chapter 46).

The F-type and the V-type ATPases transport H^+

F-type or F_oF_1 ATPases The ATP synthase of the inner membrane of mitochondria, also known as an F-type or F_oF_1 ATPase, catalyzes the final step in the ATP synthesis pathway.

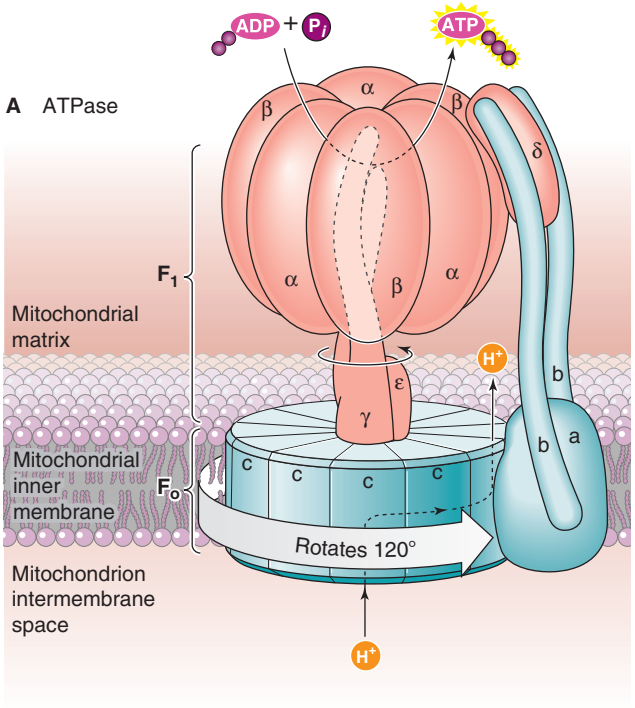
The F_oF_1 ATPase of mitochondria (Fig. 5-9A) looks a little like a lollipop held in your hand. The hand-like F_o portion is embedded in the membrane and serves as the pathway for H^+ transport. The F_o portion has at least three different subunits (a, b, and c), for an overall stoichiometry of $ab_2c_{10\text{-}12}$. The lollipop-like F_1 portion is outside the plane of the membrane and points into the mitochondrial matrix. The "stick" consists of a γ subunit, with an attached ε subunit. The "candy" portion of F_1, which has the ATPase activity, consists of three alternating pairs of α and β subunits as well as an attached δ subunit. Thus, the overall stoichiometry of F_1 is $\alpha_3\beta_3\gamma\delta\varepsilon$. The entire F_oF_1 complex has a molecular mass of ~500 kDa.

A fascinating property of the F_oF_1 ATPase is that parts of it rotate. We can think of the hand, stick, and candy portions of the F_oF_1 ATPase as having three distinct functions. (1) The hand (the c proteins of F_o) acts as a turbine that rotates in the plane of the membrane, driven by the H^+ ions that flow through the turbine—down the H^+ electrochemical gradient—into the mitochondrion. (2) The stick is an axle (γ and ε subunits of F_1) that rotates with the turbine. (3) The candy (the α and β subunits of F_1) is a stationary chemical factory—energized by the rotating axle—that synthesizes one ATP molecule for each 120-degree turn of the turbine/axle complex. In addition, the a and b subunits of F_o, and possibly the δ subunit of F_1, form a stator that holds the candy in place while the turbine/axle complex turns. Paul Boyer and John Walker shared part of the 1997 Nobel Prize in Chemistry for elucidating this "rotary catalysis" mechanism.

Under physiological conditions, the mitochondrial F_oF_1 ATPase runs as an ATP synthase (i.e., "backward" for an H^+ pump)—the final step in oxidative phosphorylation—because of a large, inwardly directed H^+ gradient across the inner mitochondrial membrane (Fig. 5-9B). The citric acid cycle captures energy as electrons and transfers these electrons to reduced nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide ($FADH_2$). NADH and $FADH_2$ transfer their high-energy electrons to the **electron transport chain**, which consists of four major complexes on the inner membrane of the mitochondrion (Fig. 5-9B). As this "respiratory chain" transfers the electrons from one electron carrier to another, the electrons gradually lose energy until they finally combine with 2 H^+ and $\frac{1}{2}$ O_2 to form H_2O. Along the way, three of the four major complexes of the respiratory chain (I, III, IV) pump H^+ across the inner membrane into the intermembrane space (i.e., the space between the inner and outer mitochondrial membranes). These "pumps" are *not* ATPases. The net result is that electron transport has established a large out-to-in H^+ gradient across the mitochondrial inner membrane.

The F_oF_1 ATPase—which is complex V in the respiratory chain—can now use this large electrochemical potential energy difference for H^+. The H^+ ions then flow backward (i.e., down their electrochemical gradient) into the mitochondrion through the F_oF_1 ATPase, which generates ATP in the matrix space of the mitochondrion from ADP and inorganic phosphate. The entire process by which electron transport generates an H^+ gradient and the F_oF_1 ATPase harnesses this H^+ gradient to synthesize ATP is known as the chemiosmotic hypothesis. Peter Mitchell, who proposed this hypothesis in 1961, received the Nobel Prize in Chemistry for his work in 1978.

The precise stoichiometry is unknown but may be one ATP molecule synthesized for every three H^+ ions flowing downhill into the mitochondrion (one H^+ for each pair of $\alpha\beta$ subunits of F_1). If the H^+ gradient across the mitochondrial inner membrane reverses, the F_oF_1 ATPase will actually function as an ATPase and use the energy of ATP hydrolysis to pump H^+ out of the mitochondrion. Similar F_oF_1 ATPases are also present in bacteria and chloroplasts.



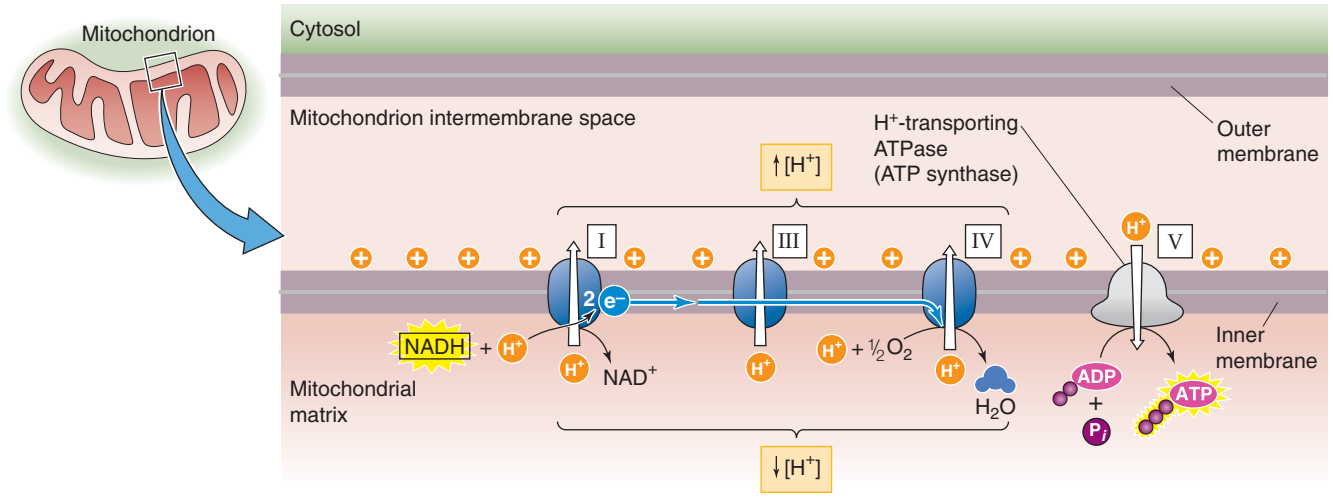


Figure 5-9 The F_oF_1 ATPase and its role as the ATP synthase in the mitochondrial synthesis of ATP. **A,** A cartoon of the F_oF_1 ATPase. The pump has two functional units, F_o (which historically stood for oligomycin-sensitive factor) and F_1 (which historically stood for factor 1). **F_o** is the transmembrane portion that contains the ion channel through which the H^+ passes. The **F_1** is the ATPase. In one complete cycle, the downhill movement of H^+ ions causes the c subunits of F_o and the axle formed by the subunits of F1 to rotate 360 degrees in three 120-degree steps, causing the α and β subunits to sequentially synthesize and release 3 ATP molecules, for a synthase stoichiometry of ~3 H^+ per ATP. However, the mitochondrion uses ~1 additional H^+ to import inorganic phosphate and to exchange cytosolic ADP for mitochondrial ATP. Thus, a total of ~4 H^+ would be needed per ATP. **B,** Complexes I, III, and IV of the respiratory chain use the energy of 1 NADH to pump H^+ out of the mitochondrial matrix; the consensus is 10 H^+ per NADH. The resulting H^+ gradient causes the mitochondrial F_oF_1 ATPase to run as an ATP synthase. Thus, the mitochondrion synthesizes (10 H^+/NADH) × (1 ATP/4 H^+) = 2.5 ATP/NADH. Similarly, the consensus is that complexes III and IV use the energy of 1 $FADH_2$ to pump 6 H^+ out of the mitochondrial matrix (not shown). Thus, the mitochondrion synthesizes (6 H^+/$FADH_2$) × (1 ATP/4 H^+) = 1.5 ATP/$FADH_2$.

V-type H^+ Pump The membranes surrounding such intracellular organelles as lysosomes, endosomes, secretory vesicles, storage vesicles, and the Golgi apparatus contain a so-called vacuolar-type (or V-type) H^+-ATPase that pumps H^+ from the cytoplasm to the interior of the organelles. The low pH generated inside these organelles is important for sorting proteins, dissociating ligands from receptors, optimizing the activity of acid hydrolases, and accumulating neurotransmitters in vesicles. The apical membranes of certain renal tubule cells as well as the plasma membranes of certain other cells also have V-type H^+ pumps that extrude H^+ from the cell. These V-type H^+ pumps, unlike the gastric H-K pump, are independent of K^+. Instead, the V-type H^+ pump is similar to the hand-held, lollipop-like structure of the F-type ATPase, with which it shares a significant—although low—level of amino acid homology. For example, the hand of the V-type pump has only six subunits, but each is twice as large as a c subunit in the F-type ATPase.

ATP-binding cassette (ABC) transporters can act as pumps, channels, or regulators

The so-called ABC proteins all have a motif in their amino acid sequence that is an ATP-binding cassette (ABC). In humans, this family includes at least 49 members in seven subfamiles named ABCA through ABCG (Table 5-6). Some are pumps that presumably hydrolyze ATP to provide energy for solute transport. Some may hydrolyze ATP, but they do not couple the liberated energy to perform active transport. In other cases, ATP regulates ABC proteins that function as ion channels or regulators of ion channels or transporters.

ABC1 Subfamily ABC1 (ABCA1) is an important transporter for mediating the efflux of phospholipids and cholesterol from macrophages and certain other cells.

MDR Subfamily The **multidrug resistance transporters (MDRs)** are ATPases and primary active transporters. The MDR proteins are tandem repeats of two structures, each of which has six membrane-spanning segments and a nucleotide-binding domain that binds ATP. MDR1, also called P-glycoprotein, extrudes cationic metabolites and drugs across the cell membrane. The substrates of MDR1 appear to have little in common structurally, except that they are hydrophobic. A wide variety of cells express MDRs, including those of the liver, kidney, and gastrointestinal tract. MDR1 plays an important and clinically antagonistic role in cancer patients in that it pumps a wide range of anticancer drugs out of cancer cells, thereby rendering cells resistant to these drugs.

MRP/CFTR Subfamily Another member of the ABC superfamily that is of physiological interest is the cystic fibrosis transmembrane regulator (CFTR), which is mutated in the hereditary disease **cystic fibrosis** (see the box on this topic in Chapter 43). CFTR is a 170-kDa glycoprotein that is present at the apical membrane of many epithelial cells. CFTR functions as a low-conductance Cl^- channel as well as a regulator of other ion channels.

Like MDR1, CFTR has two membrane-spanning domains (MSD1 and MSD2), each composed of six membrane-spanning segments (Fig. 5-10). Also like MDR1, CFTR has two nucleotide-binding domains (NBD1 and NBD2). Unlike MDR1, however, a large cytoplasmic regulatory (R) domain separates the two halves of CFTR. The regulatory domain contains multiple potential protein kinase A and protein kinase C phosphorylation sites. Phosphorylation of these sites, under the influence of neurohumoral agents that control fluid and electrolyte secretion, promotes activation of CFTR. The binding of ATP to the NBDs also controls channel opening and closing. Thus, ATP regulates the CFTR Cl^- channel by two types of mechanisms: protein

Table 5-6 ABC Transporters

Subfamily*	Alternative Subfamily Name	Examples
ABCA (12)	ABC1	ABCA1 (cholesterol transporter)
ABCB (11)	MDR (multidrug resistance)	ABCB1 (MDR1 or P-glycoprotein 1) ABCB4 (MDR2/3) ABCB11 (bile salt export pump, BSEP)
ABCC (13)	MRP/CFTR	ABCC2 (multidrug resistance–associated protein 2, MRP2) ABCC7 (cystic fibrosis transmembrane regulator, CFTR) ABCC8 (sulfonylurea receptor, SUR1) ABCC9 (SUR2)
ABCD (4)	ALD	ABCD1 (ALD, mediates uptake of fatty acids by peroxisomes)
ABCE (1)	OABP	ABCE1 (RNASELI, blocks RNase L)
ABCF (3)	GCN20	ABCF1 (lacks transmembrane domains)
ABCG (5)	White	ABCG2 (transports sulfated steroids) ABCG5/ABCG8 (heterodimer of "half" ABCs that transport cholesterol)

*Number of genes in parentheses.

phosphorylation and interaction with the nucleotide-binding domains.

Cotransporters, one class of secondary active transporters, are generally driven by the energy of the inwardly directed Na^+ gradient

Like pumps or primary active transporters, secondary active transporters can move a solute uphill (against its electrochemical gradient). However, unlike the pumps, which fuel the process by hydrolyzing ATP, the secondary active transporters fuel it by coupling the uphill movement of one or more solutes to the downhill movement of other solutes. The two major classes of secondary active transporters are cotransporters (or symporters) and exchangers (or antiporters). Cotransporters are intrinsic membrane proteins that move the "driving" solute (the one whose gradient provides the energy) and the "driven" solutes (which move uphill) in the *same* direction.

Na^+/Glucose Cotransporter The **Na^+/glucose cotransporter (SGLT)** is located at the apical membrane of the cells that line the proximal tubule and small intestine (Fig. 5-11A). The SGLTs, which belong to the SLC5 family (Table 5-4), consist of a single subunit, probably with 14 membrane-spanning segments. The SGLT2 and SGLT3 isoforms move one Na^+ ion with each glucose molecule (i.e., 1:1 stoichiometry of Na^+ to glucose), whereas the SGLT1 isoform moves two Na^+ ions with each glucose molecule.

For the Na^+/glucose cotransporter with 1:1 stoichiometry, the overall driving force is the sum of the electrochemical potential energy difference for Na^+ and the chemical potential energy difference for glucose. Thus, the highly favorable, inwardly directed Na^+ electrochemical gradient can drive the uphill accumulation of glucose from the lumen of the kidney tubule or gut into the cell. Figure 5-12 shows how the Na^+ gradient drives glucose accumulation into membrane vesicles derived from the brush border of renal proximal tubules. Equilibrium is achieved when the electrochemical potential energy difference for Na^+ in one direction is balanced by the chemical potential energy difference for glucose in the opposite direction:

$$\Delta\tilde{\mu}_{Na} = -\Delta\mu_{glucose} \qquad (5\text{-}17)$$

We can express $\Delta\tilde{\mu}_{Na}$ in terms of the Na^+ concentrations and membrane voltage and can express $\Delta\mu_{glucose}$ in terms of the glucose concentrations. If we substitute these expressions into Equation 5-17, we derive the following relationship for the maximal glucose concentration gradient that can be generated by a given electrochemical potential energy difference for Na^+:

$$\frac{[Glucose]_i}{[Glucose]_o} = \frac{[Na^+]_o}{[Na^+]_i} \times 10^{-V_m/(60mV)} \qquad (5\text{-}18)$$

In an epithelial cell that has a 10-fold Na^+ concentration gradient and a 60-mV inside-negative voltage across the apical membrane, the Na^+ electrochemical gradient can generate a 10×10^1, or 100-fold, glucose concentration gradient across the plasma membrane. In other words, the 10:1 Na^+ concentration gradient buys a 10-fold glucose gradient, and the V_m of −60 mV buys another 10-fold. However, the leakage of glucose out of the cell by other pathways at the basolateral membrane prevents the Na^+-glucose cotransporter from coming to equilibrium.

The Na^+/glucose cotransporter with 2:1 stoichiometry is capable of generating an even larger concentration gradient for glucose across the plasma membrane. Such a cotransporter would be in equilibrium when

$$2\Delta\tilde{\mu}_{Na} = -\Delta\mu_{glucose} \qquad (5\text{-}19)$$

The maximal glucose gradient is

$$\frac{[Glucose]_i}{[Glucose]_o} = \left(\frac{[Na^+]_o}{[Na^+]_i}\right)^2 \times 10^{-2V_m/(60mV)} \qquad (5\text{-}20)$$

In the same epithelial cell with a 10-fold Na^+ concentration gradient and a V_m of −60 mV, the Na^+ electrochemical gradient can generate a glucose concentration gradient of $10^2 \times 10^2$, or 10,000-fold! In other words, the 10:1 Na^+ concentration gradient—when squared for two Na^+ ions—buys a 100-fold glucose gradient, and the −60 mV membrane voltage—when multiplied by two for the effective charge on two Na^+ ions—buys another 100-fold.

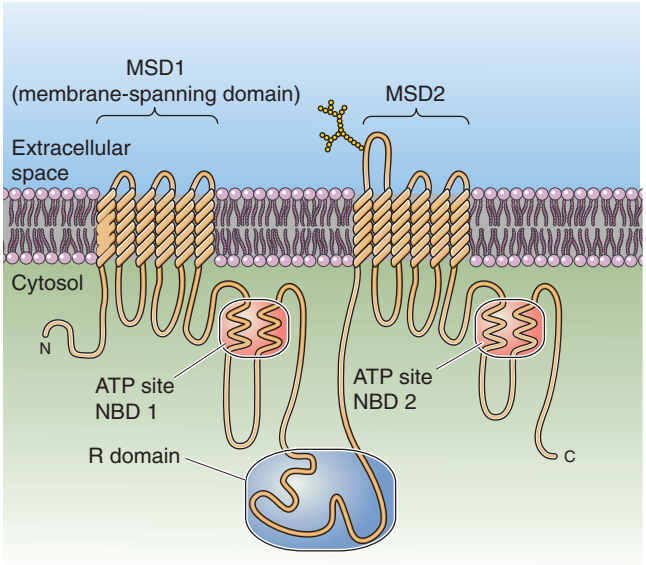


Figure 5-10 Cystic fibrosis transmembrane conductance regulator (CFTR). The CFTR Cl^- channel has two membrane-spanning domains (MSD1 and MSD2). A large cytoplasmic regulatory (R) domain separates the two halves of the molecule, each of which has an ATP-binding domain (NBD1 and NBD2). The most common mutation in cystic fibrosis is the deletion of the phenylalanine at position 508 (ΔF508) in the NBD1 domain. *(Model modified from Riordan JR, Rommens JM, Kerem B, et al: Identification of the cystic fibrosis gene: cloning and complementary DNA. Science 1989; 245: 1066-1073.)*

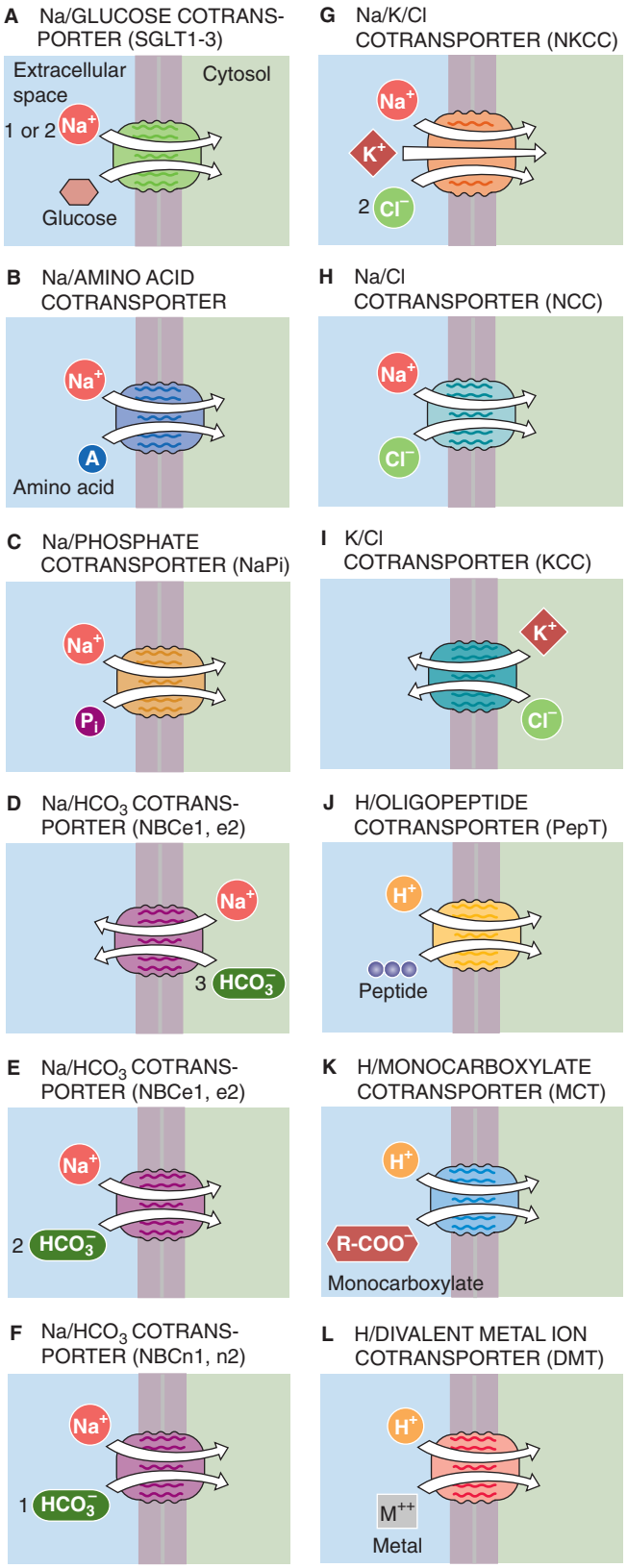


Figure 5-11 Representative cotransporters.

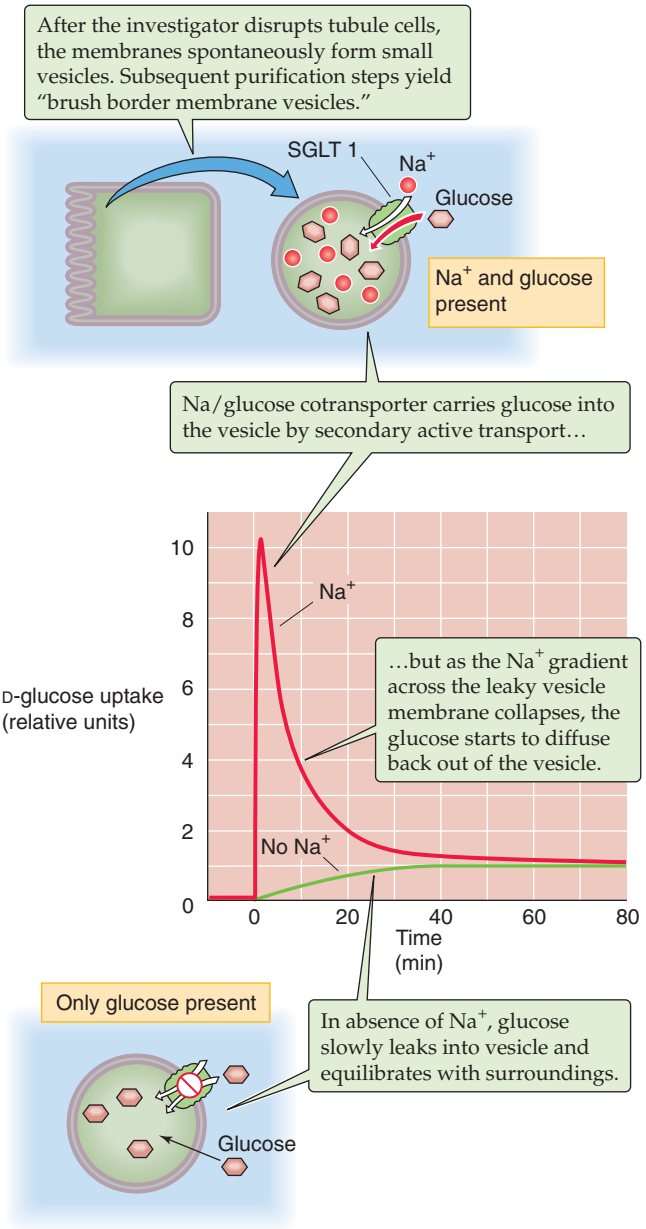


Figure 5-12 Na^+-driven glucose uptake into brush border membrane vesicles.

Because the cotransporter protein has specific sites for binding Na^+ and glucose and because the number of transporters is fixed, the rate of transport by SGLT is a saturable function of the glucose and Na^+ concentrations.

Na^+-Driven Cotransporters for Organic Solutes Functionally similar, but structurally distinct from one another, are a variety of Na^+ cotransporters in the proximal tubule and small intestine. **Na^+-driven amino acid transporters** (Fig. 5-11B) belong to both the SLC6 and SLC38 families (Table 5-4). SLC13 includes **Na^+-coupled cotransporters** for **monocarboxylates, dicarboxylates, and tricarboxylates**; SLC5 includes Na^+-coupled cotransporters for monocarboxylates.

Na/HCO_3 Cotransporters The **NBC**s belong to the SLC4 family and are a key group of acid-base transporters. In the basolateral membranes of certain epithelial cells, the **electrogenic NBCs** (NBCe1/e2, *e* for electro*genic*) operate with the Na^+:HCO_3^- stoichiometry of 1:3 (Fig. 5-11D) and—for typical ion and voltage gradients—mediate electrogenic HCO_3^- *ef*flux. Here, these NBCs mediate HCO_3^- absorption into the blood. In most other cells, these same two transporters operate with a stoichiometry of 1:2—probably because of the absence of a key protein partner—and mediate the electrogenic HCO_3^- *in*flux (Fig. 5-11E). Finally, the electroneutral NBCs (NBCn1/n2, *n* for electro*neutral*) operate with the Na^+:HCO_3^- stoichiometry of 1:1 (Fig. 5-11F) and also mediate HCO_3^- *in*flux. In these last two cases, the Na^+ electrochemical gradient drives the uphill accumulation of HCO_3^-, which is important for epithelial HCO_3^- secretion and for the regulation of intracellular pH (pH_i) to relatively alkaline values.

Na^+-Driven Cotransporters for Other Inorganic Anions Important examples include the **inorganic phosphate cotransporters** (**NaPi**; Fig. 5-11C)—which are members of the SLC17, SLC20, and SLC34 families—and sulfate cotransporter (SLC13) (Table 5-4).

Na/K/Cl Cotransporter The three types of cation-coupled Cl^- cotransporters all belong to the SLC12 family. The first is the **Na/K/Cl cotransporter** (**NKCC**), which harnesses the energy of the inwardly directed Na^+ electrochemical gradient to drive the accumulation of Cl^- and K^+ (Fig. 5-11G). One variant of this cotransporter, NKCC1 (SLC12A2), is present in a wide variety of nonepithelial cells as well as in the *basolateral* membranes of some epithelial cells. Another variant of the Na/K/Cl cotransporter, NKCC2 (SLC12A1), is present on the *apical* membrane of cells lining the thick ascending limb of the loop of Henle in the kidney (see Chapter 35). A characteristic of the NKCCs is that they are inhibited by furosemide and bumetanide, which are called loop diuretics because they increase urine flow by inhibiting transport at the loop of Henle. Because of its sensitivity to bumetanide, NKCC is sometimes called the bumetanide-sensitive cotransporter (BSC).

Na/Cl Cotransporter The second type of cation-coupled Cl^- cotransporter is found in the apical membrane of the early distal tubule of the kidney (see Chapter 35). This K^+-*independent* **Na/Cl cotransporter** (**NCC** or SLC12A3) is blocked by thiazide diuretics rather than by loop diuretics (Fig. 5-11H). For this reason, NCC has also been called the thiazide-sensitive cotransporter (TSC).

K/Cl Cotransporter The third type of cation-coupled Cl^- cotransporter is Na^+-*independent* **K/Cl cotransporter** (**KCC**1 to 4 or SLC12A4 to 7). Because the Na-K pump causes K^+ to accumulate inside the cell, the K^+ electrochemical gradient is outwardly directed across the plasma membrane (Fig. 5-11I). In addition, pathways such as the NKCC and the Cl-HCO_3 anion exchanger (see later) bring Cl^- into the cell, so that in most cells the Cl^- electrochemical gradient is also outwardly directed. Thus, the net driving force acting on the K/Cl cotransporter favors the exit of K^+ and Cl^- from the cell.

H⁺-Driven Cotransporters Although the majority of known cotransporters in animal cells are driven by the inward movement of Na^+, some are instead driven by the downhill, inward movement of H^+. The **H/oligopeptide cotransporter PepT1** and related proteins are members of the SLC15 family (Table 5-4). PepT1 is electrogenic and responsible for the uptake of small peptides (Fig. 5-11J) from the lumen into the cells of the renal proximal tubule and small intestine (see Chapters 36 and 45). The **H^+-driven amino acid cotransporters** (e.g., **PAT1**) are members of the SLC36 family. The **monocarboxylate cotransporters**, such as **MCT1**, are members of the SLC16 family. They mediate the electroneutral, H^+-coupled flux of lactate, pyruvate, or other monocarboxylates across the cell membranes of most tissues in the body (Fig. 5-11K). In the case of lactate, MCT1 can operate in either the net inward or net outward direction, depending on the lactate and H^+ gradients across the cell membrane. MCT1 probably moves lactate out of cells that produce lactate by glycolysis but into cells that consume lactate. The **divalent metal ion cotransporter (DMT1)**, a member of the SLC11 family, couples the influx of H^+ to the influx of ferrous iron (Fe^{2+}) as well as to a variety of other divalent metals, some of which (Cd^{2+}, Pb^{2+}) are toxic to cells (Fig. 5-11L). DMT1 is expressed at high levels in the kidney and proximal portions of the small intestine.

Exchangers, another class of secondary active transporters, exchange ions for one another

The other major class of secondary active transporters is the exchangers, or antiporters. Exchangers are intrinsic membrane proteins that move one or more "driving" solutes in one direction and one or more "driven" solutes in the *opposite* direction. In general, these transporters exchange cations for cations or anions for anions.

Na-Ca Exchanger The nearly ubiquitous **Na-Ca exchangers (NCX)** belong to the SLC8 family (Table 5-4). They most likely mediate the exchange of three Na^+ ions per Ca^{2+} ion (Fig. 5-13A). NCX is electrogenic and moves net positive charge in the same direction as Na^+. Under most circumstances, the inwardly directed Na^+ electrochemical gradient across the plasma membrane drives the uphill extrusion of Ca^{2+} from the cell. Thus, in concert with the plasma membrane Ca^{2+} pump, this transport system helps maintain the steep, inwardly directed electrochemical potential energy difference for Ca^{2+} that is normally present across the plasma membrane of all cells.

NCX uses the inwardly directed Na^+ electrochemical gradient to drive the secondary active efflux of Ca^{2+}. With a presumed stoichiometry of three Na^+ per Ca^{2+}, the effectiveness of the Na^+ electrochemical gradient as a driving force is magnified; thus, NCX is at equilibrium when the Ca^{2+} electrochemical gradient is balanced by three times the Na^+ electrochemical gradient:

$$\Delta\tilde{\mu}_{Ca} = 3\Delta\tilde{\mu}_{Na} \qquad (5\text{-}21)$$

Alternatively,

$$\frac{[Ca^{2+}]_o}{[Ca^{2+}]_i} = \left(\frac{[Na^+]_o}{[Na^+]_i}\right)^3 \cdot 10^{-V_m/60\,mV} \qquad (5\text{-}22)$$

In a cell with a 10-fold Na^+ concentration gradient and a V_m of −60 mV, the electrochemical potential energy difference for Na^+ can buy a Ca^{2+} concentration gradient of $10^3 \times 10^1$, or 10,000-fold, which is the Ca^{2+} gradient across most cell membranes. Thus, the effect of the 10-fold inward Na^+ concentration gradient is cubed and can account for a 10^3-fold Ca^{2+} concentration gradient across the plasma membrane. In addition, the stoichiometry of three Na^+ per Ca^{2+} produces a net inflow of one positive charge per transport cycle. Thus, the 60-mV inside-negative V_m acts as the equivalent driving force to another 10-fold concentration gradient.

Na-H Exchanger The **Na-H exchangers (NHE)**, which belong to the SLC9 family (Table 5-4), mediate the 1:1 exchange of extracellular Na^+ for intracellular H^+ across the plasma membrane (Fig. 5-13B). One or more of the nine known NHEs are present on the plasma membrane of almost every cell in the body. Through operation of NHEs, the inwardly directed Na^+ electrochemical gradient drives the uphill extrusion of H^+ from the cell and raises pH_i. The ubiquitous NHE1, which is present in nonepithelial cells as

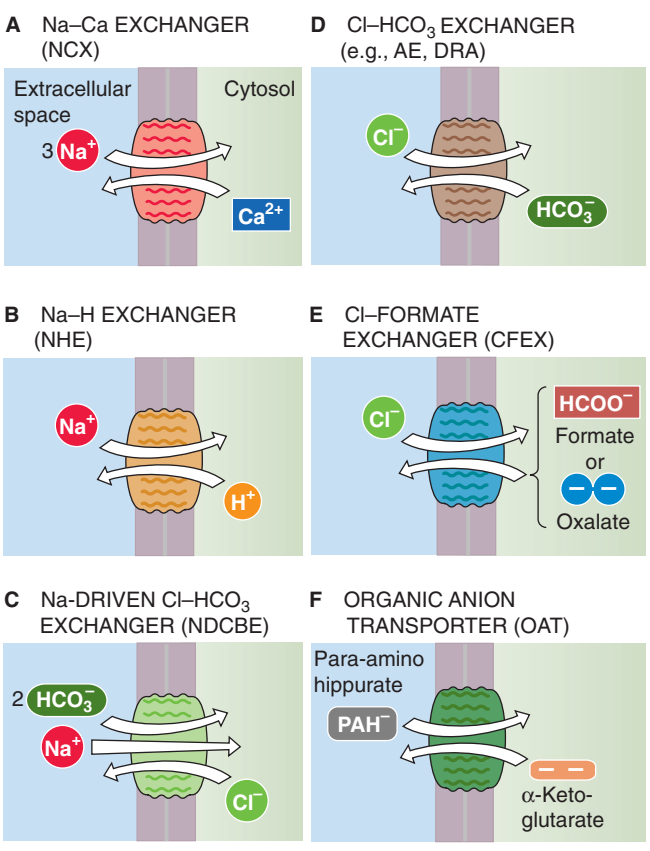


Figure 5-13 Representative exchangers.

well as on the basolateral membranes of epithelia, plays a major role in pH_i regulation and cell volume. NHE3 is present at the apical membranes of several epithelia, where it plays a major role in acid secretion (see Chapter 39) or Na^+ absorption.

Another cation exchange process that involves H^+ is the organic cation–H^+ exchanger that secretes cationic metabolites and drugs across the apical membrane of renal proximal tubule cells and hepatocytes.

Na^+-Driven Cl-HCO_3 Exchanger A second Na^+-coupled exchanger that is important for pH_i regulation is the **Na^+-driven Cl-HCO_3 exchanger (NDCBE)**, which is a member of the SLC4 family (Table 5-4). This electroneutral transporter couples the movement of one Na^+ ion and the equivalent of two HCO_3^- ions in one direction to the movement of one Cl^- ion in the opposite direction (Fig. 5-13C). NDCBE uses the inwardly directed Na^+ electrochemical gradient to drive the uphill entry of HCO_3^- into the cell. Thus, like the NHEs, NDCBE helps keep pH_i relatively alkaline.

Cl-HCO_3 Exchanger A third group of exchangers that are involved in acid-base transport are the Cl-HCO_3 exchangers (Fig. 5-13D) that function independently of Na^+. These may be members of either the SLC4 or the SLC26 families (Table 5-4). Virtually all cells in the body express one of the three electroneutral SLC4 of Cl-HCO_3 exchangers, also known as **anion exchangers (AE1–AE3)**. AE1 is important for transporting HCO_3^- into the red blood cell in the lung and out of the red blood cell in peripheral tissues (see Chapter 29). In other cells, where the inwardly directed Cl^- gradient almost always drives HCO_3^- out of the cell, AE2 and AE3 play important roles in pH_i regulation by tending to acidify the cell. Moreover, the uptake of Cl^- often plays a role in cell volume regulation.

Several members of the **SLC26 family** can function as Cl-HCO_3 exchangers and thereby play important roles in epithelial Cl^- and HCO_3^- transport. Because the stoichiometry need not be 1 : 1, SLC26 transport can be electrogenic. As described next, even SLC26 proteins that exchange Cl^- for HCO_3^- also transport a wide variety of *other* anions.

Other Anion Exchangers A characteristic of the SLC26 family is their multifunctionality. For example, **CFEX**—present in the apical membranes of renal proximal tubule cells—can mediate **Cl-formate exchange** and **Cl-oxalate exchange** (Fig. 5-13E). These activities appear to be important for the secondary active uptake of Cl^-. **Pendrin** not only mediates Cl-HCO_3 exchange but may also transport I^-, which may be important in the thyroid gland (see Chapter 49).

Anion exchangers other than those in the SLC4 and SLC26 families also play important roles. The **organic anion transporting polypeptides (OATP)** are members of the SLC21 family. In the liver, OATPs mediate the uptake of bile acids, bilirubin, and the test substrate bromosulphthalein. Another member of the SLC21 family is the **prostaglandin transporter (PGT)**, which mediates the uptake of prostanoids (e.g., prostaglandins E_2 and $F_{2\alpha}$ and thromboxane B_2).

The **organic anion transporters (OAT)** are members of the diverse SLC22 family. The OATs—by exchange or facilitated diffusion—mediate the uptake of endogenous organic anions (Fig. 5-13F) as well as drugs, including penicillin and the test substrate *p*-aminohippurate. **URAT1**, another SLC22 member, is an exchanger that mediates urate transport in the renal proximal tubule. Surprisingly, the OCT transporters that mediate the facilitated diffusion of organic *cations* are also members of SLC22.

REGULATION OF INTRACELLULAR ION CONCENTRATIONS

Figure 5-14 illustrates the tools at the disposal of a prototypic cell for managing its intracellular composition. Cells in different tissues—and even different cell types within the same tissue—have different complements of channels and transporters. Epithelial cells and neurons may segregate specific channels and transporters to different parts of the cell (e.g., apical versus basolateral membrane or axon versus soma/dendrite). Thus, different cells may have somewhat different intracellular ionic compositions.

The Na-K pump keeps $[Na^+]$ inside the cell low and $[K^+]$ high

The most striking and important gradients across the cell membrane are those for Na^+ and K^+. **Sodium** is the predominant cation in ECF, where it is present at a concentration of ~145 mM (Fig. 5-14). Na^+ is relatively excluded from the intracellular space, where it is present at only a fraction of the extracellular concentration. This Na^+ gradient is maintained primarily by active extrusion of Na^+ from the cell by the Na-K pump (Fig. 5-14, no. 1). In contrast, **potassium** is present at a concentration of only ~4.5 mM in ECF, but it is the predominant cation in the intracellular space, where it is accumulated ~25- to 30-fold above the outside concentration. Again, this gradient is the direct result of primary active uptake of K^+ into the cell by the Na-K pump. When the Na-K pump is inhibited with ouabain, $[Na^+]_i$ rises and $[K^+]_i$ falls.

In addition to generating concentration gradients for Na^+ and K^+, the Na-K pump plays an important role in generating the inside-negative membrane voltage, which is ~60 mV in a typical cell. The Na-K pump accomplishes this task in two ways. First, because the Na-K pump transports three Na^+ ions out of the cell for every two K^+ ions, the pump itself is **electrogenic**. This electrogenicity causes a net outward current of positive charge across the plasma membrane and tends to generate an inside-negative V_m. However, the pump current itself usually makes only a small contribution to the negative V_m. Second, and quantitatively much more important, the active K^+ accumulation by the Na-K pump creates a concentration gradient that favors the exit of K^+ from the cell through K^+ channels (Fig. 5-14, no. 2). The tendency of K^+ to *exit* through these channels, with unmatched negative charges left behind, is the main cause of the inside-negative membrane voltage. When K^+ channels are blocked with an inhibitor such as Ba^{2+}, V_m becomes considerably less negative (i.e., the cell depolarizes). In most cells, the principal pathway for current flow across the plasma membrane (i.e., the principal ionic conductance) is through K^+ channels. We discuss the generation of membrane voltage in Chapter 6.

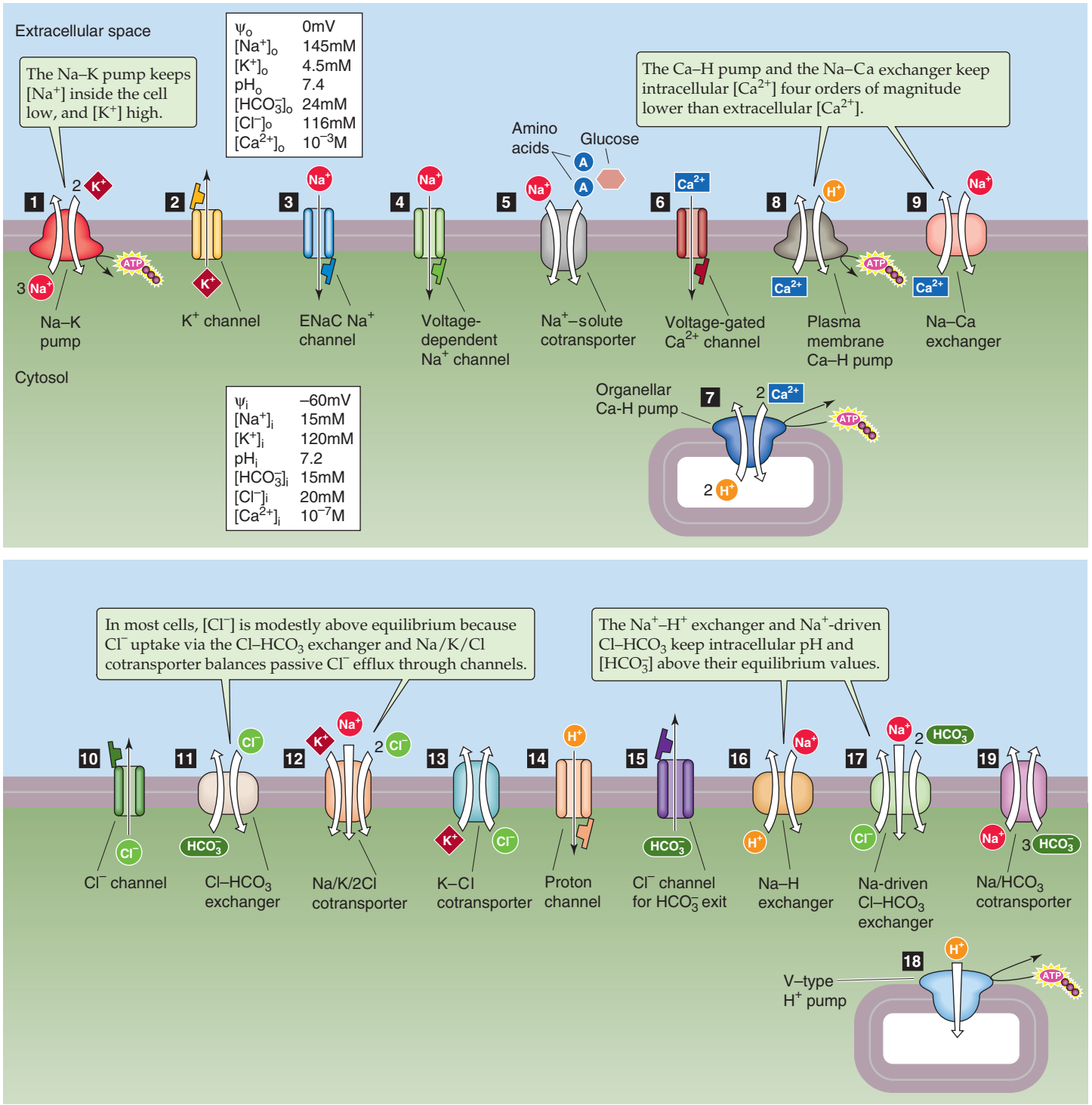


Figure 5-14 Ion gradients, channels, and transporters in a typical cell.

The inside-negative V_m, together with the large concentration gradient for Na^+, summates to create a large, inwardly directed Na^+ electrochemical gradient that strongly favors passive Na^+ entry. Given the large amount of energy that is devoted to generation of this favorable driving force for Na^+ entry, one might expect that the cell would permit Na^+ to move into the cell only through pathways serving important physiological purposes. The simple passive entry of Na^+ through channels—without harnessing of this Na^+ entry for some physiological purpose—would complete a futile cycle that culminates in active Na^+ extrusion. It would make little teleologic sense for the cell to use up considerable energy stores to extrude Na^+ only to let it passively diffuse back in with no effect. Rather, cells harness the energy of Na^+ entry for three major purposes:

1. In certain **epithelial cells**, amiloride-sensitive Na^+ channels (ENaC) are largely restricted to the *apical* or luminal surface of the cell (Fig. 5-14, no. 3), and the Na-K pumps are restricted to the *basolateral* surface of the cell. In this way, *transepithelial* Na^+ transport takes place rather than a futile cycling of Na^+ back and forth across a single plasma membrane.
2. In **excitable cells**, passive Na^+ entry occurs through voltage-dependent Na^+ channels (Fig. 5-14, no. 4) and plays a critical role in generation of the action potential. In such cells, Na^+ is cycled at high energy cost across the plasma membrane for the important physiological purpose of information transfer.
3. Virtually **every cell** in the body uses the electrochemical Na^+ gradient across the plasma membrane to drive the secondary active transport of nutrients and ions (Fig. 5-14, no. 5).

The Ca^{2+} pump and the Na-Ca exchanger keep intracellular $[Ca^{2+}]$ four orders of magnitude lower than extracellular $[Ca^{2+}]$

Whereas the concentration of Ca^{2+} in the extracellular space is ~1 mM (10^{-3} M), that in the ICF is only ~100 nM (10^{-7} M), a concentration gradient of 10^4-fold. Because of the inside-negative membrane voltage of a typical cell and the large chemical gradient for Ca^{2+}, the inwardly directed electrochemical gradient for Ca^{2+} across the plasma membrane is enormous, far larger than that for any other ion. Many cells have a variety of Ca^{2+} channels through which Ca^{2+} can enter the cell (Fig. 5-14, no. 6). In general, Ca^{2+} channels are gated by voltage (see Chapter 7) or by humoral agents (see Chapter 13) so that rapid Ca^{2+} entry into the cell occurs only in short bursts. However, given the existence of pathways for passive Ca^{2+} transport *into* cells, we may ask what transport mechanisms keep $[Ca^{2+}]_i$ low and thus maintain the enormous Ca^{2+} electrochemical gradient across the plasma membrane.

Ca^{2+} Pumps (SERCA) in Organelle Membranes Ca^{2+} pumps (ATPases) are present on the membranes that surround various intracellular organelles, such as the sarcoplasmic reticulum and endoplasmic reticulum (Fig. 5-14, no. 7). These pumps actively sequester cytosolic Ca^{2+} in intracellular stores. These stores of Ca^{2+} can later be released into the cytoplasm in bursts as part of a signal transduction process in response to membrane depolarization or humoral agents. Even though Ca^{2+} sequestration in intracellular stores is an important mechanism for regulating $[Ca^{2+}]_i$ in the short term, there is a limit to how much Ca^{2+} a cell can store. Therefore, in the steady state, Ca^{2+} extrusion across the cell membrane must balance the passive influx of Ca^{2+}.

Ca^{2+} Pump (PMCA) on the Plasma Membrane The plasma membranes of most cells contain a Ca^{2+} pump that plays a major role in extruding Ca^{2+} from the cell (Fig. 5-14, no. 8). It would seem that rising levels of intracellular Ca^{2+} would stimulate the Ca^{2+} pump to extrude Ca^{2+} and thereby return $[Ca^{2+}]_i$ toward normal. Actually, the pump *itself* is incapable of this type of feedback control; because it has such a high K_m for $[Ca^{2+}]_i$, the pump is virtually inactive at physiological $[Ca^{2+}]_i$. However, as $[Ca^{2+}]_i$ rises, the Ca^{2+} binds to a protein known as **calmodulin** (CaM, see Chapter 3), which has a high affinity for Ca^{2+}. The newly formed Ca^{2+}-CaM binds to the Ca^{2+} pump, lowers the pump's K_m for $[Ca^{2+}]_i$ into the physiological range, and thus stimulates Ca^{2+} extrusion. As $[Ca^{2+}]_i$ falls, Ca^{2+}-CaM levels inside the cell also fall so that Ca^{2+}-CaM dissociates from the Ca^{2+} pump, thereby returning the pump to its inactive state. At resting $[Ca^{2+}]_i$ levels of ~100 nM, the Ca^{2+} pump is the major route of Ca^{2+} extrusion.

Na-Ca Exchanger (NCX) on the Plasma Membrane NCX (Fig. 5-14, no. 9) plays a key role in extruding Ca^{2+} only when $[Ca^{2+}]_i$ rises substantially above normal levels. Thus, NCX is especially important in restoring low $[Ca^{2+}]_i$ when large influxes of Ca^{2+} occur. This property is most notable in excitable cells such as neurons and cardiac muscle, which may be challenged with vast Ca^{2+} influxes through voltage-gated Ca^{2+} channels during action potentials.

In most cells, $[Cl^-]$ is modestly above equilibrium because Cl^- uptake by the Cl-HCO_3 exchanger and Na/K/Cl cotransporter balances passive Cl^- efflux through channels

The $[Cl^-]$ in all cells is below the $[Cl^-]$ in the extracellular space. Virtually all cells have **anion-selective channels** (Fig. 5-14, no. 10) through which Cl^- can permeate passively. In a typical cell with a 60-mV inside-negative membrane voltage, $[Cl^-]_i$ would be a tenth that of $[Cl^-]_o$ if Cl^- were passively distributed across the plasma membrane. Such is the case for skeletal muscle. However, for most cell types, $[Cl^-]_i$ is approximately twice as high as that predicted for passive distribution alone, which indicates the presence of transport pathways that mediate the active uptake of Cl^- into the cell. Probably the most common pathway for Cl^- uptake is the **Cl-HCO_3 exchanger** (Fig. 5-14, no. 11). Because $[HCO_3^-]_i$ is several-fold higher than if it were passively distributed across the cell membrane, the outwardly directed electrochemical potential energy difference for HCO_3^- can act as a driving force for the uphill entry of Cl^- through Cl-HCO_3 exchange. Another pathway that can mediate uphill Cl^- transport into the cell is the **Na/K/Cl cotransporter** (Fig. 5-14, no. 12), which is stimulated by low $[Cl^-]_i$.

Given the presence of these transport pathways mediating Cl^- uptake, why is $[Cl^-]_i$ only ~2-fold above that predicted for passive distribution? The answer is that the passive Cl^- efflux through anion-selective channels in the plasma membrane opposes Cl^- uptake mechanisms. Another factor that tends to keep Cl^- low in some cells is the **K/Cl cotransporter.** KCC (Fig. 5-14, no. 13), driven by the outward K^+ gradient, tends to move K^+ and Cl^- out of cells. Thus, the K^+ gradient promotes Cl^- efflux both by generating the inside-negative V_m that drives Cl^- out of the cell through channels and by driving K/Cl cotransport.

The Na-H exchanger and Na^+-driven HCO_3^- transporters keep the intracellular pH and $[HCO_3^-]$ above their equilibrium values

H^+, HCO_3^-, and CO_2 within a particular compartment are generally in equilibrium with one another. Extracellular pH is normally ~7.4, $[HCO_3^-]_o$ is 24 mM, and P_{CO_2} is ~40 mm Hg. In a typical cell, intracellular pH is ~7.2. Because $[CO_2]$ is usually the same on both sides of the cell membrane, $[HCO_3^-]_i$ can be calculated to be ~15 mM. Even though the ICF is slightly more acidic than the ECF, pH_i is actually much more alkaline than it would be if H^+ and HCO_3^- were passively distributed across the cell membrane. H^+ can enter the cell passively and HCO_3^- can exit the cell passively, although both processes occur at a rather low rate. H^+ can permeate certain **cation channels** and perhaps H^+-selective channels (Fig. 5-14, no. 14), and HCO_3^- moves fairly easily through most **Cl^- channels** (Fig. 5-14, no. 15). Because a membrane voltage of −60 mV is equivalent as a driving force to a 10-fold concentration gradient of a monovalent ion, one would expect $[H^+]$ to be 10-fold higher within the cell than in the ECF, which corresponds to a pH_i that is 1 pH unit more acidic than pH_o. Similarly, one would expect $[HCO_3^-]_i$ to be only one tenth of $[HCO_3^-]_o$. The observation that pH_i and $[HCO_3^-]_i$ are maintained higher than predicted for passive distribution across the plasma membrane indicates that cells must actively extrude H^+ or take up HCO_3^-.

The transport of acid out of the cell or base into the cell is collectively termed acid extrusion. In most cells, the **acid extruders** are secondary active transporters that are energized by the electrochemical Na^+ gradient across the cell membrane. The most important acid extruders are the **Na-H** (Fig. 5-14, no. 16) and the **Na^+-driven Cl-HCO_3 exchangers** (Fig. 5-14, no. 17), as well as the **Na/HCO_3 cotransporters** with Na^+:HCO_3^- stoichiometries of 1:2 and 1:1. These transport systems are generally sensitive to changes in pH_i; they are stimulated when the cell is acidified and inhibited when the cell is alkalinized. Thus, these transporters maintain pH_i in a range that is optimal for cell functioning. Less commonly, certain epithelial cells that are specialized for acid secretion use **V-type H^+** pumps (Fig. 5-14, no. 18) or **H-K pumps** on their apical membranes to extrude acid. These epithelia include the renal collecting duct and the stomach. As noted earlier, virtually all cells have V-type H^+ pumps on the membranes surrounding such intracellular organelles as lysosomes, endosomes, and Golgi.

Because most cells have powerful acid extrusion systems, one might ask why the pH_i is not far more alkaline than ~7.2. Part of the answer is that transport processes that act as **acid loaders** balance acid extrusion. Passive leakage of H^+ and HCO_3^- through *channels,* as noted earlier, tends to acidify the cell. Cells also have *transporters* that generally move HCO_3^- out of cells. The most common is the Cl-HCO_3 exchanger (Fig. 5-14, no. 11). Another is the electrogenic NBC with the Na^+:HCO_3^- stoichiometry of 1:3 (Fig. 5-14, no. 19), which moves HCO_3^- out of the cell across the basolateral membrane of renal proximal tubules.

WATER TRANSPORT AND THE REGULATION OF CELL VOLUME

Water transport is driven by osmotic and hydrostatic pressure differences across membranes

Transport of water across biological membranes is always passive. No water pumps have ever been described. To a certain extent, single water molecules can dissolve in lipid bilayers and thus move across cell membranes at a low but finite rate by simple diffusion. The ease with which H_2O diffuses through the lipid bilayer depends on the lipid composition of the bilayer. Membranes with low fluidity (see Chapter 2), that is, those whose phospholipids have long saturated fatty acid chains with few double bonds (i.e., few kinks), exhibit lower H_2O permeability. The addition of other lipids that decrease fluidity (e.g., cholesterol) may further reduce H_2O permeability. Therefore, it is not surprising that the plasma membranes of many types of cells have specialized water channels—the aquaporins—that serve as passive conduits for water transport. The presence of aquaporins greatly increases membrane water permeability. In some cells, such as erythrocytes or the renal proximal tubule, AQP1 is always present in the membrane. The collecting duct cells of the kidney regulate the H_2O permeability of their apical membranes by inserting AQP2 water channels into their apical membranes under the control of arginine vasopressin.

Water transport across a membrane is always a linear, nonsaturable function of its net driving force. The direction of net passive transport of an uncharged solute is always down its chemical potential energy difference. For water, we must consider two passive driving forces. The first is the familiar chemical potential energy difference ($\Delta\mu_{H_2O}$), which depends on the difference in **water concentration** on the two sides of the membrane. The second is the energy difference, per mole of water, that results from the difference in **hydrostatic pressure** ($\Delta\mu_{H_2O,\ pressure}$) across the membrane. Thus, the relevant energy difference across the membrane is the sum of the chemical and pressure potential energy differences:

$$\Delta\mu_{H_2O,total} = \Delta\mu_{H_2O} + \Delta\mu_{H_2O,pressure}$$

$$\underbrace{\Delta\mu_{H_2O,total}}_{\text{Total energy difference}} = \underbrace{RT\ln\frac{[H_2O]_i}{[H_2O]_o}}_{\text{Chemical part}} + \underbrace{\bar{V}_W(P_i - P_o)}_{\text{Pressure part}} \qquad (5\text{-}23)$$

P is the hydrostatic pressure and $\bar{V}_w$ is the partial molar volume of water (i.e., volume occupied by 1 mole of water).

Because the product of pressure and volume is work, the second term in Equation 5-23 is work per mole. Dealing with water concentrations is cumbersome and imprecise because $[H_2O]$ is very high (i.e., ~56 M) and does not change substantially in the dilute solutions that physiologists are interested in. Therefore, it is more practical to work with the inverse of $[H_2O]$, namely, the concentration of osmotically active solutes, or **osmolality**. The units of osmolality are osmoles per kilogram H_2O, or Osm. In dilute solutions, the H_2O gradient across the cell membrane is roughly proportional to the difference in osmolalities across the membrane:

$$\ln\frac{[H_2O]_i}{[H_2O]_o} \cong \bar{V}_W(Osm_o - Osm_i) \qquad (5\text{-}24)$$

Osmolality is the total concentration of all osmotically active solutes in the indicated compartment (e.g., $Na^+ + Cl^- + K^+ + \ldots$). Substituting Equation 5-24 into Equation 5-23 yields a more useful expression for the total energy difference across the membrane:

$$\underbrace{\Delta\mu_{H_2O,total}}_{\frac{\text{Emergy}}{\text{mole}}} \cong \underbrace{\bar{V}_W}_{\frac{\text{Volume}}{\text{mole}}}\ \underbrace{[RT(Osm_o - Osm_i) + (P_i - P_o)]}_{\text{Pressure}} \qquad (5\text{-}25)$$

In this equation, the terms inside the brackets have the units of pressure (force/area) and thus describe the *driving force* for water movement from the inside to the outside of the cell. This driving force determines the flux of water across the membrane:

$$J_V = L_p[RT(Osm_o - Osm_i) + (P_i - P_o)] \qquad (5\text{-}26)$$

J_V is positive when water flows out of the cell and has the units liters/($cm^2 \cdot s$). The proportionality constant L_p is the **hydraulic conductivity**.

Water is in equilibrium across the membrane when the net driving force for water transport is nil. If we set $\Delta\mu_{H_2O,total}$ to zero in Equation 5-25:

$$RT(Osm_i - Osm_o) = (P_i - P_o)$$
$$\underbrace{(\pi_i - \pi_o)}_{\substack{\text{Osmotic pressure difference} \\ \Delta\pi}} = \underbrace{(P_i - P_o)}_{\substack{\text{Hydrostatic pressure difference} \\ \Delta P}} \qquad (5\text{-}27)$$

The term on the left is referred to as the **osmotic pressure difference** ($\Delta\pi$). Thus, at equilibrium, the osmotic pressure difference is equal to the hydrostatic pressure difference (ΔP). An osmotic pressure difference of 1 mosmol/kg H_2O (or 1 mOsm) is equivalent to a hydrostatic pressure difference of 19.3 mm Hg at normal body temperature.

The plasma membranes of animal cells are not so rigid (unlike the walls of plant cells) and cannot tolerate any significant hydrostatic pressure difference without deforming. Therefore, the hydrostatic pressure difference *across a cell membrane* is virtually always near zero and is therefore not a significant driving force for water transport.

Movement of water in and out of cells is driven by osmotic gradients only, that is, by differences in osmolality across the membrane. For example, if the osmolality is greater outside the cell than inside, water will flow out of the cell and the cell will shrink. Such a movement of water driven by osmotic gradients is called **osmosis**. Water is at equilibrium across *cell membranes* only when the osmolality inside and outside the cell is the same.

Hydrostatic pressure differences are an important driving force for driving fluid out across the *walls of capillaries* (see Chapter 20). Small solutes permeate freely across most capillaries. Thus, any difference in *osmotic* pressure as a result of these small solutes does not exert a driving force for water flow across that capillary. The situation is quite different for plasma proteins, which are too large to penetrate the capillary wall freely. As a result, the presence of a greater concentration of plasma proteins in the intravascular compartment than in interstitial fluid sets up a difference in osmotic pressure that tends to pull fluid back into the capillary. This difference is called the **colloid osmotic pressure** or **oncotic pressure**. Water is at equilibrium across the wall of a *capillary* when the colloid osmotic and hydrostatic pressure differences are equal. When the hydrostatic pressure difference exceeds the colloid osmotic pressure difference, the resulting movement of water out of the capillary is called **ultrafiltration**.

Because of the presence of impermeant, negatively charged proteins within the cell, Donnan forces will lead to cell swelling

NaCl, the most abundant salt in ECF, is largely excluded from the intracellular compartment by the direct and indirect actions of the Na-K pump. This relative exclusion of NaCl from the intracellular space is vital for maintaining normal cell water content (i.e., cell volume). In the absence of Na-K pumps, cells tend to swell even when both the intracellular and extracellular osmolalities are normal and identical. This statement may appear to contradict the principle that there can be no water flux without a difference in osmolality across the cell membrane (Equation 5-26). To understand this apparent paradox, consider a simplified model that illustrates the key role played by **negatively charged, impermeant macromolecules** (i.e., proteins) inside the cell (Fig. 5-15).

Imagine that a semipermeable membrane separates a left compartment (analogous to the *extra*cellular space) and a right compartment (analogous to the *intra*cellular space). The two compartments are rigid and have equal volumes throughout the experiment. The right compartment is fitted with a pressure gauge. The membrane is nondeformable and permeable to Na^+, Cl^-, and water, but it is not permeable to a negatively charged macromolecule (Y). For the sake of simplicity, assume that each Y carries 150 negative charges and is restricted to the intracellular solution. Figure 5-15A illustrates the ionic conditions at the beginning of the experiment. At this initial condition, the system is far out of equilibrium; although $[Na^+]$ is the same on both sides of the membrane, $[Cl^-]$ and $[Y^{-150}]$ have opposing concentration gradients of 150 mM.

What will happen now? The system will tend toward equilibrium. Cl^- will move down its concentration gradient into the cell. This entry of negatively charged particles will gener-

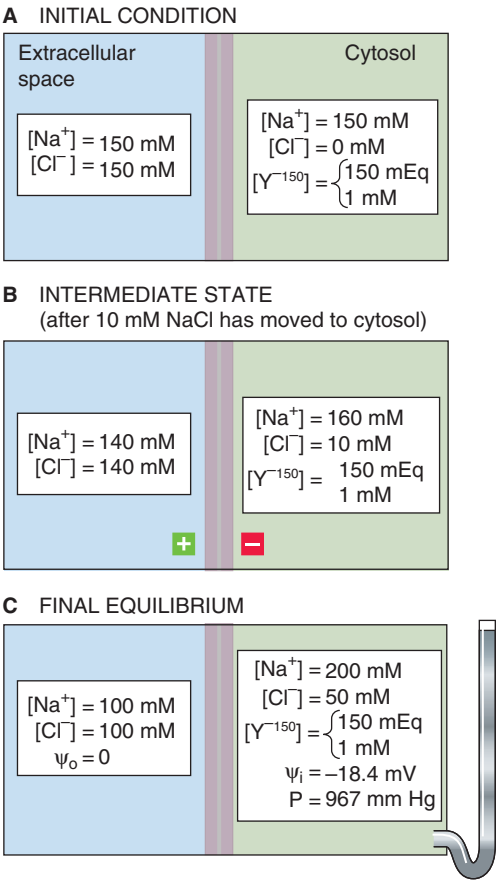


Figure 5-15 Gibbs-Donnan equilibrium. A semipermeable membrane separates two compartments that have rigid walls and equal volumes. The membrane is permeable to Na^+, Cl^-, and water but not to the macromolecule Y, which carries 150 negative charges. The calculations of ψ_i and P assume a temperature of 37°C.

ate an inside-negative membrane voltage, which in turn will attract Na^+ and cause Na^+ to move into the cell. In the final equilibrium condition, both Na^+ and Cl^- will be distributed so that the concentration of each is balanced against the same V_m, which is given by the Nernst equation (Equation 5-8):

$$V_m = -\frac{RT}{F}\ln\frac{[Na^+]_i}{[Na^+]_o} \tag{5-28}$$

$$V_m = -\frac{RT}{(-1)F}\ln\frac{[Cl^-]_i}{[Cl^-]_o} \tag{5-29}$$

Because V_m must be the same in the two cases, we combine the two equations, obtaining

$$-\ln\frac{[Na^+]_i}{[Na^+]_o} = \ln\frac{[Cl^-]_i}{[Cl^-]_o}$$
$$\frac{[Na^+]_o}{[Na^+]_i} = \frac{[Cl^-]_i}{[Cl^-]_o} = r \tag{5-30}$$

where r is the Donnan ratio because this equilibrium state is a **Gibbs-Donnan equilibrium** (often shortened to Donnan equilibrium). All the values for ionic concentrations in Equation 5-30 are *new* values. As Na^+ entered the cell, not only did $[Na^+]_i$ rise but $[Na^+]_o$ also fell, by identical amounts. The same is true for Cl^-. How much did the Na^+ and Cl^- concentrations have to change before the system achieved equilibrium? An important constraint on the system as it approaches equilibrium is that in each compartment, the total number of positive charges must balance the total number of negative charges (bulk electroneutrality) at all times. Imagine an *intermediate* state, between the initial condition and the final equilibrium state, in which 10 mM of Na^+ and 10 mM of Cl^- have moved into the cell (Fig. 5-15B). This condition is still far from equilibrium because the Na^+ ratio in Equation 5-30 is 0.875, whereas the Cl^- ratio is only 0.071; thus, these ratios are not equal. Therefore, Na^+ and Cl^- continue to move into the cell until the Na^+ ratio and the Cl^- ratio are both 0.5, the Donnan r ratio (Fig. 5-15C). This ratio corresponds to Nernst potentials of −18.4 mV for both Na^+ and Cl^-.

However, although the ions are in equilibrium, far more osmotically active particles are now on the inside than on the outside. Ignoring the osmotic effect of Y^{-150}, the sum of $[Na^+]$ and $[Cl^-]$ on the inside is 250 mM, whereas it is only 200 mM on the outside. Because of this 50-mOsm gradient (ΔOsm) across the membrane, water cannot be at equilibrium and will therefore move into the cell. In our example, the right (inside) compartment is surrounded by a rigid wall so that only a minuscule amount of water needs to enter the cell to generate a hydrostatic pressure of 967 mm Hg to oppose the additional net entry of water. This equilibrium hydrostatic pressure difference (ΔP) opposes the osmotic pressure difference ($\Delta\pi$):

$$\begin{aligned}\Delta P &= \Delta\pi \\ &= RT\Delta\text{Osm} \\ &= RT[([Na^+]_i + [Cl^-]_i) - ([Na^+]_o + [Cl^-]_o)] \\ &= RT(50\,\text{mM}) \\ &= 967\,\text{mm Hg} \\ &= 1.3\,\text{atm}\end{aligned} \tag{5-31}$$

Thus, in the rigid "cell" of our example, achieving Gibbs-Donnan equilibrium would require developing within the model cell a hydrostatic pressure that is 1.3 atm greater than the pressure in the left compartment (outside).

The Na-K pump maintains cell volume by doing osmotic work that counteracts the passive Donnan forces

Unlike in the preceding example, the plasma membranes of animal cells are not rigid but deformable, so that transmembrane hydrostatic pressure gradients cannot exist. Thus, in animal cells, the distribution of ions toward the Donnan equilibrium condition would, it appears, inevitably lead to progressive water entry, cell swelling, and ultimately bursting. Although the Donnan equilibrium model is artificial (e.g., ignoring all ions other than Na^+, Cl^-, and Y^{-150}), it nevertheless illustrates a point that is important for real cells: the negative charge on impermeant intracellular solutes (e.g., proteins and organic phosphates) will lead to bursting unless the cell does "osmotic work" to counteract the passive

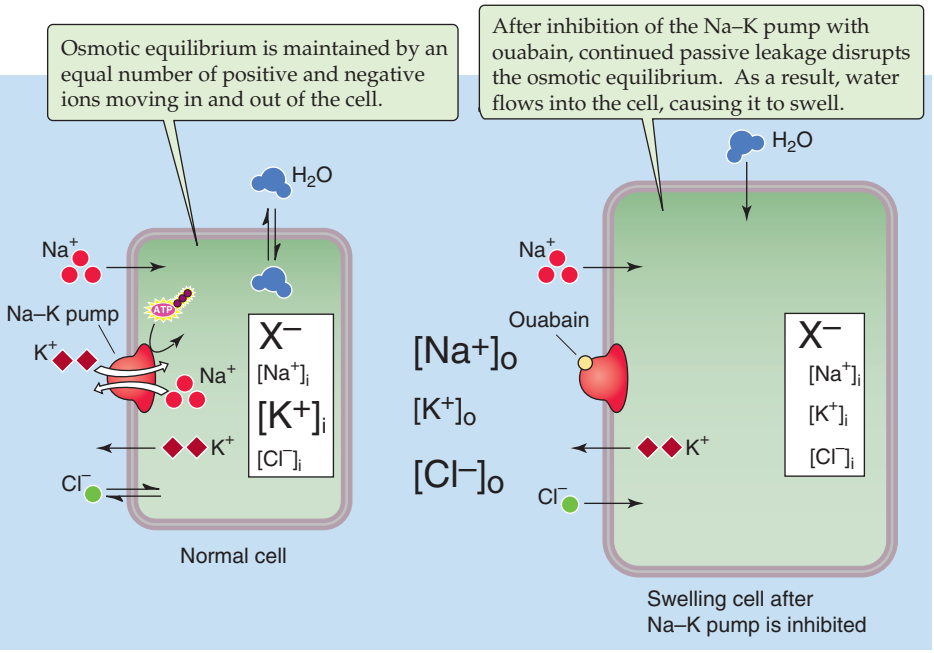


Figure 5-16 Role of the Na-K pump in maintaining cell volume.

Donnan-like swelling. The net effect of this osmotic work is to largely exclude NaCl from the cell and thereby make the cell functionally impermeable to NaCl. In a sense, NaCl acts as a functionally impermeant solute in the extracellular space that offsets the osmotic effects of intracellular negative charges. This state of affairs is not an equilibrium but a *steady state* maintained by active transport.

To illustrate the role of active transport, consider a somewhat more realistic model of a cell (Fig. 5-16). Under "normal" conditions, $[Na^+]_i$, $[K^+]_i$, and $[Cl^-]_i$ are constant because (1) the active extrusion of three Na^+ ions in exchange for two K^+ ions is balanced by the passive influx of three Na^+ ions and the passive efflux of two K^+ ions and (2) the net flux of Cl^- is zero (i.e., we assume that Cl^- is in equilibrium). When the Na-K pump is inhibited, the passive entry of three Na^+ ions exceeds the net passive efflux of two K^+ ions and thereby results in a gain of one intracellular cation and an immediate, small depolarization (i.e., cell becomes less negative inside). In addition, as intracellular $[K^+]$ slowly declines after inhibition of the Na-K pump, the cell depolarizes even further because the outward K^+ gradient is the predominant determinant of the membrane voltage. The inside-negative V_m is the driving force that is largely responsible for excluding Cl^- from the cell, and depolarization of the cell causes Cl^- to enter through anion channels. Cl^- influx results in the gain of one intracellular anion. The net gain of one intracellular cation and one anion increases the number of osmotically active particles and in so doing creates the inward osmotic gradient that leads to cell swelling. Thus, in the normal environment in which cells are bathed, the action of the Na-K pump is required to prevent the cell swelling that would otherwise occur.

A real cell, of course, is far more complex than the idealized cell in Figure 5-16, having myriad interrelated channels and transporters (Fig. 5-14). These other pathways, together with the Na-K pump, have the net effect of excluding NaCl and other solutes from the cell. Because the solute gradients that drive transport through these other pathways ultimately depend on the Na-K pump, inhibiting the Na-K pump will de-energize these other pathways and lead to cell swelling.

Cell volume changes trigger rapid changes in ion channels or transporters, returning volume toward normal

The joint efforts of the Na-K pump and other transport pathways are necessary for maintaining normal cell volume. What happens if cell volume is acutely challenged? A subset of "other pathways" respond to the cell volume change by transferring solutes across the membrane, thereby returning the volume toward normal.

Response to Cell Shrinkage If we increase extracellular osmolality by adding an impermeant solute such as mannitol (Fig. 5-17A), the extracellular solution becomes **hyperosmolal** and exerts an osmotic force that draws water out of the cell. The cell continues to shrink until the osmolality inside and out becomes the same. Many types of cells respond to this shrinkage by activating solute uptake processes to increase cell solute and water content. This response is known as a **regulatory volume increase (RVI)**. Depending on the cell type, cell shrinkage activates different types of solute uptake mechanisms. In many types of cells, shrinkage activates the ubiquitous NHE1 isoform of the *Na-H exchanger*. In addition to mediating increased uptake of Na^+, extrusion of H^+ alkalinizes the cell and consequently activates *Cl-HCO_3 exchange*. The net effect is thus the entry of Na^+ and Cl^-. The resulting increase in intracellular osmoles then draws water into the cell to restore cell volume toward normal. Alternatively, the RVI response may be mediated by activation of the NKCC1 isoform of the *Na/K/Cl cotransporter*.

Response to Cell Swelling If extracellular osmolality is decreased by the addition of water (Fig. 5-17B), the extracellular solution becomes **hypo-osmolal** and exerts a lesser

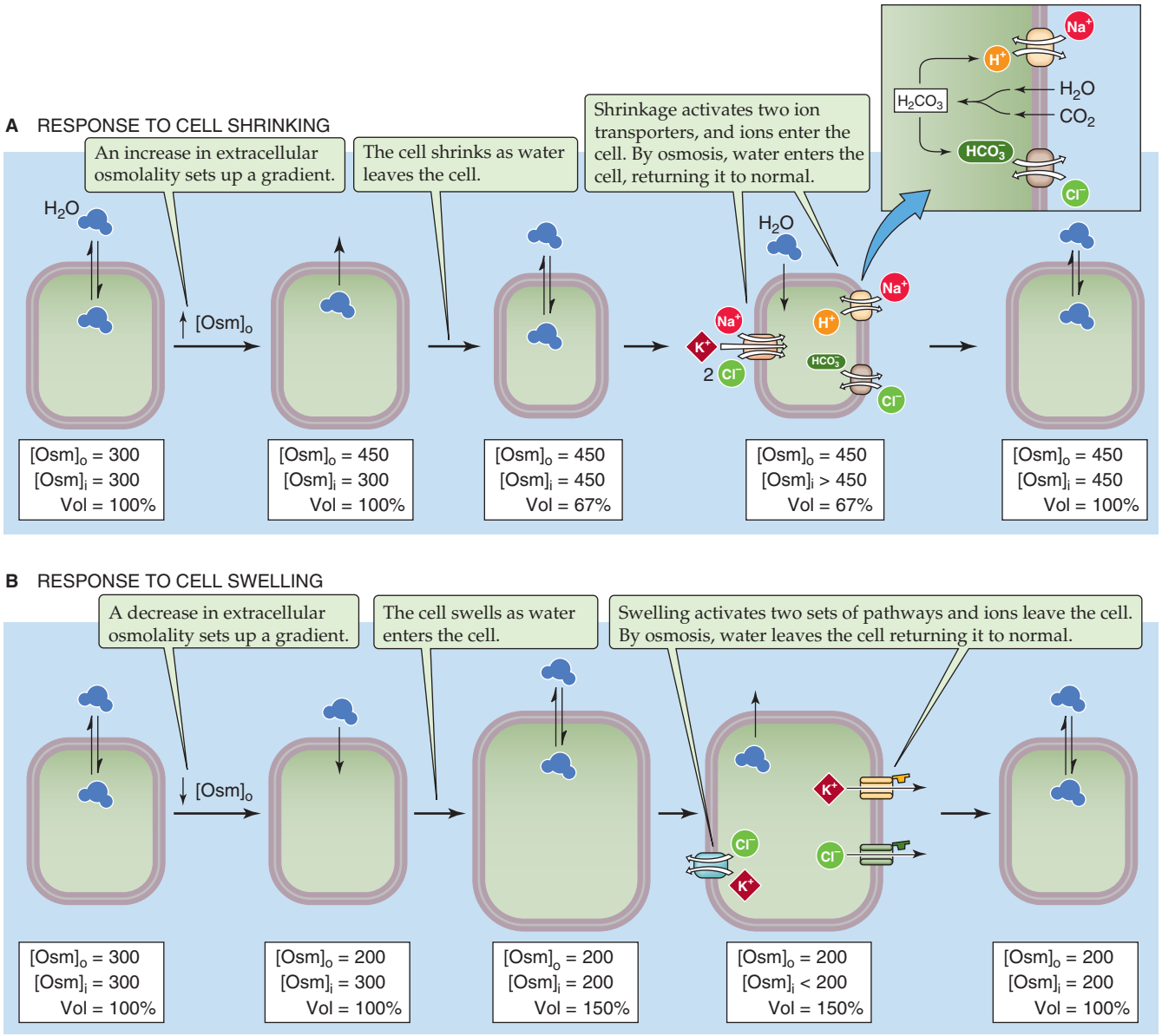


Figure 5-17 Short-term regulation of cell volume.

Disorders of Extracellular Osmolality

Regulatory adjustments in cell volume can be extremely important clinically. In major disorders of extracellular osmolality, the principal signs and symptoms arise from abnormal brain function, which can be fatal. For example, it is all too common for the elderly or infirm, unable to maintain proper fluid intake because of excessive heat or disability, to be brought to the emergency department in a state of severe dehydration. The hyperosmolality that results from dehydration can lead to brain shrinkage, which in extreme cases can cause intracerebral hemorrhage from tearing of blood vessels. If the brain cells compensate for this hyperosmolality by the long-term mechanisms discussed (e.g., manufacturing of idiogenic osmoles), cell shrinkage may be minimized. However, consider the consequence if an unsuspecting physician, unaware of the nuances of cell volume regulation, rapidly corrects the elevated extracellular hyperosmolality back down to normal. Rapid water entry into the brain cells will cause cerebral edema (i.e., brain swelling) and may result in death from herniation of the brainstem through the tentorium. For this reason, severe disturbances in ECF osmolality must usually be corrected slowly.

osmotic force so that water moves into the cell. The cell continues to swell until the osmolality inside and out becomes the same. Many cell types respond to this swelling by activating solute efflux pathways to decrease cell solute and water content and thereby return cell volume toward normal. This response is known as a **regulatory volume decrease (RVD)**. Depending on the cell type, swelling activates different types of solute efflux mechanisms. In many types of cells, swelling activates Cl^- or K^+ channels (or both). Because the electrochemical gradients for these two ions are generally directed outward across the plasma membrane, activating these channels causes a net efflux of K^+ and Cl^-, which lowers the intracellular solute content and causes water to flow out of the cell. The result is restoration of cell volume toward normal. Alternatively, the RVD response may be initiated by activating the *K/Cl cotransporter*.

In the normal steady state, the transport mechanisms that are responsible for RVI and RVD are usually not fully quiescent. Not only does cell shrinkage activate the transport pathways involved in RVI (i.e., solute loaders), it also appears to inhibit at least some of the transport pathways involved in RVD (i.e., solute extruders). The opposite is true of cell swelling. In all cases, it is the Na-K pump that ultimately generates the ion gradients that drive the movements of NaCl and KCl that regulate cell volume in response to changes in extracellular osmolality.

Cells respond to long-term hyperosmolality by accumulating new intracellular organic solutes

Whereas the acute response (seconds to minutes) to hyperosmolality (i.e., RVI) involves the uptake of salts, chronic adaptation (hours to days) to hyperosmolality involves accumulating organic solutes *(osmolytes)* within the cell. Examples of such intracellularly accumulated osmolytes include two relatively impermeant alcohol derivatives of common sugars (i.e., sorbitol and inositol) as well as two amines (betaine and taurine). Generation of organic solutes *(idiogenic osmoles)* within the cell plays a major role in raising intracellular osmolality and restoring cell volume during chronic adaptation to hyperosmolality—a response that is particularly true in brain cells. Sorbitol is produced from glucose by a reaction that is catalyzed by the enzyme **aldose reductase**. Cell shrinkage is a powerful stimulus for the synthesis of aldose reductase.

In addition to synthesizing organic solutes, cells can also transport them into the cytosol from the outside. For example, cells use distinct Na^+-coupled cotransport systems to accumulate inositol, betaine, and taurine. In some types of cells, shrinkage induces greatly enhanced expression of these transporters, thereby leading to the accumulation of these intracellular solutes.

The gradient in tonicity—or effective osmolality—determines the osmotic flow of water across a cell membrane

Total body water is distributed among blood plasma, the interstitial, intracellular, and transcellular fluids. The mechanisms by which water exchanges between interstitial fluid and ICF, and between interstitial fluid and plasma, rely on the principles that we have just discussed.

Water Exchange Across Cell Membranes Because cell membranes are not rigid, hydrostatic pressure differences never arise between cell water and interstitial fluid. Increasing the *hydrostatic* pressure in the interstitial space will cause the cell to compress so that the intracellular hydrostatic pressure increases to a similar extent. Thus, water does not enter the cell under these conditions. However, increasing the interstitial *osmotic* pressure, thus generating a $\Delta\pi$, is quite a different matter. If we suddenly increase ECF osmolality by adding an *impermeant* solute such as mannitol, the resulting osmotic gradient across the cell membrane causes water to move out of the cell. If the cell does not have an RVI mechanism or if the RVI mechanism is blocked, cell volume will remain reduced indefinitely.

On the other hand, consider what would happen if we suddenly increase ECF osmolality by adding a *permeant* solute such as urea. Urea can rapidly penetrate cell membranes by facilitated diffusion through members of the UT family of transporters; however, cells have no mechanism for extruding urea. Because urea penetrates the membrane more slowly than water does, the initial effect of applying urea is to shrink the cell (Fig. 5-18). However, as urea gradually equilibrates across the cell membrane and abolishes the initially imposed osmotic gradient, the cell reswells to its initial volume. Thus, sustained changes in cell volume do not occur with a change in the extracellular concentration of a permeant solute.

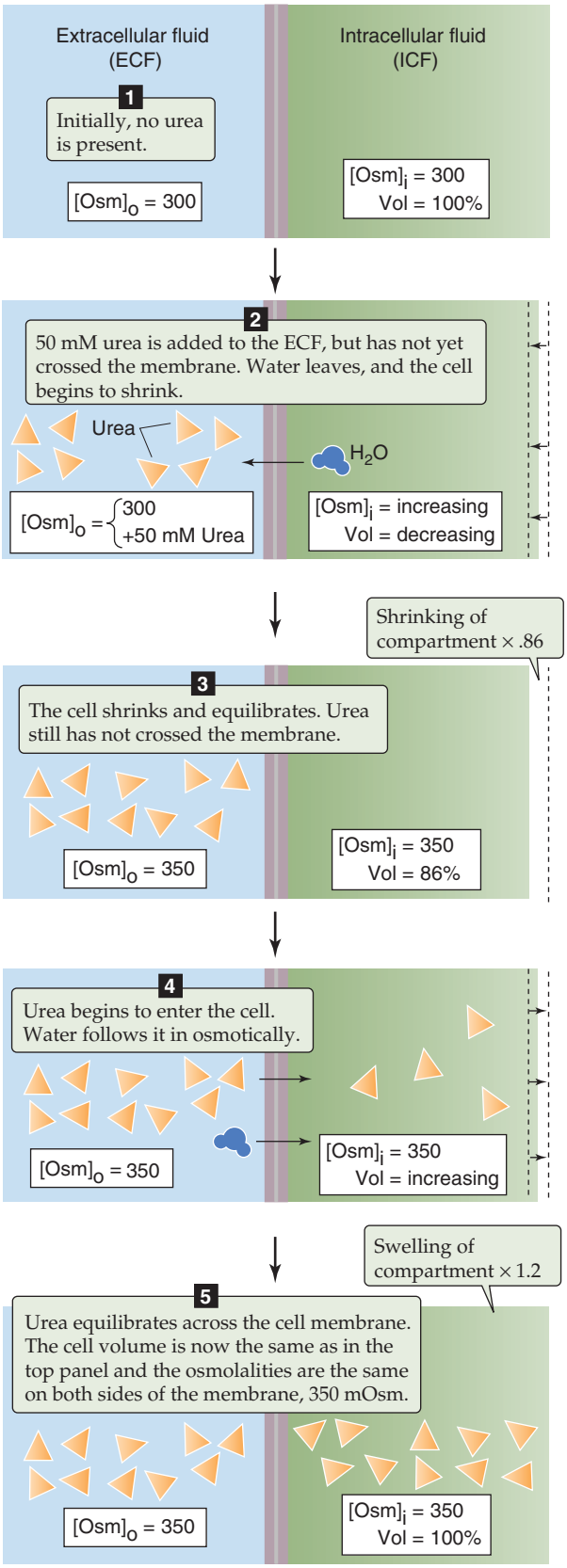


Figure 5-18 Effect of urea on the volume of a single cell bathed in an infinite volume of extracellular fluid. We assume that the cell membrane is permeable only to water during the initial moments in steps 2 and 3. Later, during steps 4 and 5, we assume that the membrane is permeable to both water and urea.

The difference between the effects of mannitol and urea on the final cell volume illustrates the need to distinguish between total osmolality and **effective osmolality** (also known as **tonicity**). In terms of clinically measured solutes, total and effective osmolality of the ECF can be approximated as

$$\text{Total osmolality (mOsm)} \cong 2\cdot[Na^+] + \frac{\text{Glucose (mg/dL)}}{18} + \frac{\text{BUN (mg/dL)}}{2.8}$$

$$\text{Tonicity or effective osmolality (mOsm)} \cong 2\cdot[Na^+] + \frac{\text{Glucose (mg/dL)}}{18} \quad (5\text{-}32)$$

BUN stands for blood urea nitrogen, that is, the concentration of the nitrogen that is contained in the plasma as urea. In Equation 5-32, the clinical laboratory reports $[Na^+]$ in milliequivalents per liter. Because the laboratory reports the glucose and BUN concentrations in terms of milligrams per deciliter, we divide glucose by one tenth of the molecular weight of glucose and BUN by one tenth of the summed atomic weights of the two nitrogen atoms in urea. The computed tonicity does not include BUN because—as we saw earlier—urea easily equilibrates across most cell membranes. On the other hand, the computed tonicity includes both Na^+ and glucose. It includes Na^+ because Na^+ is *functionally* impermeant owing to its extrusion by the Na-K pump. Tonicity includes glucose because this solute does not appreciably accumulate in most cells as a result of metabolism. In some clinical situations, the infusion of impermeant solutes, such as radiographic contrast agents or mannitol, can also contribute to tonicity of the ECF.

Osmolality describes the number of osmotically active solutes in a single solution. If we regard a plasma osmolality of 290 mOsm as being normal, solutions having an osmolality of 290 mOsm are **isosmolal**, solutions with osmolalities above 290 mOsm are hyperosmolal, and those with osmolalities below 290 mOsm are hypo-osmolal. On the other hand, when we use the terms *isotonic, hypertonic,* and *hypotonic,* we are comparing one solution with another solution (e.g., ICF) across a well-defined membrane (e.g., a cell membrane). A solution is **isotonic** when its *effective* osmolality is the same as that of the reference solution, which for our purposes is the ICF. A **hypertonic** solution is one that has a higher effective osmolality than the reference solution, and a **hypotonic** solution has a lower effective osmolality.

Shifts of water between the intracellular and interstitial compartments result from alterations in *effective* ECF osmolality, or *tonicity*. Clinically, such changes in tonicity are usually caused by decreases in $[Na^+]$ in the plasma and ECF (hyponatremia), increases in $[Na^+]$ (hypernatremia), or increases in glucose concentration (hyperglycemia). Changes in the concentration of a highly permeant solute such as urea, which accumulates in patients with kidney failure, have no effect on tonicity.

Water Exchange Across the Capillary Wall The barrier separating the blood plasma and interstitial compartments, the capillary wall, is—to a first approximation—freely permeable to solutes that are smaller than plasma proteins. Thus, the only *net* osmotic force that acts across the capillary

wall is that caused by the asymmetric distribution of proteins in plasma versus interstitial fluid. Several terms may be used for the osmotic force that is generated by these impermeant plasma proteins, such as protein osmotic pressure, colloid osmotic pressure, and oncotic pressure. These terms are synonymous and can be represented by the symbol $\pi_{oncotic}$. The **oncotic pressure difference** ($\Delta\pi_{oncotic}$), which tends to pull water from the interstitium to the plasma, is opposed by the hydrostatic pressure difference across the capillary wall (ΔP), which drives fluid from plasma into the interstitium. All net movements of water across the capillary wall are accompanied by the small solutes dissolved in this water, at their ECF concentrations; that is, the pathways taken by the water across the capillary wall are so large that small solutes are not *sieved* out.

To summarize, fluid shifts between plasma and the interstitium respond only to changes in the balance between ΔP and $\Delta\pi_{oncotic}$. Small solutes such as Na^+, which freely cross the capillary wall, do not contribute significantly to osmotic driving forces across this barrier and move along with the water in which they are dissolved. We will return to this subject when we discuss the physiology of capillaries in Chapter 20.

Adding isotonic saline, pure water, or pure NaCl to the ECF will increase ECF volume but will have divergent effects on ICF volume and ECF osmolality

Adding various combinations of NaCl and **solute-free water** to the ECF will alter the volume and composition of the body fluid compartments. Three examples illustrate the effects seen with intravenous therapy. In Figure 5-19A, we start with a total body water of 42 liters (60% of a 70-kg person), subdivided into an ICF volume of 25 liters (60% of total body water) and an ECF volume of 17 liters (40% of total body water). These numerical values are the same as those in Figure 5-1 and Table 5-1.

Infusion of Isotonic Saline Consider the case in which we infuse or ingest 1.5 liters of isotonic saline, which is a 0.9% solution of NaCl in water (Fig. 5-19B). This solution has an effective osmolality of 290 mOsm in the ECF. This 1.5 liters is initially distributed throughout the ECF and raises ECF volume by 1.5 liters. Because the effective osmolality of the ECF is unaltered, no change occurs in the effective osmotic gradient across the cell membranes, and the added water moves neither into nor out of the ICF. This outcome is, of course, in accord with the definition of an *isotonic* solution. Thus, we see that adding isotonic saline to the body is an efficient way to expand the ECF without affecting the ICF. Similarly, if it were possible to remove isotonic saline from the body, we would see that this measure would efficiently contract the ECF and again have no effect on the ICF.

Infusion of "Solute-Free" Water Now consider a case in which we either ingest 1.5 liters of pure water or infuse 1.5 liters of an isotonic (5%) glucose solution (Fig. 5-19C). Infusing the glucose solution intravenously is equivalent, in the long run, to infusing pure water because the glucose is metabolized to CO_2 and water, with no solutes left behind in the ECF. Infusing pure water would be unwise inasmuch as it would cause the cells near the point of infusion to burst.

How do the effects of adding 1.5 liters of pure water compare with those of the previous example? At first, the 1.5 liters of pure water will be rapidly distributed throughout the ECF and increase its volume from 17 to 18.5 liters (Fig. 5-19C, Early). This added water will also dilute the preexisting solutes in the ECF, thereby lowering ECF osmolality to 290 mOsm × 17/18.5 = 266 mOsm. Because intracellular osmolality remains at 290 mOsm at this imaginary, intermediate stage, a large osmotic gradient is created that favors the entry of water from the ECF into the ICF. Water will move into the ICF and consequently lower the osmolality of the ICF and simultaneously raise the osmolality of the ECF until osmotic equilibrium is restored (Fig. 5-19C, Final). Because the added water is distributed between the ICF and ECF according to the initial ICF/ECF ratio of 60%/40%, the final ECF volume is 17.6 liters (i.e., 17 liters expanded by 40% of 1.5 liters). Thus, infusion of solute-free water is a relatively *ineffective* means of expanding the ECF. More of the added water has ended up intracellularly (60% of 1.5 liters = 0.9 L of expansion). The major effect of the water has been to dilute the osmolality of body fluids. The initial total body solute content was 290 mOsm × 42 L = 12,180 milliosmoles. This same solute has now been diluted in 42 + 1.5 or 43.5 liters, so the final osmolality is 12,180/43.5 = 280 mOsm.

Ingestion of Pure NaCl Salt The preceding two "experiments" illustrate two extremely important principles that govern fluid and electrolyte homeostasis, namely, that adding or removing Na^+ will mainly affect ECF volume (Fig. 5-19B), whereas adding or removing solute-free water will mainly affect the *osmolality* of body fluids (Fig. 5-19C). The first point can be further appreciated by considering a third case, one in which we add the same amount of NaCl that is contained in 1.5 liters of isotonic (i.e., 0.9%) saline: 1.5 L × 290 mOsm = 435 mosmol. However, we will not add any water. At first, these 435 milliosmoles of NaCl will rapidly distribute throughout the 17 liters of ECF and increase the osmolality of the ECF (Fig. 5-19D, Early). The initial, total osmolal content of the ECF was 290 mOsm × 17 L = 4930 mosmol. Because we added 435 milliosmoles, we now have 5365 milliosmoles in the ECF. Thus, the ECF osmolality is 5365/17 = 316 mOsm. The resulting hyperosmolality draws water out of the ICF into the ECF until osmotic equilibrium is re-established. What is the final osmolality? The total number of milliosmoles dissolved in total body water is the original 12,180 milliosmoles plus the added 435 milliosmoles, for a total of 12,615 milliosmoles. Because these milliosmoles are dissolved in 42 liters of total body water, the final osmolality of the ICF and ECF is 12,615/42 = 300 mOsm. In the new equilibrium state, the ECF volume has increased by 0.9 liter even though no water at all was added to the body. Because the added ECF volume has come from the ICF, the ICF shrinks by 0.9 liter. This example further illustrates the principle that the total body content of Na^+ is the major determinant of ECF volume.

Whole-body Na^+ content determines ECF volume, whereas whole-body water content determines osmolality

Changes in ECF volume are important because they are accompanied by proportional changes in the volume of

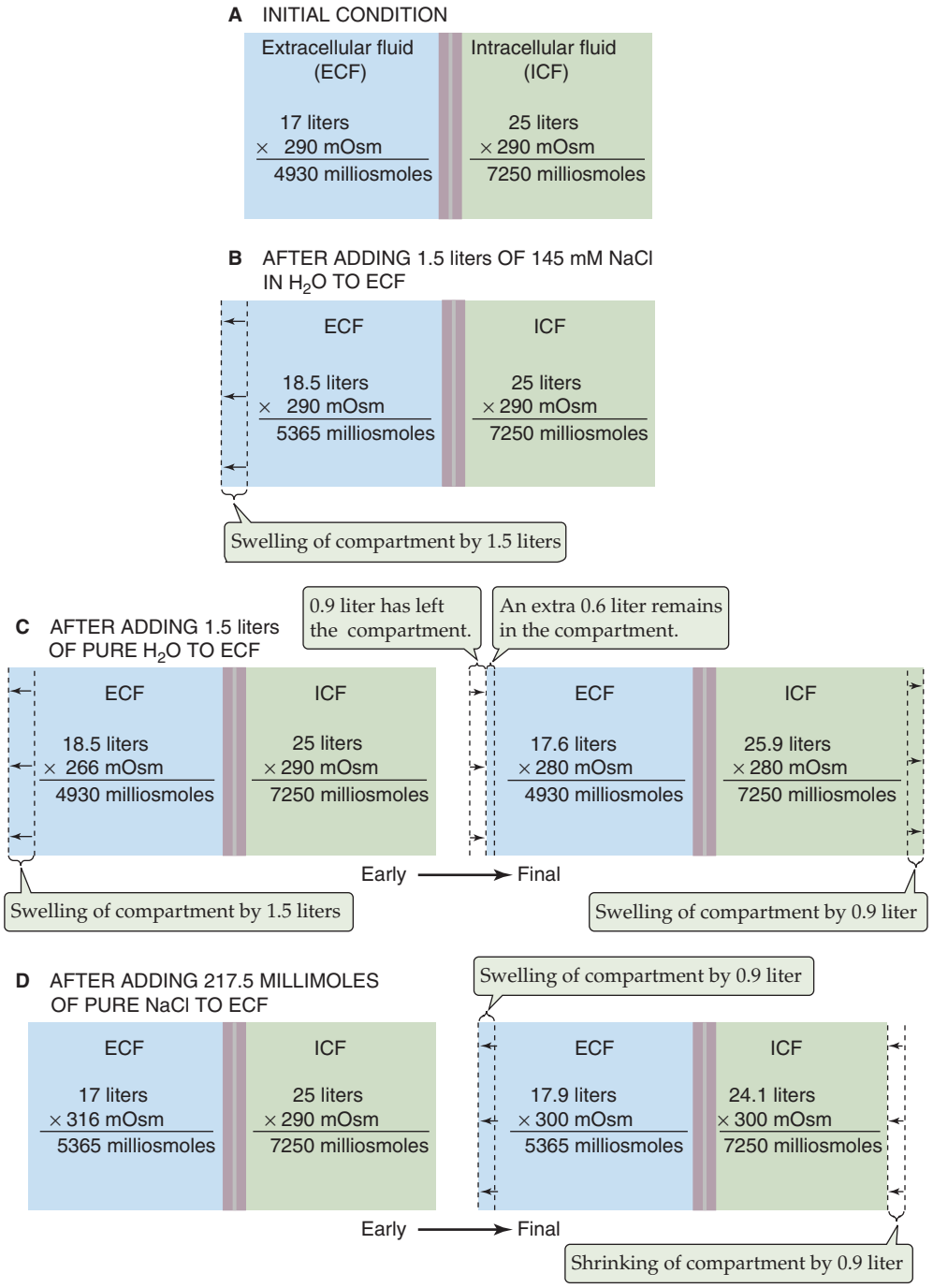


Figure 5-19 Effect on body fluid compartments of infusing different solutions.

blood plasma, which in turn affects the adequacy with which the circulatory system can perfuse vital organs with blood. The blood volume that is necessary to achieve adequate perfusion of key organs is sometimes referred to as the **effective circulating volume**. Because the body generally stabilizes osmolality, an increase in extracellular Na^+ content will increase ECF volume:

$$\underbrace{\text{Extracellular } Na^+ \text{ content}}_{\text{millimoles}} = \underbrace{[Na^+]_o}_{\text{millimoles/liter}} \times \underbrace{\text{ECF volume}}_{\text{liters}}$$
$$\cong \left(\frac{\text{Osmolality}}{2}\right) \times \underbrace{\text{ECF volume}}_{\text{liters}} \quad (5\text{-}33)$$

Because cells contain very little Na^+, extracellular Na^+ content is nearly the same as **total body Na^+** content.

We will see in Chapter 40 how the body regulates effective circulating volume; increases in effective circulating volume, which reflect increases in ECF volume or total body Na^+ *content*, stimulate the renal excretion of Na^+. In contrast, the plasma Na^+ *concentration* does *not* regulate renal excretion of Na^+. It makes sense that regulation of Na^+ excretion is not sensitive to the plasma Na^+ concentration because the concentration is not an indicator of ECF volume.

As discussed, when we hold osmolality constant, Na^+ content determines ECF volume. What would happen if we held constant the Na^+ content, which is a major part of total body osmoles? An increase in **total body water** would decrease **osmolality**.

$$\underbrace{\text{Total body osmoles}}_{\text{milliosmoles}} = \underbrace{[\text{Osmolality}]}_{\text{milliosmoles/liter}} \times \underbrace{\text{Total body water}}_{\text{liters}} \quad (5\text{-}34)$$

Thus, a net gain or loss of solute-free water has a major impact on the *osmolality* and $[Na^+]$ of the ECF. Moreover, because a large part (~60%) of the added solute-free water distributes into the ICF, a gain or loss of solute-free water affects ICF more than ECF. We will see in Chapter 40 how the body regulates osmolality; a small decrease in osmolality triggers osmoreceptors to diminish thirst (resulting in diminished intake of solute-free water) and increase renal water excretion. In emergency states of very low ECF and effective circulating volume, some crosstalk occurs between the volume and osmolality control systems. As a result, the body not only will try to conserve Na^+ but will also seek water (by triggering thirst) and conserve water (by concentrating the urine). Although water (in comparison to saline) is not a very good expander of plasma and ECF volume, it is better than nothing.

TRANSPORT OF SOLUTES AND WATER ACROSS EPITHELIA

Thus far we have examined how cells transport solutes and water across their membranes and thereby control their intracellular composition. We now turn our attention to how the body controls the milieu intérieur, namely, the ECF that bathes the cells. Just as the cell membrane is the barrier between the ICF and ECF, epithelia are the barriers that separate the ECF from the outside world. In this subchapter, we examine the fundamental principles of how epithelial cells transport solutes and water across epithelial barriers.

An epithelium is an uninterrupted sheet of cells that are joined together by junctional complexes (see Chapter 2). These junctions serve as a selectively permeable barrier between the solutions on either side of the epithelium and demarcate the boundary between the apical and basolateral regions of the cell membrane. The apical and basolateral membranes are remarkably different in their transport function. This polarization allows the epithelial cell to transport water and selected solutes from one compartment to another. In other words, the epithelium is capable of vectorial transport. In many cases, transport of solutes across an epithelium is an active process.

Membranes may be called by different names in different epithelia. The **apical membrane** can be known as the brush border, the mucosal membrane, or the luminal membrane. The **basolateral membrane** is also known as the serosal or peritubular membrane.

The epithelial cell generally has different electrochemical gradients across its apical and basolateral membranes

Imagine an artificial situation in which an epithelium separates two identical solutions. Furthermore, imagine that there is no difference in voltage across the epithelium and no difference in hydrostatic pressure. Under these circumstances, the driving forces for the *passive movement* of solutes or water across the epithelium would be nil. Because the apical and basolateral membranes of the cell share the same cytosol, the electrochemical gradients across the apical and basolateral membranes would be identical.

However, this example is virtually never realistic for two reasons. First, because the composition of the "outside world" is not the same as that of ECF, transepithelial concentration differences occur. Second, transepithelial voltage is not zero. Thus, the electrochemical gradients across the apical and basolateral membranes of an epithelial cell are generally very different.

Electrophysiological methods provide two major types of information about ion transport by epithelial cells. First, electrophysiological techniques can define the electrical driving forces that act on ions either across the entire epithelium or across the individual apical and basolateral cell membranes. Second, these electrical measurements can define the overall electrical resistance of the epithelium or the electrical resistance of the individual apical and basolateral cell membranes.

The voltage difference between the solutions on either side of the epithelium is the **transepithelial voltage** (V_{te}). We can measure V_{te} by placing one microelectrode in the lumen of the organ or duct of which the epithelium is the wall and a second reference electrode in the blood or interstitial space (Fig. 5-20A). If we instead insert the first microelectrode directly into an epithelial cell (Fig. 5-20A), the voltage difference between this cell and the reference electrode in blood or the interstitial space measures the **basolateral cell membrane voltage** (V_{bl}). Finally, if we compare the intracellular electrode with a reference electrode in the lumen (Fig. 5-20A), the voltage difference is the **apical cell membrane voltage** (V_a). Obviously, the sum of V_a and V_{bl} is equal to the transepithelial voltage (Fig. 5-20B). It is also possible to insert ion-sensitive microelectrodes into the lumen or the epithelial cells and thereby determine the local activity of ions such as Na^+, K^+, H^+, Ca^{2+}, and Cl^-.

By using the same voltage electrodes that we introduced in the preceding paragraph, we can pass electrical current across either the whole epithelium or the individual apical and basolateral membranes. From Ohm's law, it is thus possible to calculate the **electrical resistance** of the entire wall of the epithelium, or transepithelial resistance (R_{te}); that of the apical membrane, or apical resistance (R_a); or that of the basolateral membrane, or basolateral resistance (R_{bl}).

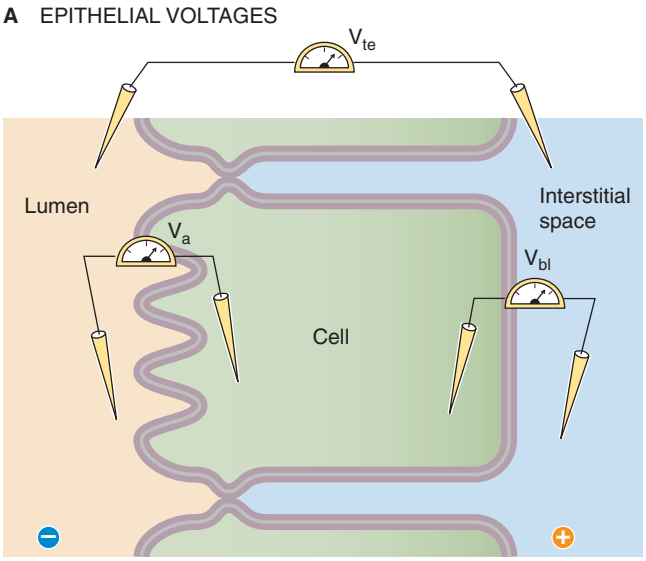


Figure 5-20 Measurement of voltages in an epithelium. **A,** The transepithelial voltage difference between electrodes placed in the lumen and interstitial space (or blood) is V_{te}. The basolateral voltage difference between electrodes placed in the cell and interstitial space is V_{bl}. The apical voltage difference between electrodes placed in the lumen and cell is V_a. **B,** Relative to the reference voltage of zero in the interstitial space, the voltage inside the cell in this example is –70 mV, and the voltage in the lumen is –3 mV. These values are typical of a cell in the renal proximal tubule or a small intestine.

Tight and leaky epithelia differ in the permeabilities of their tight junctions

One measure of how tightly an epithelium separates one compartment from another is its resistance to the flow of electrical current. The range of transepithelial electrical resistance is quite large. For example, 1 cm^2 of a rat proximal tubule has a resistance of only 6 Ω, whereas 1 cm^2 of a rabbit urinary bladder has a resistance of 70,000 Ω. Why is the range of R_{te} values so great? The cells of these epithelia do not differ greatly in either their apical or basolateral membrane resistances. Instead, the epithelia with low electrical resistances have a low-resistance pathway located in their tight junctions. Epithelia are thus classified as either "tight" (high electrical resistance) or "leaky," depending on the relative resistance of their tight junctions. In other words, the tight junctions of leaky epithelia are relatively more permeant to the diffusion of ions than the tight junctions of tight epithelia.

Now that we have introduced the concept that solutes and water can move between epithelial cells through tight junctions, we can define two distinct pathways by which substances can cross epithelia. First, a substance can cross through the cell by sequentially passing across the apical and then the basolateral membranes, or vice versa. This route is called the **transcellular pathway**. Second, a substance can bypass the cell entirely and cross the epithelium through the tight junctions and lateral intercellular spaces. This route is called the **paracellular pathway**.

As might be expected, leaky epithelia are not so good at maintaining large transepithelial ion or osmotic gradients. In general, **leaky epithelia** perform bulk transepithelial transport of solutes and water in a nearly isosmotic fashion (i.e., the transported fluid has about the same osmolality as the fluid from which it came). Examples include the small intestine and the proximal tubule of the kidney. As a general rule, **tight epithelia** generate or maintain large transepithelial ion concentration or osmotic gradients. Examples include the distal nephron of the kidney, the large intestine, and the tightest of all epithelia, the urinary bladder (whose function is to be an absolutely impermeable storage vessel).

In addition to tight junctions, epithelia share a number of basic properties First, the Na-K pump is located exclusively on the basolateral membrane (Fig. 5-21). The only known exception is the choroid plexus, where the Na-K pump is located on the apical membrane.

Second, most of the K^+ that is taken up by the Na^+ pump generally recycles back out across the basolateral membrane through K^+ channels (Fig. 5-21). A consequence of the abundance of these K^+ channels is that the K^+ gradient predominantly determines V_{bl}, which is usually 50 to 60 mV, inside negative.

Third, as in other cells, $[Na^+]_i$, typically 10 to 30 mM, is much lower in an epithelial cell than in the ECF. This low $[Na^+]_i$ is a consequence of the active extrusion of Na^+ by the Na-K pump. The large, inwardly directed Na^+ electrochemical gradient serves as a driving force for Na^+ entry through apical Na^+ channels and for the secondary active transport of other solutes across the apical membrane (e.g., by Na^+/glucose cotransport, Na-H exchange, Na/K/Cl cotransport) or basolateral membrane (e.g., by Na-Ca exchange).

Epithelial cells can absorb or secrete different solutes by inserting specific channels or transporters at either the apical or basolateral membrane

By placing different transporters at the apical and basolateral membranes, epithelia can accomplish net transepithelial transport of different solutes in either the absorptive or secretory direction. For example, the renal proximal tubule moves glucose from the tubule lumen to the blood by using the Na^+/glucose cotransporter (SGLT) to move glucose into

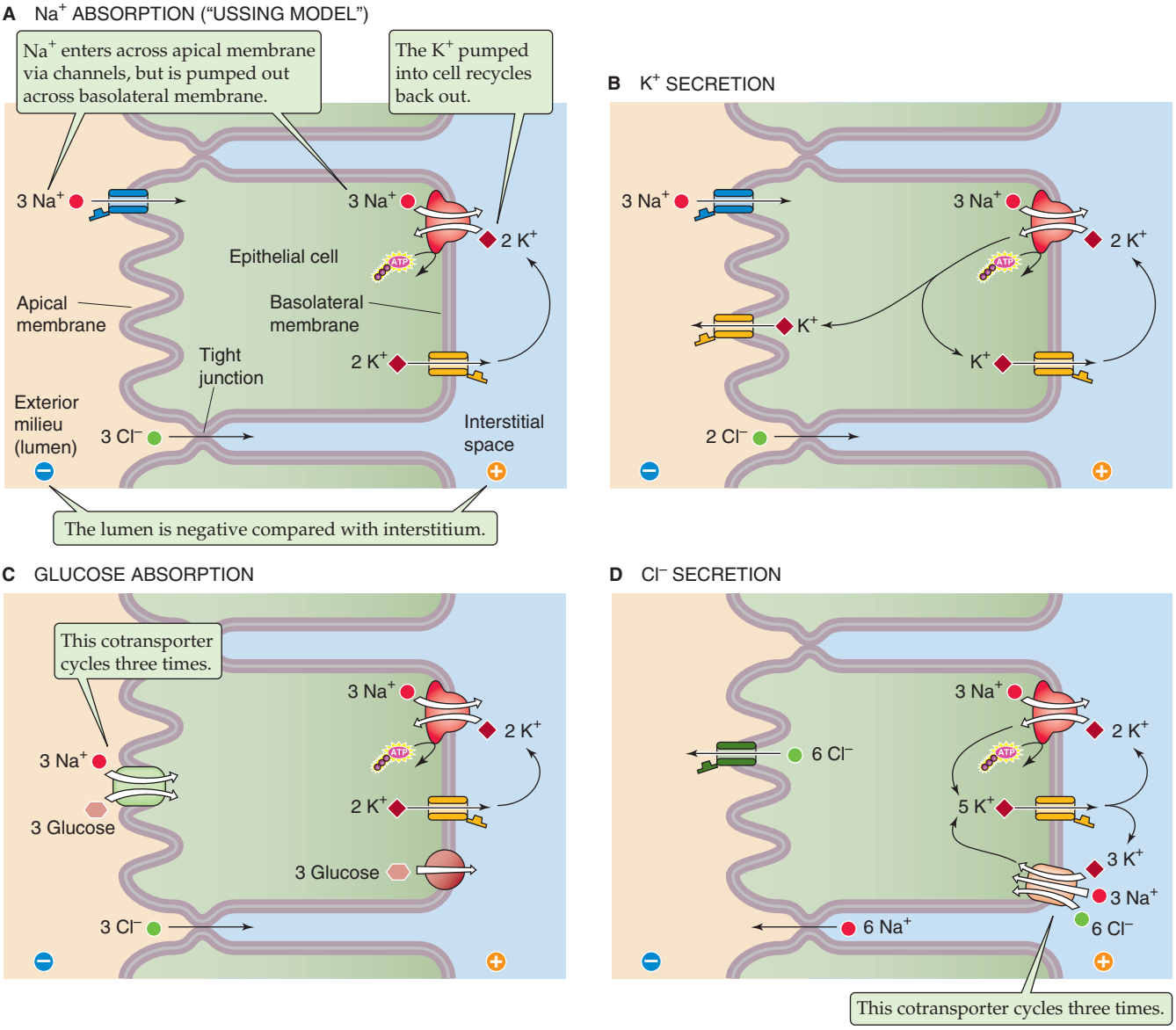


Figure 5-21 Models of epithelial solute transport.

the cell across the apical membrane, but it uses facilitated diffusion of glucose (GLUT) to move glucose out of the cell across the basolateral membrane. Clearly, the proximal tubule cell could not use the same Na^+/glucose cotransporter at both the apical and basolateral membranes because the electrochemical Na^+ gradient is similar across both membranes.

We will now look at four examples to illustrate how epithelia can absorb or secrete various solutes by using the transporters discussed earlier in this chapter.

Na^+ Absorption Consider the model in Figure 5-21A, which is similar to that first proposed by Hans Ussing and coworkers to explain NaCl absorption across the frog skin. The basolateral Na-K pump pumps Na^+ out of the cell, thereby lowering $[Na^+]_i$ and generating an inward Na^+ electrochemical gradient across the apical membrane. This apical Na^+ gradient in turn provides the driving force for Na^+ to enter the cell passively across the apical membrane through ENaC Na^+ channels. The Na^+ that enters the cell in this way is pumped out across the basolateral membrane in exchange for K^+, which recycles back out across the basolateral membrane. Note that the Na-K pump generates a current of positive charge across the cell from lumen to interstitium. This current, in turn, creates a lumen-negative transepithelial voltage that can then provide a driving force for passive Cl^- absorption across the tight junctions—through the so-called paracellular pathway. The net result is NaCl absorption. This process is the mechanism for NaCl reabsorption in the collecting tubule of the kidney.

K^+ Secretion With slight alterations, the same basic cell model can perform K^+ secretion as well as Na^+ absorption (Fig. 5-21B). Adding K^+ channels to the apical membrane allows some of the K^+ that is taken up by the Na-K pump across the basolateral membrane to be secreted across the apical membrane. This mechanism is the basis of K^+ secre-

tion in the collecting tubule of the kidney. Such a model accurately predicts that drugs such as amiloride, which blocks apical ENaC Na^+ channels in these cells, will inhibit K^+ secretion as well as Na^+ reabsorption.

Glucose Absorption The small intestine and proximal tubule absorb nutrients that are present in the luminal compartment by secondary active cotransport of Na^+ with organic solutes. An example is Na^+ cotransport with glucose by SGLT (Fig. 5-21C). The inwardly directed electrochemical Na^+ gradient across the apical membrane, generated by the Na-K pump, now drives the entry of both Na^+ and glucose. Glucose, which has accumulated in the cell against its concentration gradient, exits passively across the basolateral membrane by a carrier-mediated transporter (GLUT) that is not coupled to Na^+. Again, the flow of positive current across the cell generates a lumen-negative transepithelial voltage that can drive passive Cl^- absorption across the tight junctions. The net effect is to absorb both NaCl and glucose.

Cl^- Secretion If the cell places the Na^+-coupled Cl^- entry mechanism on the basolateral membrane, the same basic cell model can mediate NaCl *secretion* into the lumen (Fig. 5-21D). The inwardly directed Na^+ electrochemical gradient now drives secondary active Cl^- uptake across the basolateral membrane by the Na/K/Cl cotransporter NKCC1. Cl^- accumulated in the cell in this way can then exit across the apical membrane passively through Cl^- channels such as CFTR. Notice that negative charges now move across the cell from interstitium to lumen and generate a lumen-negative voltage that can drive passive Na^+ secretion across the tight junctions (paracellular pathway). The net process is NaCl secretion, even though the primary active transporter, the Na-K pump, is pumping Na^+ from the cell to the interstitium. Secretory cells in the intestine and pulmonary airway epithelium use this mechanism for secreting NaCl.

Water transport across epithelia passively follows solute transport

In general, water moves passively across an epithelium in response to osmotic gradients. An epithelium that secretes salt will secrete fluid, and one that absorbs salt will absorb fluid. The finite permeability of the bare lipid bilayer to water and the presence of aquaporins in most cell membranes ensure that osmotic equilibration for most cells is rapid. In addition, particularly in leaky epithelia, tight junctions provide a pathway for water movement between the epithelial cells. However, epithelial water permeability (hydraulic conductivity) varies widely because of differences in membrane lipid composition and in abundance of aquaporins. The presence of aquaporins in the plasma membrane may be either constitutive or highly regulated.

Absorption of a Hyperosmotic Fluid If the epithelium absorbs more salt than its isotonic equivalent volume of water, the absorbate is hyperosmotic. An example is the thick ascending limb of the loop of Henle in the kidney, which reabsorbs a large amount of salt but relatively little water. As a result, dilute fluid is left behind in the lumen, and the renal interstitium is rendered hyperosmotic.

Absorption of an Isosmotic Fluid In certain epithelia, such as the renal proximal tubule and small intestine, net water movement occurs with no detectable osmotic gradients across the epithelium (Fig. 5-22). Moreover, the reabsorbed fluid appears to be isosmotic with respect to luminal fluid. Of course, fluid absorption could not really occur without the requisite solute driving force across the epithelium. Two explanations, which are not exclusive, have been offered.

First, the water permeability of epithelia performing isosmotic water reabsorption might be extremely high because of the high constitutive expression of aquaporins in the apical and basolateral membranes. Thus, modest transepithelial osmotic gradients (perhaps only 1 to 2 mOsm), which are the product of solute absorption, are sufficient to drive water transport at the rates observed. Measurements cannot distinguish such small osmotic gradients from no gradient at all.

Second, the lateral intercellular spaces between the epithelial cells (lateral interspaces; see Fig. 5-22, option 1) as well as the spaces between the infoldings of the basal membrane (basal labyrinth; see Fig. 5-22, option 2) might be modestly hyperosmotic as a consequence of the accumulation of absorbed solutes in a localized region. The resulting localized osmotic gradient would pull water into the lateral interspaces from the cell (across the lateral portion of the basolateral membrane) or from the lumen (across the tight junction). Similarly, a localized osmotic gradient would pull water into the basal labyrinth from the cell (across the basal portion of the basolateral membrane). By the time that the fluid emerges from these spaces and reaches the interstitium, it would have become nearly isosmotic.

Absorption of a Hypo-osmotic Fluid If both sides of an epithelium are bathed by isosmotic solutions, it is not possible to concentrate the fluid in the lumen. You might think that you could accomplish the task by absorbing a hypo-osmotic fluid. However, this would require absorbing more water than solute, which would require water transport to "lead" rather than to follow solute transport. Indeed, active transport of water does not occur, and water cannot move against an osmotic gradient. Hypo-osmotic fluid absorption does indeed occur in the body but requires that the osmolality of the basolateral compartment exceed that of the apical compartment. As we will see in Chapter 38, the medullary collecting duct uses this approach to concentrate the urine. The collecting duct absorbs a hypo-osmotic fluid because (1) the interstitial fluid in the renal medulla is hyperosmotic and (2) the water permeability of the renal collecting duct is high due to the insertion of AQP2—under hormonal control—into the apical membrane.

Epithelia can regulate transport by controlling transport proteins, tight junctions, and the supply of the transported substances

A large range of physiological stimuli regulate the rates at which specific epithelia transport specific solutes. Virtually all known intracellular signaling cascades (see Chapter 3) have been implicated in mediating these regulatory effects.

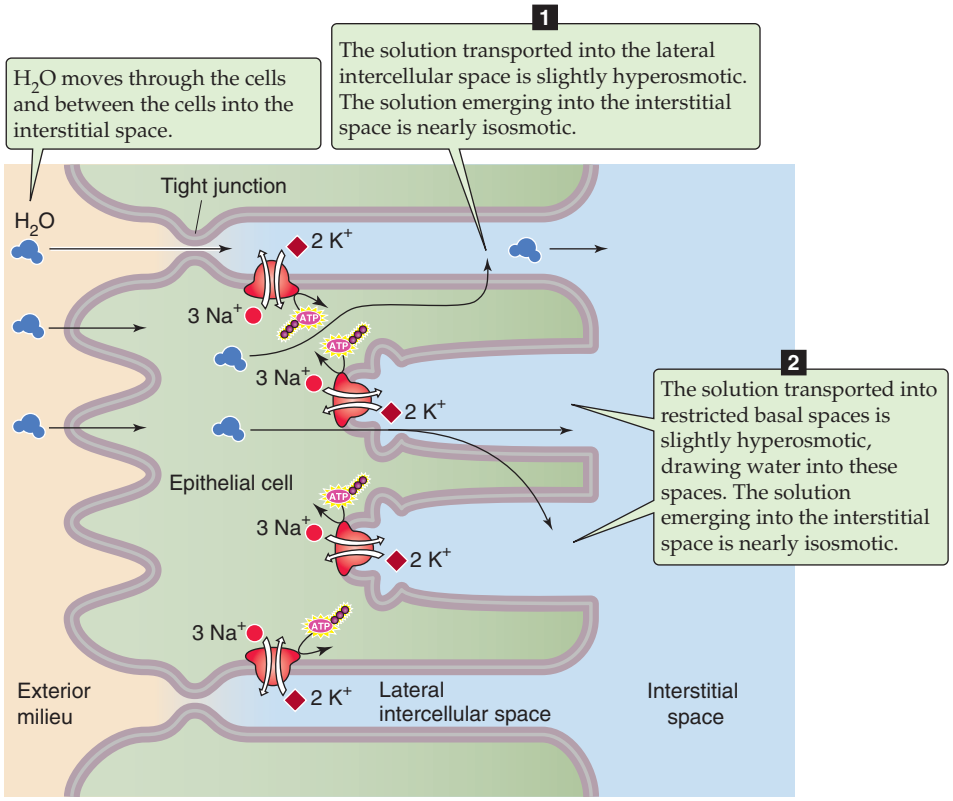


Figure 5-22 Model of isotonic water transport in a leaky epithelium. Na-K pumps present on the lateral and basal membrane pump Na^+ into two restricted spaces: the lateral intercellular space and restricted spaces formed by infoldings of the basal membrane. The locally high osmolality in the lateral intercellular space pulls water from the lumen and the cell. Similarly, the locally high osmolality in the restricted basal spaces pulls water from the cell. The solution that emerges from these two restricted spaces—and enters the interstitial space—is only slightly hypertonic (virtually isotonic) compared with the luminal solution.

Ultimately, these cascades must affect the rates at which specific solutes move through transporters or channels.

Increased Synthesis (or Degradation) of Transport Proteins One approach for modifying transport activity is to change the number of transport molecules in the cell. For example, the hormone aldosterone directly or indirectly increases the transcription rate of genes that encode Na-K pump subunits, thereby increasing Na-K pump synthesis in the distal nephron of the kidney.

Recruitment of Transport Proteins to the Cell Membrane Cells can also change the functional activity of transporters by storing some of them in an intracellular organelle "pool" and then inserting them into the cell membrane. For example, histamine causes cytoplasmic "tubulovesicles" containing H-K pumps (the pool) to fuse with the apical membrane of gastric parietal cells, thereby initiating gastric acid secretion.

Post-translational Modification of Preexisting Transport Proteins Another approach for modulating the transporter rate is to change the activity of preexisting transport proteins. For example, increases in the level of intracellular cyclic adenosine monophosphate (cAMP) enhance the phosphorylation of apical membrane Cl^- channels that are involved in NaCl secretion by intestinal and airway epithelia. The cystic fibrosis gene product (CFTR) is a Cl^- channel whose function is regulated by phosphorylation. A defect in the regulation of apical membrane Cl^- channels is the primary physiological abnormality in cystic fibrosis.

Changes in the Paracellular Pathway The passive movement of solutes across the tight junction can contribute to either "forward" transepithelial movement of the solute or backleak of the solute, depending on the solute gradients. Thus, the epithelium can modulate net transport by changing the permeability of the paracellular pathway. For example, the Na^+ permeability of the tight junctions of the proximal tubule increases when ECF volume increases. This increase in the permeability of the paracellular pathway may lower net Na^+ reabsorption because of increased backleak of absorbed Na^+ from the lateral interspace, across the tight junction, and into the lumen.

Luminal Supply of Transported Species Changes in the concentration of transported solutes can have profound effects on rates of net solute transport. As fluid moves along the renal proximal tubule, for example, the very process of glucose absorption depletes glucose from the lumen, thereby slowing further glucose absorption. Increasing the rate at which fresh, high-glucose fluid enters the proximal tubule lumen raises the glucose concentration at the site of glucose uptake and thus increases the rate of glucose absorption.

REFERENCES

Books and Reviews

Alper SL: The band 3–related anion exchanger (AE) gene family. Annu Rev Physiol 1991; 53:549-564.

Boyer PD: The ATP synthase—a splendid molecular machine. Annu Rev Biochem 1997; 66:717-749.

Frizzell RA (guest editor of special issue): Physiology of cystic fibrosis. Physiol Rev 1999; 79.
Gadsby DC, Dousmanis AG, Nairn AC: ATP hydrolysis cycles the gating of CFTR Cl^- channels. Acta Physiol Scand Suppl 1998; 43:247-256.
Kaplan MR, Mount DB, Delpire E, et al: Molecular mechanisms of NaCl cotransport. Annu Rev Physiol 1996; 58:649-668.
Lang F, Busch GL, Ritter M, et al: Functional significance of cell volume regulatory mechanisms. Physiol Rev 1998; 78:247-306.
Palacin M, Estevez R, Bertran J, Zorzano A: Molecular biology of mammalian plasma membrane amino acid transporters. Physiol Rev 1998; 78:969-1054.
Steel A, Hediger MA: The molecular physiology of sodium- and proton-coupled solute transporters. News Physiol Sci 1998; 13:123-131.

Journal Articles

Canessa CM, Schild L, Buell G, et al: Amiloride-sensitive epithelial Na^+ channel is made of three homologous subunits. Nature 1994; 367:463-467.
Hediger MA, Coady MJ, Ikeda IS, Wright EM: Expression cloning and cDNA sequencing of the Na^+/glucose transporter. Nature 1987; 330:379-381.
Preston GM, Agre P: Isolation of the cDNA for erythrocyte integral membrane protein of 28 kilodaltons: Member of an ancient channel family. Proc Natl Acad Sci USA 1991; 88:1110-1114.
Skou JC: The influence of some cations on an adenosine triphosphatase from peripheral nerves. Biochim Biophys Acta 1957; 23:394-401.