CHAPTER 6

ELECTROPHYSIOLOGY OF THE CELL MEMBRANE

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Physics is concerned with the fundamental nature of matter and energy, whereas the goal of medical physiology is to understand the workings of living tissue. Despite their different perspectives, physics and physiology share common historical roots in the early investigations of charge and electricity. In the late 1700s, Luigi Galvani, a professor of anatomy in Bologna, Italy, used the leg muscles of a dissected frog to assay the presence of electrical charge stored in various ingenious devices that were the predecessors of modern capacitors and batteries. He observed that frog legs vigorously contracted when electrical stimulation was applied either directly to the leg muscle or to the nerves leading to the muscle (Fig. 6-1). Such early physiological experiments contributed to the development of electromagnetic theory in physics and electrophysiological theory in biology.

The phenomenon of "animal electricity" is central to the understanding of physiological processes. Throughout this book, we will describe many basic functions of tissues and organs in terms of electrical signals mediated by cell membranes. Whereas electrical currents in a metal wire are conducted by the flow of electrons, electrical currents across cell membranes are carried by the major inorganic ions of physiological fluids: Ca^{2+}, Na^+, K^+, Cl^-, and HCO_3^-. Many concepts and terms used in cellular electrophysiology are the same as those used to describe electrical circuits. At the molecular level, electrical current across cell membranes flows through three unique classes of integral membrane proteins (see Chapter 2): ion channels, electrogenic ion transporters, and electrogenic ion pumps. The flow of ions through specific types of channels is the basis of electrical signals that underlie neuronal activity and animal behavior. Opening and closing of such channels is the fundamental process behind electrical phenomena such as the nerve impulse, the heartbeat, and sensory perception. Channel proteins are also intimately involved in hormone secretion, ionic homeostasis, osmoregulation, and regulation of muscle contractility.

This chapter begins with a review of basic principles of electricity and introduces the essentials of electrophysiology. We also discuss the molecular biology of ion channels and provide an overview of channel structure and function.

IONIC BASIS OF MEMBRANE POTENTIALS

Principles of electrostatics explain why aqueous pores formed by channel proteins are needed for ion diffusion across cell membranes

The plasma membranes of most living cells are electrically polarized, as indicated by the presence of a transmembrane voltage—or a **membrane potential**—in the range of 0.1 V. In Chapter 5, we discussed how the energy stored in this miniature battery can drive a variety of transmembrane transport processes. Electrically excitable cells such as brain neurons and heart myocytes also use this energy for signaling purposes. The brief electrical impulses produced by such cells are called **action potentials**. To explain these electrophysiological phenomena, we begin with a basic review of electrical energy.

Atoms consist of negatively (–) and positively (+) charged elementary particles, such as electrons (e^-) and protons (H^+), as well as electrically neutral particles (neutrons). Charges of the same sign repel each other, and those of opposite sign attract. Charge is measured in units of **coulombs** (C). The **unitary charge** of one electron or proton is denoted by e_0 and is equal to 1.6022×10^{-19} C. Ions in solution have a charge valence (z) that is an integral number of elementary charges; for example, $z = +2$ for Ca^{2+}, $z = +1$ for K^+, and $z = -1$ for Cl^-. The charge of a single ion (q_0), measured in coulombs, is the product of its valence and the elementary charge:

$$q_0 = ze_0 \qquad (6\text{-}1)$$

In an aqueous solution or a bulk volume of matter, the number of positive and negative charges is always equal. Charge is also conserved in any chemical reaction.

The attractive electrostatic force between two ions that have valences of z_1 and z_2 can be obtained from Coulomb's law. This force ($\boldsymbol{F}$) is proportional to the product of these valences and inversely proportional to the square of the distance (r) between the two. The force is also inversely proportional to a dimensionless term called the **dielectric constant** (ε):

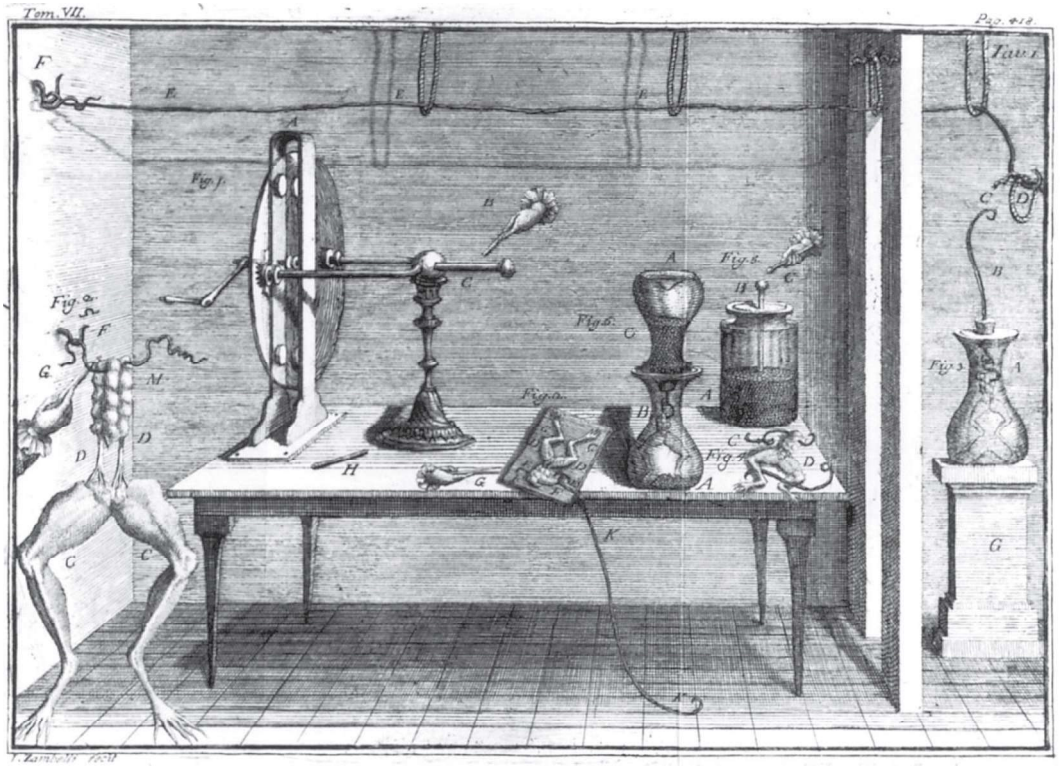
Tom. VII.
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Fig. 1.
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A

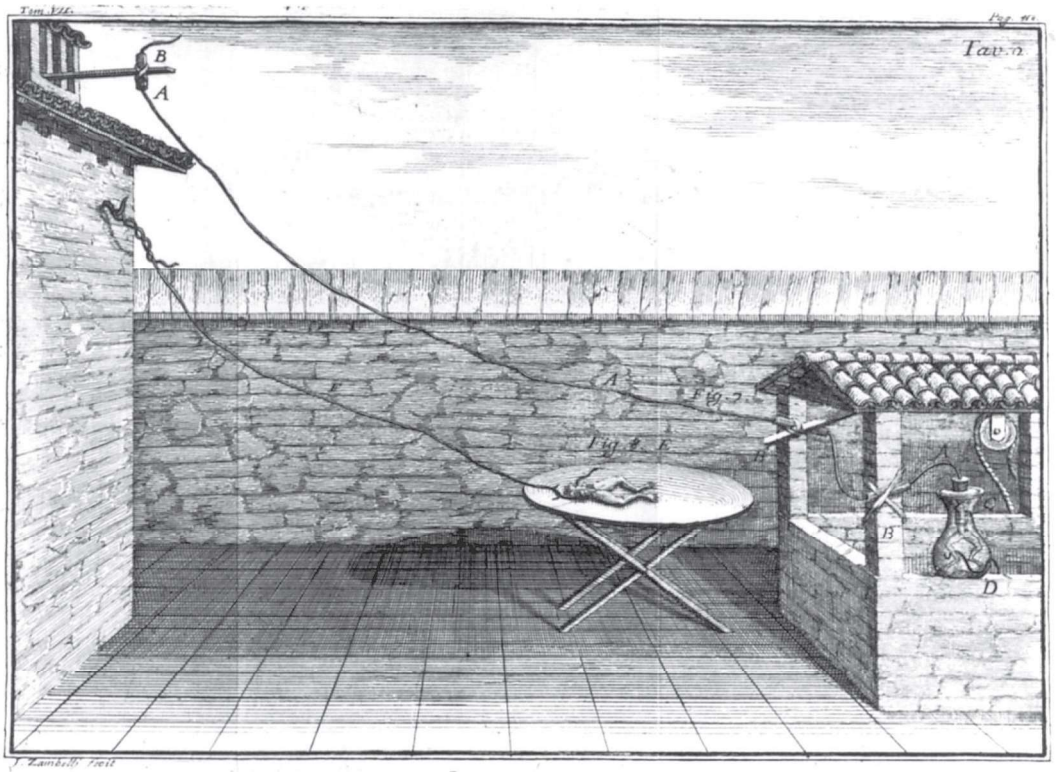
Tom. VII.
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Fig. 1.
Fig. 2.

B

$$F \propto \frac{z_1 \cdot z_2}{\varepsilon r^2} \qquad (6\text{-}2)$$

Because the dielectric constant of water is ~40-fold greater than that of the hydrocarbon interior of the cell membrane, the electrostatic force between ions is reduced by a factor of ~40 in water compared with membrane lipid.

If we were to move the Na^+ ion from the extracellular to the intracellular fluid without the aid of any proteins, the Na^+ would have to cross the membrane by "dissolving" in the lipids of the bilayer. However, the energy required to transfer the Na^+ ion from water (high ε) to the interior of a phospholipid membrane (low ε) is ~36 kcal/mol. This value is 60-fold higher than molecular thermal energy at room temperature. Thus, the probability that an ion would dissolve in the bilayer (i.e., partition from an aqueous solution into the lipid interior of a cell membrane) is essentially zero. This analysis explains why inorganic ions cannot readily cross a phospholipid membrane without the aid of other molecules such as specialized transporters or channel proteins, which provide a favorable polar environment for the ion as it moves across the membrane (Fig. 6-2).

Membrane potentials can be measured by use of microelectrodes and voltage-sensitive dyes

The voltage difference across the cell membrane, or the **membrane potential** (V_m), is the difference between the electrical potential in the cytoplasm (Ψ_i) and the electrical potential in the extracellular space (Ψ_o). Figure 6-3A shows how to measure V_m with an intracellular electrode. The sharp tip of a microelectrode is gently inserted into the cell and measures the transmembrane potential with respect to the electrical potential of the extracellular solution, defined as ground (i.e., $\Psi_o = 0$). If the cell membrane is not damaged by electrode impalement and the impaled membrane seals tightly around the glass, this technique provides an accurate measurement of V_m. Such a voltage measurement is called an **intracellular recording**.

For an amphibian or mammalian skeletal muscle cell, resting V_m is typically about −90 mV, meaning that the interior of the resting cell is ~90 mV more negative than the exterior. There is a simple relationship between the electrical potential difference across a membrane and another parameter, the **electrical field** (***E***):

$$E = \frac{V_m \leftarrow \text{Electrical potential difference across the membrane}}{a \leftarrow \text{Distance across the membrane}} \qquad (6\text{-}3)$$

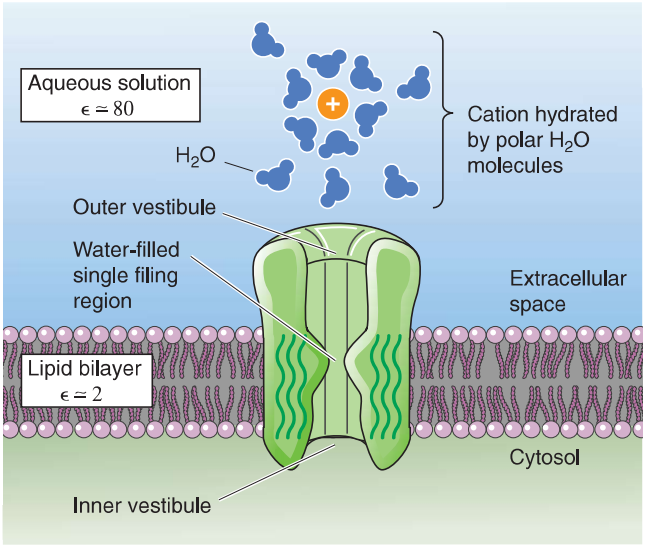

Figure 6-2 Formation of an aqueous pore by an ion channel. The dielectric constant of water (ε = 80) is ~40-fold higher than the dielectric constant of the lipid bilayer (ε = 2).

Figure 6-1 Early electrophysiological experiments of Galvani. **A,** Electrical stimulation of a dissected frog with diverse sources of electricity. On the center of the table is a board with a dissected frog that has been prepared for an experiment (Fig. Ω). A hand with a charged metal rod (G) is about to touch the sacral nerves (D), contracting the limbs (C). A metal wire (F) penetrates the spinal cord; a second metal wire (K) grounds the first wire to the floor. On the left side of the table (Fig. 1) is a large "electrical machine" with a rotating disk (A), a conductor (C), and a hand holding a metal rod (B) that is about to be charged. On the extreme left of the room (Fig. 2), a dissected frog is suspended from an iron wire that penetrates the spinal cord (F); the wire is attached to the wall by a hook. A hand with a charged metal rod (G) is touching the wire, stimulating the sacral nerves (D) and causing the legs (C) to twitch. Outside the room on the extreme right side (Fig. 3) is a frog in a glass jar (A). Emerging from the glass jar is an iron wire (B) that is attached at one end to a hook on the frog and ends in a hook (C) in the air. A silk loop (D) near this hook connects to a long conductor (F) that runs near the ceiling to a hook in the wall at the extreme left of the main room. At the far right/front of the table in the main room (Fig. 4) is a dissected frog with one conductor connected to a nerve (C) and another connected to a muscle (D). Just behind this frog (Fig. 5) is a "Leiden jar" (A) containing small lead shot used by hunters. A hand with a charged metal rod (C) is about to touch a conductor (B) emerging from the jar. To the left of the Leiden jar (Fig. 6) is an inverted jar (A) with lead shot (C). This jar sits on top of a similar jar (B) containing a suspended, dissected frog and is connected by a conductor to the lead shot in the upper jar. The legs of the frog are grounded to lead shot near the bottom of the jar. **B,** Electrical stimulation of the leg muscles of a dissected frog by "natural electricity" (i.e., lightning). In one experiment (Fig. 7), an iron wire (A) runs from near the roof, through several insulating glass tubes (B), to a flask (C) that contains a freshly dissected frog. A second wire (D) grounds the frog's legs to the water in the well. In a second experiment (Fig. 8), a noninsulated wire extends from an iron hook fastened to the wall and to the spinal cord of a frog (E), which is on a table coated with oil. *(From Galvani L: De viribus electricitatis in motu musculari commentarius Aloysii Galvani, Bononiae. New Haven, CT: Yale University, Harvey Cushing/John Hay Whitney Medical Library, 1791.)*

Accordingly, for a V_m of −0.1 V and a membrane thickness of $a = 4$ nm (i.e., 40×10^{-8} cm), the magnitude of the electrical field is ~250,000 V/cm. Thus, despite the small transmembrane *voltage,* cell membranes actually sustain a very large electrical *field.* Later, we discuss how this electrical field influences the activity of a particular class of membrane signaling proteins called voltage-sensitive ion channels (see Chapter 7).

Skeletal muscle cells, cardiac cells, and neurons typically have resting membrane potentials of approximately −60 to −90 mV; smooth muscle cells have membrane potentials in the range of −55 mV; and the V_m of the human erythrocyte is only about −9 mV. However, certain bacteria and plant cells have transmembrane voltages as large as −200 mV. For very small cells such as erythrocytes, small intracellular organelles such as mitochondria, and fine processes such as the synaptic endings of neurons, V_m cannot be directly measured with a microelectrode. Instead, spectroscopic techniques allow the membrane potentials of such inaccessible membranes to be measured indirectly (Fig. 6-3B). This technique involves labeling of the cell or membrane with an appropriate organic dye molecule and monitoring of the absorption or fluorescence of the dye. The optical signal of the dye molecule can be independently calibrated as a function of V_m. Whether V_m is measured directly by a microelectrode or indirectly by a spectroscopic technique, virtually all biological membranes have a nonzero membrane potential. This transmembrane voltage is an important determinant of any physiological transport process that involves the movement of charge.

Measurements of V_m have shown that many types of cells are electrically excitable. Examples of excitable cells are neurons, muscle fibers, heart cells, and secretory cells of the pancreas. In such cells, V_m exhibits characteristic time-dependent changes in response to electrical or chemical stimulation. When the cell body, or soma, of a neuron is electrically stimulated, electrical and optical methods for measuring V_m detect an almost identical response at the cell body (Fig. 6-3C). The optical method provides the additional insight that the V_m changes are similar but delayed in the more distant neuronal processes that are inaccessible to a microelectrode (Fig. 6-3D). When the cell is not undergoing such active responses, V_m usually remains at a steady value that is called the **resting potential**. In the next section, we discuss the origin of the membrane potential and lay the groundwork for understanding its active responses.

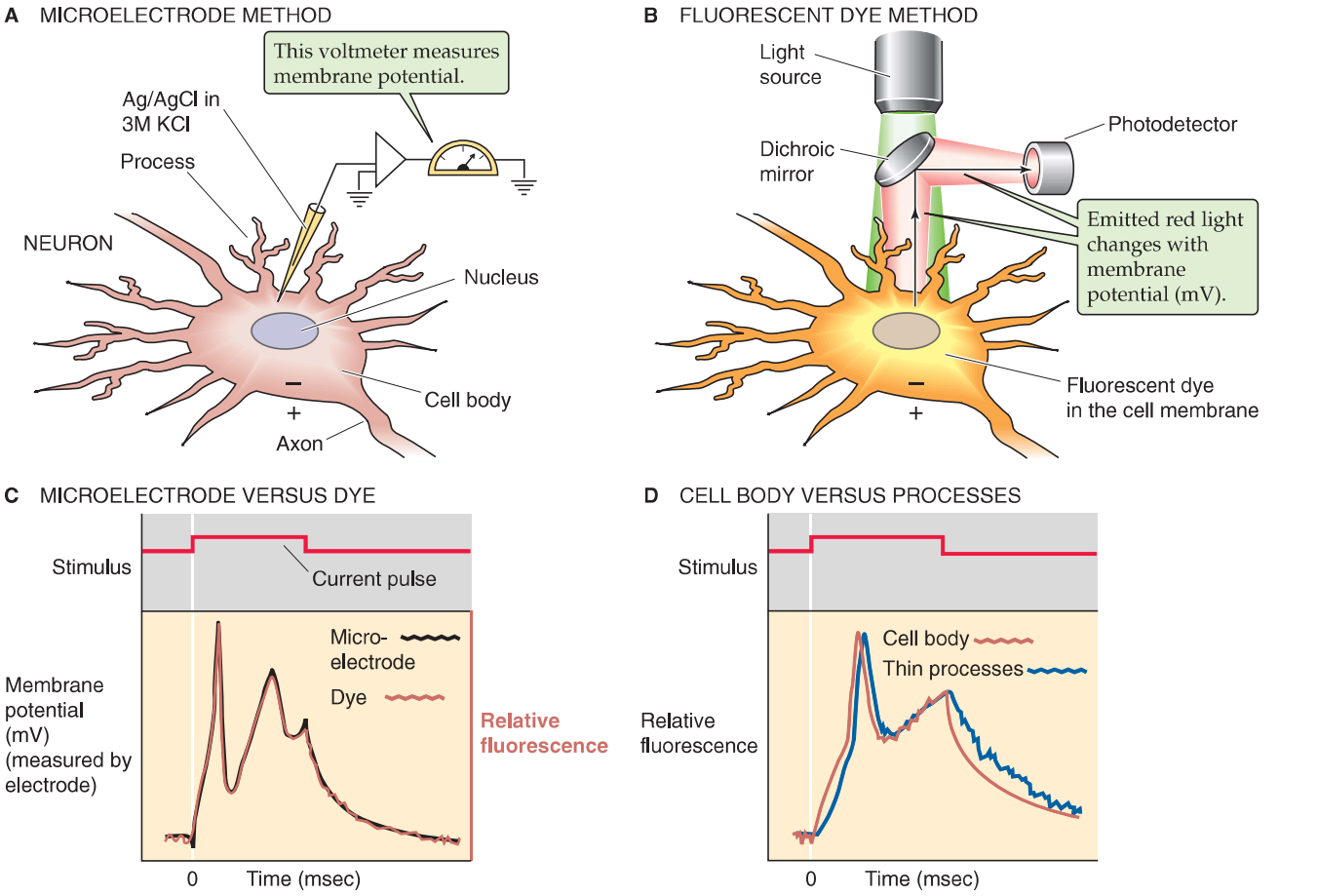

Figure 6-3 Recording of membrane potential. (***C*** *and* ***D,*** *Data modified from Grinvald A: Real-time optical mapping of neuronal activity: From single growth cones to the intact mammalian brain. Annu Rev Neurosci 1985; 8:263-305. © Annual Reviews www.annualreviews.org.)*

Membrane potential is generated by ion gradients

In Chapter 5, we introduced the concept that some integral membrane proteins are electrogenic transporters in that they generate an electrical current that sets up an electrical potential across the membrane. One class of electrogenic transporters includes the adenosine triphosphate (ATP)–dependent *ion pumps*. These proteins use the energy of ATP hydrolysis to produce and to maintain concentration gradients of ions across cell membranes. In animal cells, the Na-K pump and Ca^{2+} pump are responsible for maintaining normal gradients of Na^+, K^+, and Ca^{2+}. The reactions catalyzed by these ion transport enzymes are electrogenic because they lead to separation of charge across the membrane. For example, enzymatic turnover of the Na-K pump results in the translocation of three Na^+ ions out of the cell and two K^+ ions into the cell, with a net movement of one positive charge out of the cell. In addition to electrogenic pumps, cells may express *secondary active transporters* that are electrogenic, such as the Na^+/glucose cotransporter (see Chapter 5).

It may seem that the inside negative V_m originates from the continuous pumping of positive charges out of the cell by the electrogenic Na-K pump. The resting potential of large cells—whose surface-to-volume ratio is so large that ion gradients run down slowly—is maintained for a long time even when metabolic poisons block ATP-dependent energy metabolism. This finding implies that an ATP-dependent pump is not the immediate energy source underlying the membrane potential. Indeed, the squid giant axon normally has a resting potential of –60 mV. When the Na-K pump in the giant axon membrane is specifically inhibited with a cardiac glycoside (see Chapter 5), the immediate positive shift in V_m is only 1.4 mV. Thus, in most cases, the direct contribution of the Na-K pump to the resting V_m is very small.

In contrast, many experiments have shown that cell membrane potentials depend on ionic **concentration gradients**. In a classic experiment, Paul Horowicz and Alan Hodgkin measured the V_m of a frog muscle fiber with an intracellular microelectrode. The muscle fiber was bathed in a modified physiological solution in which SO_4^{2-} replaced Cl^-, a manipulation that eliminates the contribution of anions to V_m. In the presence of normal extracellular concentrations of K^+ and Na^+ for amphibians ($[K^+]_o$ = 2.5 mM and $[Na^+]_o$ = 120 mM), the frog muscle fiber has a resting V_m of approximately –94 mV. As $[K^+]_o$ is increased above 2.5 mM by substitution of K^+ for Na^+, V_m shifts in the positive direction. As $[K^+]_o$ is decreased below 2.5 mM, V_m becomes more negative (Fig. 6-4). For $[K^+]_o$ values greater than 10 mM, the V_m measured in Figure 6-4 is approximately a linear function of the *logarithm* of $[K^+]_o$. Numerous experiments of this kind have demonstrated that the *immediate* energy source of the membrane potential is not the active pumping of ions but rather the potential energy stored in the ion concentration gradients themselves. Of course, it is the ion pumps—and the secondary active transporters that derive their energy from these pumps—that are responsible for generating and maintaining these ion gradients.

One way to investigate the role of ion gradients in determining V_m is to study this phenomenon in an in vitro (cell-free) system. Many investigators have used an artificial model of a cell membrane called a **planar lipid bilayer**. This system consists of a partition with a hole ~200 µm in diameter that separates two chambers filled with aqueous solutions (Fig. 6-5). It is possible to paint a planar lipid bilayer having a thickness of only ~4 nm across the hole, thereby sealing the partition. By incorporating membrane proteins and other molecules into planar bilayers, one can study the essential characteristics of their function in isolation from the complex metabolism of living cells. Transmembrane voltage can be measured across a planar bilayer with a voltmeter connected to a pair of Ag/AgCl electrodes that are in electrical contact with the solution on each side of the membrane through salt bridges. This experimental arrangement is much like an intracellular voltage recording, except that both sides of the membrane are completely accessible to manipulation.

The ionic composition of the two chambers on opposite sides of the bilayer can be adjusted to simulate cellular concentration gradients. Suppose that we put 4 mM KCl on the left side of the bilayer and 155 mM KCl on the right side to mimic, respectively, the external and internal concentrations of K^+ for a mammalian muscle cell. To eliminate the osmotic flow of water between the two compartments (see Chapter 5), we also add a sufficient amount of a nonelectrolyte (e.g., mannitol) to the side with 4 mM KCl. We can make the membrane selectively permeable to K^+ by introducing purified K^+ channels or K^+ ionophores into the membrane. Assuming that the K^+ channels are in an open state and are impermeable to Cl^-, the right ("internal") compartment quickly becomes electrically negative with respect to the left ("external") compartment because positive charge (i.e., K^+) diffuses from high to low concentration. However, as the

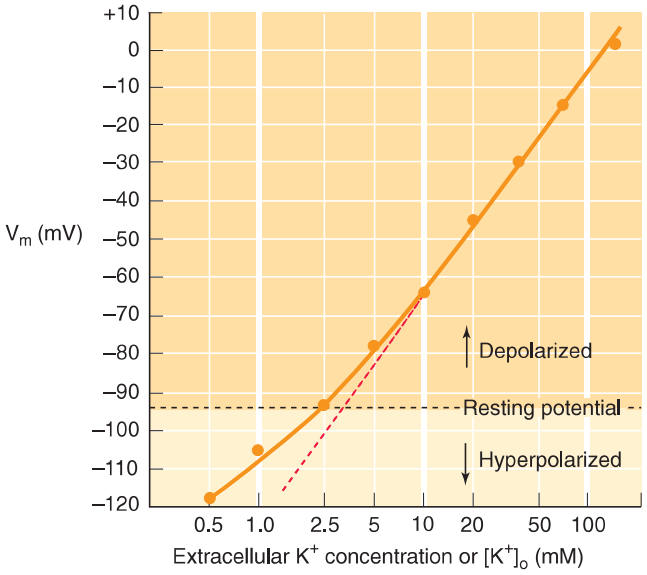

Figure 6-4 Dependence of resting potential on extracellular K^+ concentration in a frog muscle fiber. The slope of the linear part of the curve is 58 mV for a 10-fold increase in $[K^+]_o$. Note that the horizontal axis for $[K^+]_o$ is plotted using a logarithmic scale. *(Data from Hodgkin AL, Horowicz P: The influence of potassium and chloride ions on the membrane potential of single muscle fibers. J Physiol [Lond] 1959; 148:127-160.)*

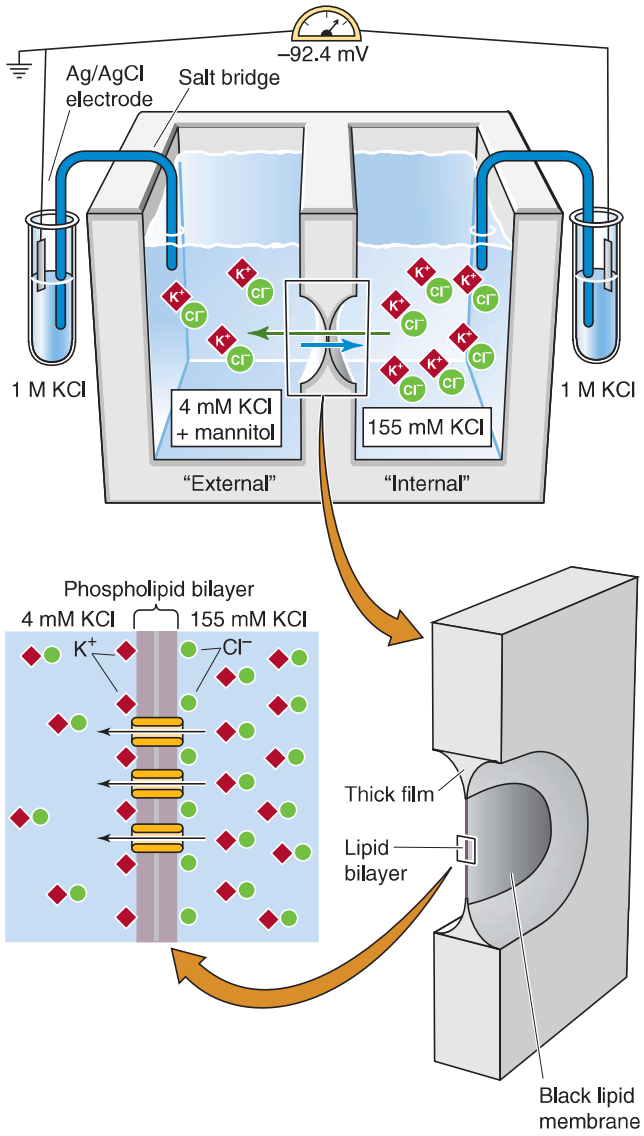

Figure 6-5 Diffusion potential across a planar lipid bilayer containing a K^+-selective channel.

negative voltage develops in the right compartment, the negativity opposes further K^+ efflux from the right compartment. Eventually, the voltage difference across the membrane becomes so negative as to halt further net K^+ movement. At this point, the system is in **equilibrium**, and the transmembrane voltage reaches a value of 92.4 mV, right-side negative. In the process of generating the transmembrane voltage, a separation of charge has occurred in such a way that the excess *positive* charge on the left side (low $[K^+]$) balances the same excess *negative* charge on the right side (high $[K^+]$). Thus, the stable voltage difference (–92.4 mV) arises from the separation of K^+ ions from their counterions (in this case Cl^-) across the bilayer membrane.

For mammalian cells, nernst potentials for ions typically range from –100 mV for K^+ to +100 mV for Ca^{2+}

The model system of a planar bilayer (impermeable membrane), unequal salt solutions (ionic gradient), and an ion-selective channel (conductance pathway) contains the minimal components essential for generating a membrane potential. The hydrophobic membrane bilayer is a formidable barrier to inorganic ions and is also a poor conductor of electricity. Poor conductors are said to have a high **resistance** to electrical current, in this case, ionic current. On the other hand, ion channels act as molecular conductors of ions. They introduce a **conductance pathway** into the membrane and lower its resistance.

In the planar bilayer experiment of Figure 6-5, V_m originates from the diffusion of K^+ down its concentration gradient. Membrane potentials that arise by this mechanism are called **diffusion potentials**. At equilibrium, the diffusion potential of an ion is the same as the equilibrium potential (E_X) given by the **Nernst equation** previously introduced as Equation 5-8.

$$E_X = -\frac{RT}{z_X F}\ln\frac{[X]_i}{[X]_o} \qquad (6\text{-}4)$$

The Nernst equation predicts the equilibrium membrane potential for any concentration gradient of a particular ion across a membrane. E_X is often simply referred to as the **Nernst potential**. The Nernst potentials for K^+, Na^+, Ca^{2+}, and Cl^-, respectively, are written as E_K, E_{Na}, E_{Ca}, and E_{Cl}.

The linear portion of the plot of V_m versus the logarithm of $[K^+]_o$ for a frog muscle cell (Fig. 6-4) has a **slope** that is ~58.1 mV for a 10-fold change in $[K^+]_o$, as predicted by the Nernst equation. Indeed, if we insert the appropriate values for R and F into Equation 6-4, select a temperature of 20°C, and convert the logarithm base e (ln) to the logarithm base 10 ($\log_{10}$), we obtain a coefficient of –58.1 mV, and the Nernst equation becomes

$$E_K = (-58.1\text{mV})\log_{10}\frac{[K^+]_i}{[K^+]_o} \qquad (6\text{-}5)$$

For a negative ion such as Cl^-, where $z = -1$, the sign of the slope is positive:

$$E_{Cl} = (+58.1\text{mV})\log_{10}\frac{[Cl^-]_i}{[Cl^-]_o} \qquad (6\text{-}6)$$

For Ca^{2+} ($z = +2$), the slope is half of –58.1 mV, or approximately –30 mV. Note that a Nernst slope of 58.1 mV is the value for a univalent ion at 20°C. For mammalian cells at 37°C, this value is 61.5 mV.

At $[K^+]_o$ values above ~10 mM, the magnitude of V_m and the slope of the plot in Figure 6-4 are virtually the same as those predicted by the Nernst equation (Equation 6-5), suggesting that the resting V_m of the muscle cell is almost equal to the K^+ diffusion potential. When V_m follows the Nernst

TABLE 6-1 Ion Concentration Gradients in Mammalian Cells

Ion (X)	$[X]_{out}$ (mM)	$[X]_{in}$ (mM)	$[X]_{out}/[X]_{in}$	V_x* (mV)
Skeletal muscle				
K^+	4.5	155	0.026	–95
Na^+	145	12	12	+67
Ca^{2+}	1.0	10^{-4}	10,000	+123
Cl^-	116	4.2	29	–89
HCO_3^-	24	12	2	–19
Most other cells				
K^+	4.5	120	0.038	–88
Na^+	145.4	15	9.67	+61
Ca^{2+}	1.0	10^{-4}	10,000	+123
Cl^-	116	20	5.8	–47
HCO_3^-	24	15	1.6	–13

*Nernst equilibrium potential for ion X at 37°C.

equation for K^+, the membrane is said to behave like a potassium electrode because ion-specific electrodes monitor ion concentrations according to the Nernst equation.

Table 6-1 lists the expected Nernst potentials for K^+, Na^+, Ca^{2+}, Cl^-, and HCO_3^- as calculated from the known concentration gradients of these physiologically important inorganic ions for mammalian skeletal muscle and a typical non-muscle cell. For a mammalian muscle cell with a V_m of –80 mV, E_K is ~15 mV more negative than V_m, whereas E_{Na} and E_{Ca} are about +67 and +123 mV, respectively, far more positive than V_m. E_{Cl} is ~9 mV more negative than V_m in muscle cells but slightly more positive than the typical V_m of –60 mV in most other cells.

What determines whether the cell membrane potential follows the Nernst equation for K^+ or Cl^- rather than that for Na^+ or Ca^{2+}? As we shall see in the next two sections, the membrane potential is determined by the relative permeabilities of the cell membrane to the various ions.

Currents carried by ions across membranes depend on the concentration of ions on both sides of the membrane, the membrane potential, and the permeability of the membrane to each ion

Years before ion channel proteins were discovered, physiologists devised a simple but powerful way to predict the membrane potential, even if several different kinds of permeable ions are present at the same time. The first step, which we discuss in this section, is to compute an ionic current, that

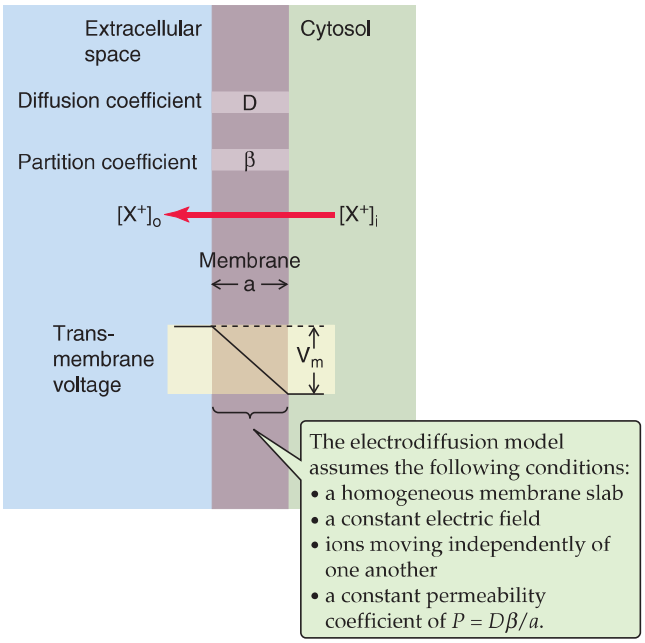

Figure 6-6 Electrodiffusion model of the cell membrane.

is, the movement of a single ion species through the membrane. The second step, which we describe in the following section, is to obtain V_m by summating the currents carried by each species of ion present, assuming that each species moves independently of the others.

The process of ion permeation through the membrane is called **electrodiffusion** because both electrical and concentration gradients are responsible for the ionic current. To a first approximation, the permeation of ions through most channel proteins behaves as though the flow of these ions follows a model based on the Nernst-Planck electrodiffusion theory, which was first applied to the diffusion of ions in simple solutions. This theory leads to an important equation in medical physiology called the constant-field equation, which predicts how V_m will respond to changes in ion concentration gradients or membrane permeability. Before introducing this equation, we first consider some important underlying concepts and assumptions.

Without knowing the molecular basis for ion movement through the membrane, we can treat the membrane as a "black box" characterized by a few fundamental parameters (Fig. 6-6). We must assume that the rate of ion movement through the membrane depends on (1) the external and internal concentrations of the ion X ($[X]_o$ and $[X]_i$, respectively), (2) the transmembrane voltage (V_m), and (3) a permeability coefficient for the ion X (P_X). In addition, we make four major assumptions about how the ion X behaves in the membrane:

The membrane is a homogeneous medium with a thickness *a*.

The voltage difference varies linearly with distance across the membrane (Fig. 6-6). This assumption is equivalent to stating that the *electrical field*—that is, the change in voltage with distance—is constant throughout the thick-

ness of the membrane. This requirement is therefore called the **constant-field assumption**.

The movement of an ion through the membrane is independent of the movement of any other ions. This assumption is called the **independence principle**.

The permeability coefficient P_X is a constant (i.e., it does not vary with the chemical or electrical driving forces). P_X (*units:* cm/s) is defined as $P_X = D_X\beta/a$. D_X is the diffusion coefficient for the ion in the membrane, β is the membrane/water partition coefficient for the ion, and a is the thickness of the membrane. Thus, P_X describes the ability of an ion to dissolve in the membrane (as described by β) and to diffuse from one side to the other (as described by D_X) over the distance a.

With these assumptions, we can calculate the current carried by a single ion X (I_X) through the membrane by using the basic physical laws that govern (1) the movement of molecules in solution (Fick's law of diffusion; see Equation 5-13), (2) the movement of charged particles in an **electrical field** (electrophoresis), and (3) the direct proportionality of current to voltage (Ohm's law). The result is the **Goldman-Hodgkin-Katz (GHK) *current* equation**, named after the pioneering electrophysiologists who applied the constant-field assumption to Nernst-Planck electrodiffusion:

$$I_X = \frac{z^2F^2V_mP_X}{RT}\left(\frac{[X]_i - [X]_o e^{(-zFV_m/RT)}}{1 - e^{(-zFV_m/RT)}}\right) \qquad (6\text{-}7)$$

I_X, or the rate of ions moving through the membrane, has the same units as electrical current: amperes (coulombs per second). Thus, the GHK current equation relates the current of ion X through the membrane to the internal and external concentrations of X, the transmembrane voltage, and the permeability of the membrane to X. The GHK equation thus allows us to predict how the current carried by X depends on V_m. This **current-voltage (*I-V*) relationship** is important for understanding how ionic currents flow into and out of cells.

Figure 6-7A shows how the K^+ current (I_K) depends on V_m, as predicted by Equation 6-7 for the normal internal (155 mM) and external (4.5 mM) concentrations of K^+. By convention, a current of ions flowing into the cell (**inward current**) is defined in electrophysiology as a negative-going current, and a current flowing out of the cell (**outward current**) is defined as a positive current. (As in physics, the direction of current is always the direction of movement of positive charge. This convention means that an inward flow of Cl^- is an outward current.) For the case of 155 mM K^+ inside the cell and 4.5 mM K^+ outside the cell, an inward current is predicted at voltages that are more negative than –95 mV, and an outward current is predicted at voltages that are more positive than –95 mV (Fig. 6-7A). The value of –95 mV is called the **reversal potential** (V_{rev}) because it is precisely at this voltage that the direction of current reverses (i.e., the net current equals zero). If we set I_K equal to zero in Equation 6-7 and solve for V_{rev}, we find that the GHK current equation reduces to the Nernst equation for K^+ (Equation 6-5). Thus, the GHK current equation for an ion X predicts a reversal potential (V_{rev}) equal to the Nernst potential (E_X) for that ion; that is, the current is zero when the ion is in electrochemical equilibrium. At V_m values more negative than V_{rev}, the net driving force on a cation is inward; at voltages that are more positive than V_{rev}, the net driving force is outward.

Figure 6-7B shows the analogous *I-V* relationship predicted by Equation 6-7 for physiological concentrations of Na^+. In this case, the Na^+ current (I_{Na}) is inward at V_m values

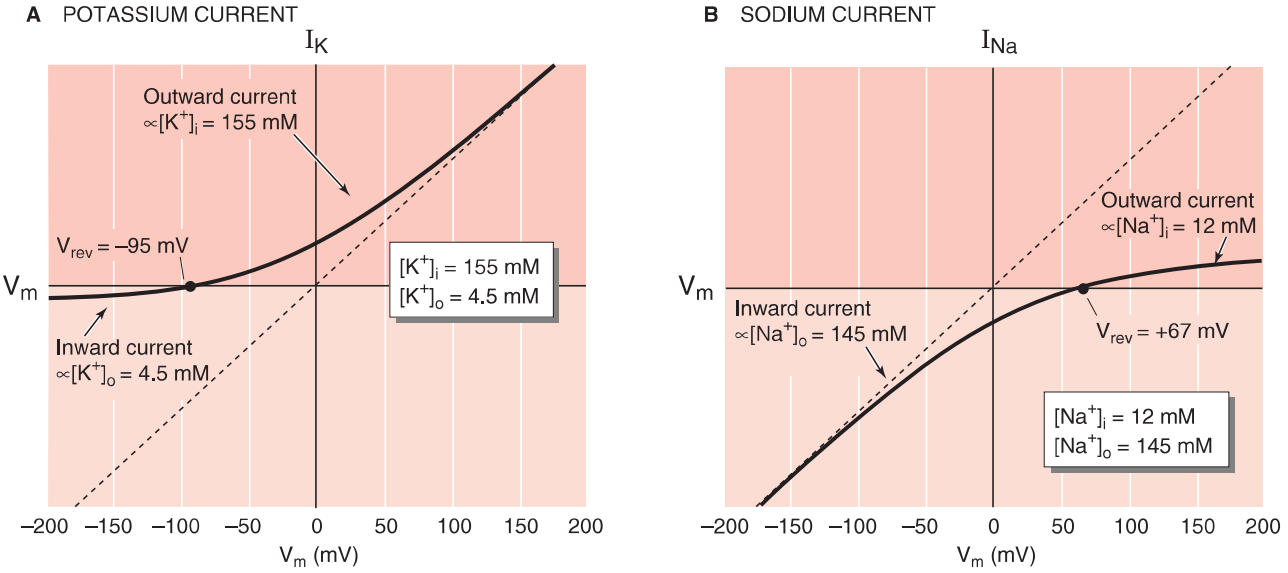

Figure 6-7 Current-voltage relationships predicted by the GHK current equation. **A,** The curve is the K^+ current predicted from the GHK equation (Equation 7)—assuming that the membrane is perfectly selective for K^+—for a $[K^+]_i$ of 155 mM and a $[K^+]_o$ of 4.5 mM. The *dashed line* represents the current that can be expected if both $[K^+]_i$ and $[K^+]_o$ were 155 mM (Ohm's law). **B,** The curve is the Na^+ current predicted from the GHK equation—assuming that the membrane is perfectly selective for Na^+—for a $[Na^+]_i$ of 12 mM and a $[Na^+]_o$ of 145 mM. The *dashed line* represents the current that can be expected if both $[Na^+]_i$ and $[Na^+]_o$ were 145 mM.

more negative than V_{rev} (+67 mV) and outward at voltages that are more positive than this reversal potential. Here again, V_{rev} is the same as the Nernst potential, in this case, E_{Na}.

Membrane potential depends on ionic concentration gradients and permeabilities

In the preceding section, we discussed how to use the GHK current equation to predict the current carried by any *single* ion, such as K^+ or Na^+. If the membrane is permeable to the monovalent ions K^+, Na^+, and Cl^-—and only to these ions—the **total ionic current** carried by these ions across the membrane is the sum of the individual ionic currents:

$$I_{total} = I_K + I_{Na} + I_{Cl} \qquad (6\text{-}8)$$

The individual ionic currents given by Equation 6-7 can be substituted into the right-hand side of Equation 6-8. Note that for the sake of simplicity, we have not considered currents carried by electrogenic pumps or other ion transporters; we could have added extra "current" terms for such electrogenic transporters. At the resting membrane potential (i.e., V_m is equal to V_{rev}), the sum of all ion currents is zero (i.e., $I_{total} = 0$). When we set I_{total} to zero in the expanded Equation 6-8 and solve for V_{rev}, we get an expression known as the **GHK *voltage* equation** or the **constant-field equation**:

$$V_{rev} = \frac{RT}{F}\ln\left(\frac{P_K[K^+]_o + P_{Na}[Na^+]_o + P_{Cl}[Cl^-]_i}{P_K[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_o}\right) \qquad (6\text{-}9)$$

Because we derived Equation 6-9 for the case of $I_{total} = 0$, it is valid only when zero net current is flowing across the membrane. This zero net current flow is the steady-state condition that exists for the cellular *resting* potential, that is, when V_m equals V_{rev}. The logarithmic term of Equation 6-9 indicates that resting V_m depends on the concentration gradients and the permeabilities of the various ions. However, resting V_m depends primarily on the concentrations of the most permeant ion.

The principles underlying Equation 6-9 show why the plot of V_m versus $[K^+]_o$ in Figure 6-4, which summarizes data obtained from a frog muscle cell, bends away from the idealized Nernst slope at very low values of $[K^+]_o$. Imagine that we expose a mammalian muscle cell to a range of $[K^+]_o$ values, always substituting extracellular K^+ for Na^+, or vice versa, so that the sum of $[K^+]_o$ and $[Na^+]_o$ is kept fixed at its physiological value of 4.5 + 145 = 149.5 mM. To simplify matters, we assume that the membrane permeability to Cl^- is very small (i.e., $P_{Cl} \cong 0$). We can also rearrange Equation 6-9 by dividing the numerator and denominator by P_K and representing the ratio P_{Na}/P_K as α. At 37°C, this simplified equation becomes

$$V_{rev} = (61.5\,mV)\times\log_{10}\left(\frac{[K^+]_o + \alpha[Na^+]_o}{[K^+]_i + \alpha[Na^+]_i}\right) \qquad (6\text{-}10)$$

Figure 6-8 shows that when α is zero—that is, when the membrane is impermeable to Na^+—Equation 6-10 reduces to the Nernst equation for K^+ (Equation 6-4), and the plot

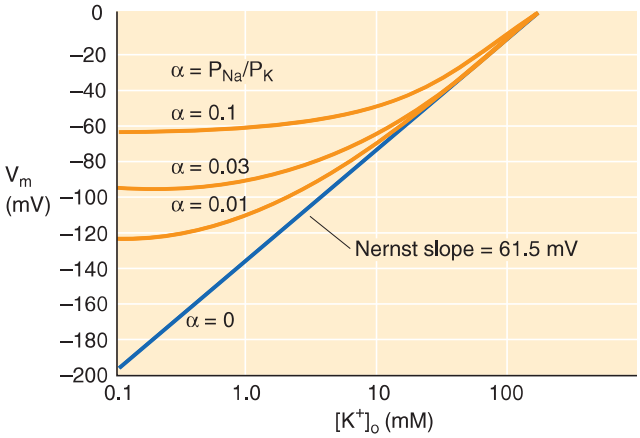

Figure 6-8 Dependence of the resting membrane potential on $[K^+]_o$ and on the P_{Na}/P_K ratio, α. The *blue line* describes an instance in which there is no Na^+ permeability (i.e., $P_{Na}/P_K = 0$). The three *orange curves* describe the V_m predicted by Equation 6-10 for three values of α greater than zero and assumed values of $[Na^+]_o$, $[Na^+]_i$, and $[K^+]_i$ for skeletal muscles, as listed in Table 6-1. The deviation of these orange curves from linearity is greater at low values of $[K^+]_o$, where the $[Na^+]_o$ is relatively larger.

of V_m versus the logarithm of $[K^+]_o$ is linear. If we choose an α of 0.01, however, the plot bends away from the ideal at low $[K^+]_o$ values. This bend reflects the introduction of a slight permeability to Na^+. As we increase this P_{Na} further by increasing α to 0.03 and 0.1, the curvature becomes even more pronounced. Thus, as predicted by Equation 6-10, increasing the permeability of Na^+ relative to K^+ tends to shift V_m in a positive direction, toward E_{Na}. In some skeletal muscle cells, an α of 0.01 best explains the experimental data.

The constant-field equation (Equation 6-9) and simplified relationships derived from it (e.g., Equation 6-10) show that steady-state V_m depends on the concentrations of all permeant ions, weighted according to their relative permeabilities. Another very useful application of the constant-field equation is determination of the ionic selectivity of channels. If the *I-V* relationship of a particular channel is determined in the presence of known gradients of two different ions, one can solve Equation 6-10 to obtain the permeability ratio, α, of the two ions from the measured value of the reversal potential, V_{rev}.

In general, the resting potential of most vertebrate cells is dominated by high permeability to K^+, which accounts for the observation that the resting V_m is typically close to E_K. The resting permeability to Na^+ and Ca^{2+} is normally very low. Skeletal muscle cells, cardiac cells, and neurons typically have resting membrane potentials ranging from –60 to –90 mV. As discussed in Chapter 7, excitable cells generate action potentials by transiently increasing Na^+ or Ca^{2+} permeability and thus driving V_m in a positive direction toward E_{Na} or E_{Ca}. A few cells, such as vertebrate skeletal muscle fibers, have high permeability to Cl^-, which therefore contributes to the resting V_m. This high permeability also explains why the Cl^- equilibrium potential in skeletal muscle is essentially equivalent to the resting potential (Table 6-1).