

CHAPTER 6

Ionic Basis of the Resting Potential

At rest, a neuron has a stable electrical potential across its outer cell membrane, the inside being negative with respect to the outside. In the neuron, the intracellular potassium concentration is high compared with that in the extracellular fluid, whereas the intracellular concentrations of sodium and chloride are low. As a result, potassium tends to diffuse out of the cell and sodium and chloride tend to diffuse in. The tendency for potassium ions to move out of the cell and for chloride ions to move in is opposed by the membrane potential.

In this chapter we discuss the relations between concentration and potential, first for a model cell whose membrane is permeable only to potassium and chloride. In this cell, the concentration gradients and the membrane potential can be balanced exactly, so that there is no net flux of either ion across the membrane. The membrane potential is then equal to the equilibrium potential for both potassium and chloride. In the model cell, changing the extracellular potassium concentration changes the potassium equilibrium potential, and hence the membrane potential. In contrast, changing extracellular chloride concentration eventually leads to an equivalent change in intracellular chloride, so that the chloride equilibrium potential and the membrane potential are unchanged.

Real cells are also permeable to sodium. At rest, sodium ions constantly move into the cell, reducing the internal negativity of the membrane. As a result, potassium, being no longer in equilibrium, leaks out. If there were no compensation, these fluxes would lead to changes in the internal concentrations of sodium and potassium. However, the concentrations are maintained by the sodium–potassium exchange pump, which transports sodium out and potassium in across the cell membrane in a ratio of three parts sodium to two parts potassium. The resting membrane potential depends on the potassium equilibrium potential, the sodium equilibrium potential, the relative permeability of the cell membrane to the two ions, and the pump ratio. At the resting potential, the passive fluxes of sodium and potassium are exactly matched by the rates at which they are transported in the opposite direction. Because the sodium–potassium exchange pump transports more positive ions outward than inward across the membrane, it makes a direct contribution of several millivolts to the membrane potential.

The chloride equilibrium potential may be positive or negative relative to the resting membrane potential, depending on the chloride transport processes. Although the chloride distribution plays little role in determining the resting membrane potential, substantial chloride permeability is important in some cells for electrical stability.

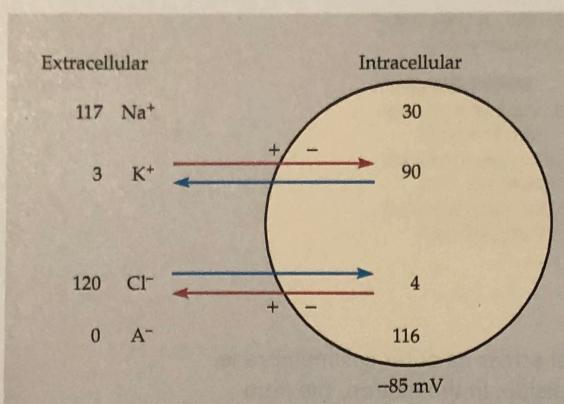


FIGURE 6.1 Ion Distributions in a Model Cell. The cell membrane is impermeable to Na^+ and the internal anion (A^-) and permeable to K^+ and Cl^- . The concentration gradient for K^+ tends to drive it out of the cell (upper blue arrow), while the potential gradient tends to attract K^+ into the cell (upper red arrow). In a cell at rest, the two forces are exactly in balance. Concentration and electrical gradients for Cl^- are in the reverse directions. Ion concentrations are expressed in millimolar (mM).

Electrical signals are generated in nerve cells and muscle fibers primarily by changes in permeability of the cell membrane to ions such as sodium and potassium. Increases in permeability allow ions to move inward or outward across the cell membrane down their electrochemical gradients. As we discussed in Chapter 4, permeability increases are due to activation of ion channels. Ions moving through the open channels change the charge on the cell membrane, and hence change the membrane potential. In order to understand how signals are generated, it is necessary first to understand how the standing ion gradients across the cell membrane determine the resting membrane potential.

A Model Cell

It is useful to begin with the idealized model cell shown in Figure 6.1. This cell contains potassium, sodium, chloride, and a large anion species, and it is bathed in a solution of sodium and potassium chloride. Other ions present in real cells (e.g., calcium or magnesium) are ignored for the moment, as their direct contributions to the resting membrane potential are negligible. The extracellular and intracellular ion concentrations in the model cell are similar to those found in frogs. In birds and mammals, ion concentrations are somewhat higher, while in marine invertebrates (e.g., squid) they are very much higher (Table 6.1). The model cell membrane is permeable to potassium and chloride, but not to sodium or to the internal anion. There are three major requirements for a cell to remain in a stable condition:

1. The intracellular and extracellular solutions must each be electrically neutral. For example, a solution of chloride ions alone cannot exist; their negative charges must be balanced by an equal number of positive charges on cations such as sodium or potassium.
2. The cell must be in osmotic balance. Otherwise water will enter or leave the cell, causing it to swell or shrink. Osmotic balance is achieved when the total concentration of solute particles inside the cell is equal to that on the outside.
3. There must be no net movement of any particular ion into or out of the cell.

Ionic Equilibrium

How are the concentrations of the permeant ions maintained in the model cell, and what electrical potential is developed across the cell membrane? Figure 6.1 shows that the two ions are distributed in reverse ratios—potassium is more concentrated on the inside of the cell, chloride on the outside. Imagine first that the membrane is permeable only to potassium. The question that arises immediately is why potassium ions do not diffuse out of the cell until the concentrations on either side of the cell membrane are equal. The answer is that the process is self-limiting. As the potassium ions diffuse outward, positive charges accumulate on the outer surface of the membrane and excess negative charges are left on the inner surface. As a result, an electrical potential develops across the membrane, with the inside being negative with respect to the outside.

The electrical gradient slows the efflux of positively charged potassium ions, and when the potential becomes sufficiently large, further net efflux of potassium is stopped. The potential at which this occurs is called the **potassium equilibrium potential (E_K)**. At E_K , the effects of the concentration gradient and the potential gradient on ion flux through the membrane balance one another exactly. Individual potassium ions still enter and leave the cell, but no net movement occurs. The potassium ions are in equilibrium.

The conditions for potassium to be in equilibrium across the cell membrane are the same as those described in Chapter 4 for

TABLE 6.1

Concentrations of ions inside and outside freshly isolated axons of squid

Ion	Concentration (mM)		
	Axoplasm	Blood	Seawater
Potassium	400	20	10
Sodium	50	440	460
Chloride	60	560	540
Calcium	0.1 μM^a	10	10

Source: After A. L. Hodgkin, 1964. *The Conduction of the Nervous Impulse*. Liverpool University Press, Liverpool, based on A. L. Hodgkin, 1951. *Biol. Rev.* 26: 339-409.

^aIonized intracellular calcium from P. F. Baker et al., 1971. *J. Physiol.* 218: 709-755.

maintaining zero net flux through an individual channel in a membrane patch. There, a concentration gradient was balanced by a potential applied to the patch pipette. The important difference here is that the ion flux itself produces the required transmembrane potential. In other words, equilibrium in the model cell is automatic and inevitable. Recall from Chapter 4 that the potassium equilibrium potential is given by the Nernst equation:

$$E_K = \frac{RT}{zF} \ln \frac{[K]_o}{[K]_i} = 58 \log \frac{[K]_o}{[K]_i}$$

where $[K]_o$ and $[K]_i$ are the external and internal potassium ion concentrations, respectively. For the cell shown in Figure 6.1, at 25°C E_K is $58 \log (1/30) = -85$ mV. Suppose now that, in addition to potassium channels, the membrane has chloride channels. Because for an anion $z = -1$, the equilibrium potential for chloride is:

$$E_{Cl} = -58 \log \frac{[Cl]_o}{[Cl]_i}$$

or from the properties of logarithmic ratio

$$E_{Cl} = 58 \log \frac{[Cl]_i}{[Cl]_o}$$

In our model cell, the chloride concentration ratio is again 1/30 and E_{Cl} is also -85 mV. As with potassium, the membrane potential of -85 mV balances exactly the tendency for chloride to move down its concentration gradient, in this case *into* the cell.

In summary, the tendency both for potassium ions to leave the cell and for chloride ions to diffuse inward is opposed by the membrane potential. Because the concentration ratios for the two ions are of exactly the same magnitude (1:30), their equilibrium potentials are exactly the same. As potassium and chloride are the only two ions that can move across the membrane and both are in equilibrium at -85 mV, the model cell can exist indefinitely without any net gain or loss of ions.

Electrical Neutrality

The charge separation across the membrane of our model cell means there is an excess of anions inside the cell and of cations outside. This appears to violate the principle of electrical neutrality but, in fact, does not. Potassium ions diffusing outward collect as excess cations against the outer membrane surface, leaving excess anions closely attracted to the inner surface. Both the potassium ions and the counter ions they leave behind are, in effect, removed from the intracellular bulk solution, leaving it neutral. Similarly, chloride ions diffusing inward add to the collection of excess anions on the inner surface of the membrane and leave counterions in the outer charged layer, so the extracellular solution remains neutral as well. The outer layer of cations and inner layer of anions, of equal and opposite charges, are not in free solution but are held to the membrane surface by mutual attraction. Thus, the membrane acts as a capacitor, separating and storing charge. This does not mean that any given anion or cation is locked in position against the membrane. Ions in the charged layer interchange freely with those in the bulk solution. The point is that although the identities of the ions in the layer are constantly changing, their total number remains constant, and the bulk solution stays neutral.

Another question we might ask about charge separation is whether or not the number of ions accumulated in the charged layer represents a significant fraction of the total number of ions in the cell. The answer is that it does not. If we consider our model cell to have a radius of 25 μm, then at a concentration of 120 millimolar (mM) there are roughly 4×10^{12} cations and an equal number of anions in the cytoplasm. At a membrane potential of -85 mV the amount of charge separated by the membrane is about 5×10^{11} univalent ions/cm² (see Chapter 8). Our cell has a surface area of about 8×10^{-5} cm², so there are approximately 4×10^7 negative ions collected at the inner surface of the membrane, which is 1/100,000 the number in free solution.

The Effect of Extracellular Potassium and Chloride on Membrane Potential

In neurons and in many other cells, the steady-state resting membrane potential is sensitive to changes in extracellular potassium concentration but is relatively unaffected by changes in extracellular chloride. To understand how this comes about, it is useful to consider the consequences of such changes in the model cell. We will assume throughout this discussion that the volume of the extracellular fluid is infinitely large relative to the volume of the cell. Thus, movements of ions and water into or out of the cell have no significant effect on extracellular concentrations.

Figure 6.2A shows the changes in intracellular composition and membrane potential that result from increasing extracellular potassium from 3 mM to 6 mM. The extracellular sodium concentration is reduced by 3 mM to keep the osmolarity unchanged. The increase in extracellular potassium reduces the concentration gradient for outward potassium movement, while initially leaving the electrical gradient unchanged. As a result, there will be a net inward movement of potassium ions. As positive charges accumulate on its inner surface, the membrane is depolarized. This in turn means that chloride ions are no longer in equilibrium and they move into the cell as well. Potassium and chloride entry continue until a new equilibrium is established, and both ions are at a new concentration ratio consistent with the new membrane potential, in this example -68 mV.

Potassium and chloride entry is accompanied by entry of water to maintain osmotic balance, resulting in a slight increase in cell volume. When the new equilibrium is reached, intracellular potassium has increased in concentration from 90 mM to 91 mM, intracellular chloride from 4 mM to 7.9 mM, and the cell volume has increased by 3.5%.

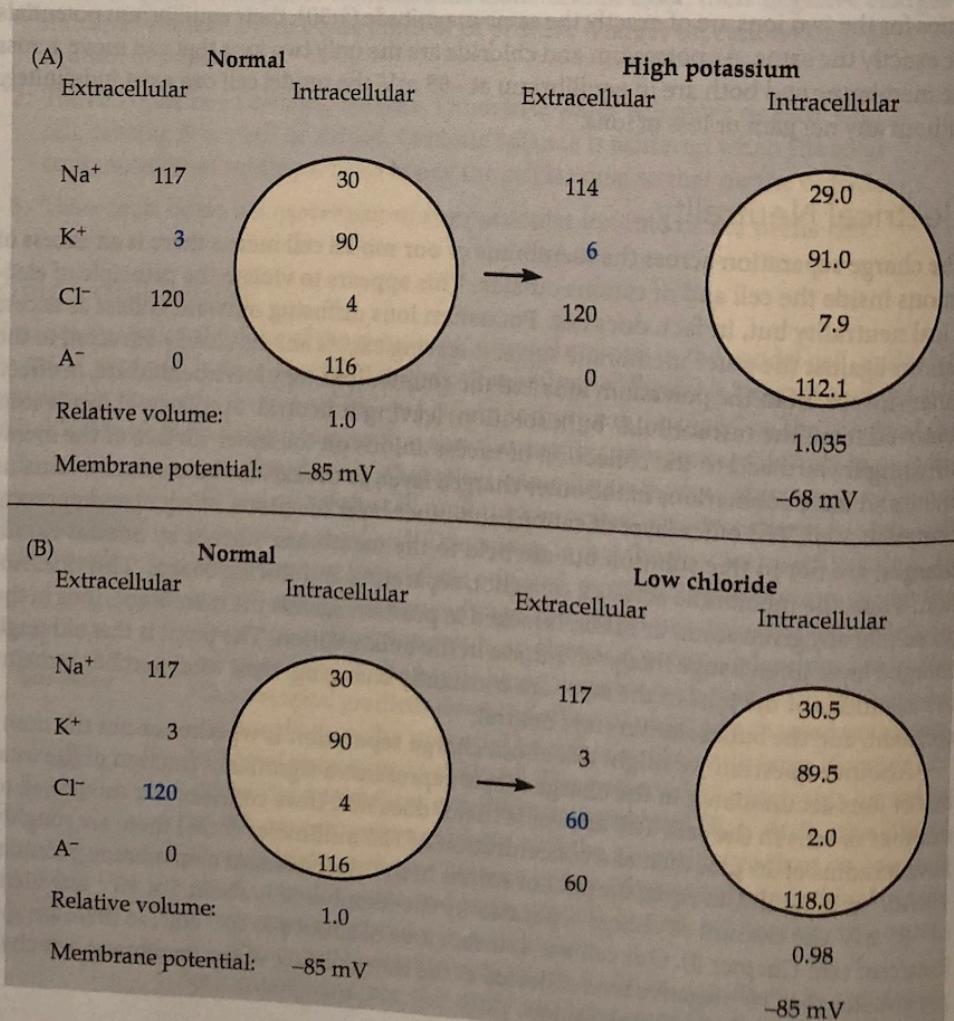


FIGURE 6.2 Effects of Changing Extracellular Ion Composition
on intracellular ion concentrations and membrane potential. (A) When extracellular K^+ is doubled, a corresponding reduction in extracellular Na^+ keeps osmolarity constant. (B) Half the extracellular Cl^- is replaced by an impermeant anion, A^- . Ion concentrations are mM, and extracellular volumes are assumed to be very large with respect to cell volumes so that fluxes into and out of the cell do not change extracellular concentrations.

At first glance it seems that more chloride than potassium has entered the cell, but think what the concentrations would be if the cell did *not* increase in volume: The concentrations of both ions would be greater than the indicated values by 3.5%. Thus, the intracellular chloride concentration would be about 8.2 mM (instead of 7.9 mM after the entry of water), and intracellular potassium would be about 94.2 mM—both being 4.2 mM higher than in the original solution. In other words, we can think first of potassium and chloride entering in equal quantities (except for the trivial difference required to change the charge on the membrane) and then of water following to achieve the final concentrations shown in the figure.

Similar considerations apply to changes in extracellular chloride concentration, but with a marked difference: When the new steady state is finally reached, the membrane potential is essentially unchanged. The consequences of a 50% reduction in extracellular chloride concentration are shown in Figure 6.2B, in which 60 mM of chloride in the solution bathing the cell is replaced by an impermeant anion. Chloride leaves the cell, depolarizing the membrane toward a new chloride equilibrium potential (-68 mV). Potassium, no longer being in equilibrium, leaves as well. As in the previous example, potassium and chloride leave the cell in equal quantities (accompanied by water). Because the intracellular concentration of potassium is high, the fractional change in concentration produced by the efflux is relatively small. However, the efflux of chloride causes a sizable fractional change in the intracellular chloride concentration, and hence in the chloride equilibrium potential. As chloride continues to leave the cell, the equilibrium potential returns toward its original value of -85 mV. The process continues until the chloride and potassium equilibrium potentials are again equal and the membrane potential is restored.

¹Bernstein, J. 1902. *Pflügers Arch.* 92: 521–562.

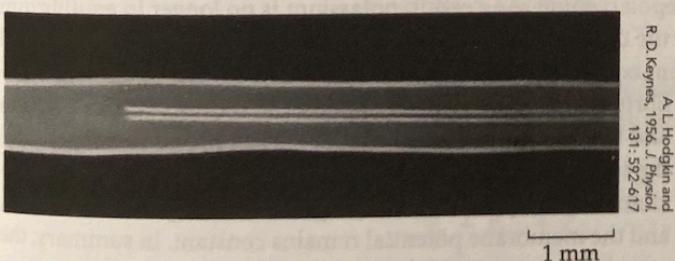
²Young, J. Z. 1937. *Q. J. Microsc. Sci.* 78: 367–387.

Membrane Potentials in Squid Axons

The idea that the resting membrane potential is the result of an unequal distribution of potassium ions between the extracellular and intracellular fluids was first proposed by Julius Bernstein¹ in 1902. He could not test this hypothesis directly, however, because there was no satisfactory way of measuring membrane potential. It is now possible to measure membrane potential accurately and to see whether changes in external and internal potassium concentrations produce the potential changes predicted by the Nernst relation. The first such experiments were done on giant axons that innervate the mantle of the squid. The axons are up to 1 mm in diameter,² and their large size permits the insertion of recording electrodes into their cytoplasm to measure transmembrane potential directly (Figure 6.3A). Furthermore,

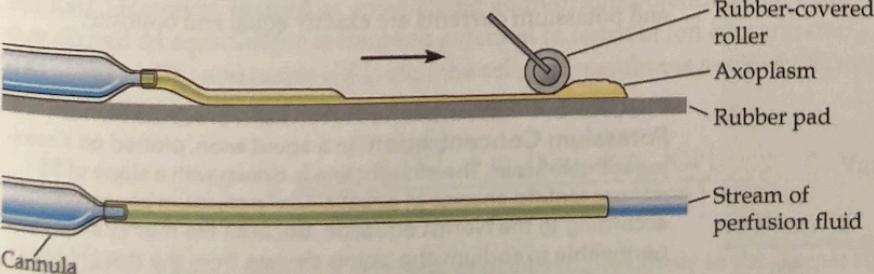
FIGURE 6.3 Recording from a Squid Axon. (A) Isolated squid giant axon with axial recording electrode inside. (B) Extrusion of axoplasm from the axon, which is then cannulated and perfused internally. (C) Comparison of recordings before perfusion ("Intact") and after perfusion ("Perfused") shows that the resting and action potentials are unaffected by removal of the axoplasm. (B and C after P. F. Baker et al., 1962. *J. Physiol.* 164: 330–354.)

(A)

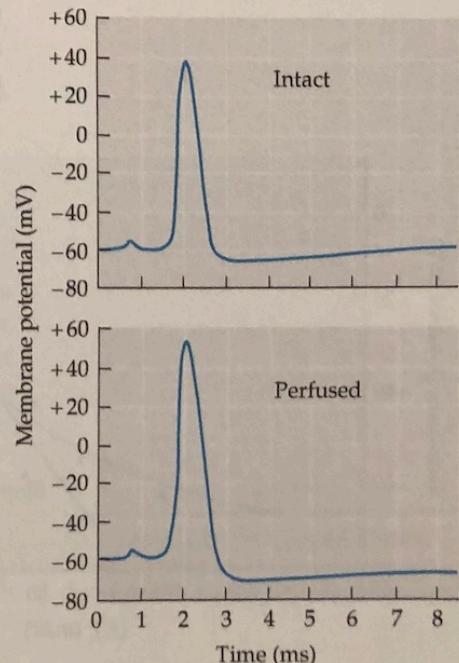


R. D. Keynes, 1956. *J. Physiol.* 131: 592–617
A. L. Hodgkin and

(B)



(C)



they are remarkably resilient and continue to function even when their axoplasm has been squeezed out with a rubber roller and replaced with an internal perfusate (Figure 6.3B,C)! Thus their internal as well as external ionic composition can be controlled.

The concentrations of some of the major ions in squid blood and in the axoplasm of the squid nerves are given in Table 6.1 (several ions, including magnesium and internal anions, are omitted). Experiments on isolated axons are usually done in seawater, with the ratio of intracellular to extracellular potassium concentrations being 40:1. In these conditions the membrane potential is -65 to -70 mV, considerably less negative than the potassium equilibrium potential of -93 mV, but more negative than the chloride equilibrium potential, which is about -55 mV.

Bernstein's hypothesis was tested by measuring the resting membrane potential and comparing it with the potassium equilibrium potential at various extracellular potassium concentrations. As with our model cell, these changes would not be expected to produce a significant change in internal potassium concentration. From the Nernst equation, changing the concentration ratio by a factor of ten should change the membrane potential by 58 mV at room temperature. The results of an experiment on squid axon in which the external potassium concentration was changed are shown in Figure 6.4. The external concentration is plotted on a logarithmic scale on the abscissa and the membrane potential on the ordinate. The expected slope of 58 mV per tenfold change in extracellular potassium concentration is realized only at relatively high concentrations (solid straight line), with the slope becoming less and less steep as external potassium is reduced. This result indicates that the potassium ion distribution is not the only factor contributing to the membrane potential.

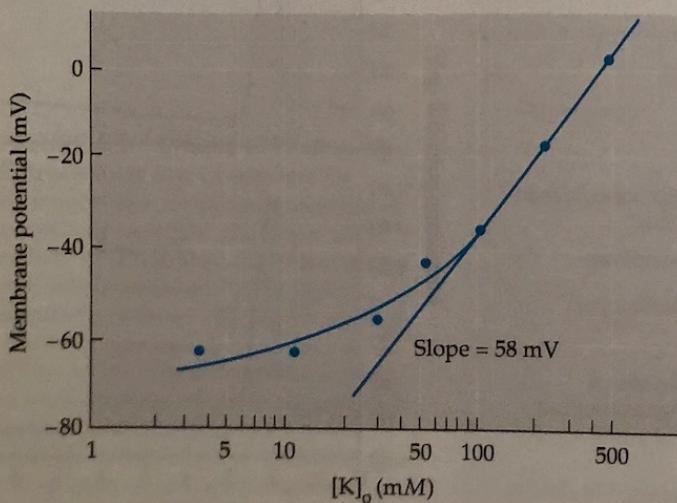
The Effect of Sodium Permeability

From the experiments on squid axon we can conclude that the hypothesis put forward by Bernstein in 1902 is almost correct; the membrane potential is strongly, but not exclusively, dependent on the potassium concentration ratio. We can explain the deviation from the Nernst relation shown in Figure 6.4 simply by abandoning the notion that the membrane is impermeable to sodium. Real nerve cell membranes, in fact, have a permeability to sodium that ranges between 1% and 10% of their permeability to potassium.

To consider the effect of sodium permeability, we begin with our model cell (see Figure 6.1) and, for the moment, ignore any movement of chloride ions. The Nernst equation tells us that sodium would be in equilibrium at a membrane potential of $+34$ mV (E_{Na}), far from the actual membrane potential of -85 mV. So if we make the membrane permeable to sodium, both the concentration gradient and the membrane potential tend to drive sodium into the cell. As sodium ions enter the cell, they accumulate on the inner surface of the membrane, causing depolarization. As a result, potassium is no longer in equilibrium and potassium ions leave the cell. As depolarization progresses, the membrane potential moves closer to the sodium equilibrium potential and farther from the potassium equilibrium potential.

As this happens, the sodium influx decreases and the potassium efflux increases. The process continues until the influx of sodium is exactly balanced by the efflux of potassium. At that point there is no further charge accumulation, and the membrane potential remains constant. In summary, the membrane potential lies between the potassium and sodium equilibrium potentials and is the potential at which the sodium and potassium currents are exactly equal and opposite.

FIGURE 6.4 Membrane Potential versus External Potassium Concentration in a squid axon, plotted on a semi-logarithmic scale. The straight line is drawn with a slope of 58 mV per tenfold change in extracellular potassium concentration, according to the Nernst equation. Because the membrane is also permeable to sodium, the points deviate from the straight line, especially at low potassium concentrations. (After A. L. Hodgkin and P. Horowicz, 1959. *J. Physiol.* 145: 405-432.)



Chloride ions participate in the process as well, but as we have already seen, there is ultimately an adjustment in intracellular chloride concentration in the model cell, so that the chloride equilibrium potential matches the new membrane potential. As the cation fluxes reach a balance, the intracellular chloride concentration increases until there is no net chloride flux across the membrane.

The Constant Field Equation

To determine the exact membrane potential in our model cell, we have to consider the individual ion currents across the membrane. The inward sodium current (I_{Na}) depends on the driving force for sodium, which is the difference between the membrane potential and the sodium equilibrium potential, $V_m - E_{Na}$ (see Chapter 4). The sodium current also depends on the sodium conductance of the membrane, or g_{Na} . The sodium conductance is a measure of the ease with which sodium can pass through the membrane and depends on the number of open sodium channels—the more open channels, the greater the conductance. So the sodium current is:

$$I_{Na} = g_{Na}(V_m - E_{Na})$$

The same relationship holds for potassium and chloride:

$$I_K = g_K(V_m - E_K)$$

$$I_{Cl} = g_{Cl}(V_m - E_{Cl})$$

If we assume that chloride is in equilibrium, so that $I_{Cl} = 0$, then for the membrane potential to remain constant, the potassium and sodium currents must be equal and opposite:

$$g_K(V_m - E_K) = -g_{Na}(V_m - E_{Na})$$

It is useful to examine this relationship in more detail. Suppose g_K is much larger than g_{Na} . Then, if the currents are to be equal, the driving force for potassium efflux must be much smaller than that for sodium entry. In other words, the membrane potential must be much closer to E_K than to E_{Na} . Conversely, if g_{Na} is relatively large, the membrane potential will be closer to E_{Na} .

By rearranging the equation we arrive at an expression for the membrane potential:

$$V_m = \frac{g_K E_K + g_{Na} E_{Na}}{g_K + g_{Na}}$$

If, for some reason, chloride is not at equilibrium, then chloride currents across the membrane must be considered and the equation becomes slightly more complicated:

$$V_m = \frac{g_K E_K + g_{Na} E_{Na} + g_{Cl} E_{Cl}}{g_K + g_{Na} + g_{Cl}}$$

These ideas were developed originally by Goldman,³ and independently by Hodgkin and Katz.⁴ However, instead of considering equilibrium potentials and conductances, they derived an equation for membrane potential in terms of ion concentrations outside ($[K]_o$, $[Na]_o$, $[Cl]_o$) and inside ($[K]_i$, etc.) the cell, and membrane permeability to each ion (p_K , p_{Na} , and p_{Cl}):

$$V_m = 58 \log \frac{p_K [K]_o + p_{Na} [Na]_o + p_{Cl} [Cl]_o}{p_K [K]_i + p_{Na} [Na]_i + p_{Cl} [Cl]_o}$$

Note that the chloride ratios are reversed, as occurred previously in the Nernst equation, because the chloride valence is -1.

³Goldman, D. E. 1943. *J. Gen. Physiol.* 27: 37-60.

⁴Hodgkin, A. L., and Katz, B. 1949. *J. Physiol.* 108: 37-77.

As before, if chloride is in equilibrium, the chloride terms disappear. This equation is sometimes called the GHK equation for its originators, and is also known as the **constant field equation**, because one of the assumptions made in arriving at the expression was that the voltage gradient, or “field,” across the membrane is uniform. The constant field equation is entirely analogous to the previous equation and makes the same predictions: When the permeability to potassium is very high relative to the sodium and chloride permeabilities, the sodium and chloride terms become negligible and the membrane potential approaches the equilibrium potential for potassium: $V_m = 58 \log ([K]_i / [K]_o)$. Increasing sodium permeability causes the membrane potential to move toward the sodium equilibrium potential.

The constant field equation provides us with a useful general principle to remember: The membrane potential depends on the relative conductances (or permeabilities) of the membrane to the major ions, and on the equilibrium potentials for those ions. In real cells the resting permeabilities to potassium and chloride are relatively high. Hence, the resting membrane potential is close to the potassium and chloride equilibrium potentials. When sodium permeability is increased, as occurs during an action potential (see Chapter 7) or an excitatory postsynaptic potential (see Chapter 11), the membrane potential moves toward the sodium equilibrium potential.

The Resting Membrane Potential

As useful as the constant field equation is, it does not provide us with an accurate description of the resting membrane potential, because the requirement for zero net current across the membrane is not, in itself, adequate. Instead, for the cell to remain in a stable condition, *each* individual ion current must be zero. As a result, under the conditions of the constant field equation, the cell would gradually fill up with sodium and chloride and lose potassium. In real cells, intracellular sodium and potassium concentrations are kept constant by a sodium-potassium exchange pump (sodium-potassium ATPase; see Chapter 9). As sodium and potassium leak into and out of the cell, the pump transports a matching amount of each ion in the opposite direction (Figure 6.5). Thus, metabolic energy is used to maintain the cell in a **steady state**.

In order to have a more complete and accurate description of the resting membrane potential, we must consider both the passive ion fluxes and the activity of the pump. Again, we first consider the currents carried by passive fluxes of sodium and potassium across the membrane:

$$I_{Na} = g_{Na}(V_m - E_{Na})$$

$$I_K = g_K(V_m - E_K)$$

We no longer assume that the sodium and potassium currents are equal and opposite, but if we know how they are related we can, as before, obtain an equation for the membrane potential in terms of the sodium and potassium equilibrium potentials and their relative conductances. The relationship between the sodium and potassium currents is given by the characteristics of the pump. Because it keeps intracellular sodium and potassium concentrations constant by transporting the ions in the ratio of 3 Na to 2 K (see Chapter 9), it follows that the passive ion fluxes must be in the same ratio: $I_{Na}:I_K = 3:2$. So we can write:

$$\frac{I_{Na}}{I_K} = \frac{g_{Na}(V_m - E_{Na})}{g_K(V_m - E_K)} = -1.5$$

The ratio is negative because the sodium and potassium currents are flowing in opposite directions. By rearranging the equation we get an expression for the membrane potential:

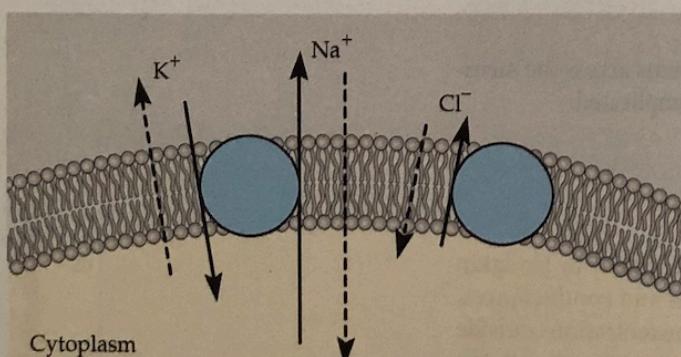


FIGURE 6.5 Passive Ion Fluxes and Pumps in a Steady State.

State. Net passive ion movements across the membrane are indicated by dashed arrows, and transport systems by solid arrows and circles. Lengths of arrows indicate the magnitudes of net ion movements. Total flux is zero for each ion. For example, net inward leak of Na^+ is equal to the rate of outward transport. $Na^+ : K^+$ transport is coupled with a ratio of 3:2. In any particular cell, Cl^- transport may be outward (as shown) or inward.

$$V_m = \frac{1.5g_K E_K + g_{Na} E_{Na}}{1.5g_K + g_{Na}}$$

The equation is similar to the expression derived previously for the model cell and makes the same kinds of predictions. As before, the membrane potential depends on the relative magnitudes of g_K and g_{Na} . The difference is that the potassium term is multiplied by a factor of 1.5. Because of this factor, the membrane potential is closer to E_K than would otherwise be the case. Thus, the driving force for sodium entry is increased and that for potassium influx reduced. As a result, the passive fluxes are in a ratio of 3 Na to 2 K rather than 1:1.

In summary, the real cell differs from the model cell in that the resting membrane potential is the potential at which the passive influx of sodium is 1.5 times the passive efflux of potassium, rather than the potential at which the two fluxes are equal and opposite. The passive inward and outward currents are determined by the equilibrium potentials and conductances for the two ions; the required ratio of 3:2 is determined by the transport characteristics of the pump.

The problem of finding an expression for the resting membrane potential of real cells, taking into account the transport activity, was first considered by Mullins and Noda,⁵ who used intracellular microelectrodes to study the effects of ionic changes on membrane potential in muscle. Like Goldman, Hodgkin, and Katz, they derived an expression for membrane potential in terms of permeabilities and concentrations. The result is equivalent to the constant field equation we have just derived using conductances and equilibrium potentials:

$$V_m = 58 \log \frac{rp_K [K]_o + p_{Na} [Na]_o}{rp_K [K]_i + p_{Na} [Na]_i}$$

where r is the absolute value of the transport ratio (3:2). The equation provides an accurate description of the membrane potential when the cell is at rest—that is, when all the permeant ions are in a steady state.

Chloride Distribution

How do these considerations apply to chloride? As for all other ions, there must be no net chloride current across the resting membrane. As already discussed (see Figure 6.2B), chloride is able to reach equilibrium simply by an appropriate adjustment in internal concentration, without affecting the steady-state membrane potential. In many cells, however, there are transport systems for chloride as well (see Chapter 9). In squid axon and in muscle, chloride is transported actively into the cells; in many nerve cells, active transport is outward (see Figure 6.5). The effect of inward transport is to add an increment to the equilibrium concentration such that there is an outward leak of chloride equal to the rate of transport in the opposite direction.⁶ Outward transport has the reverse effect.

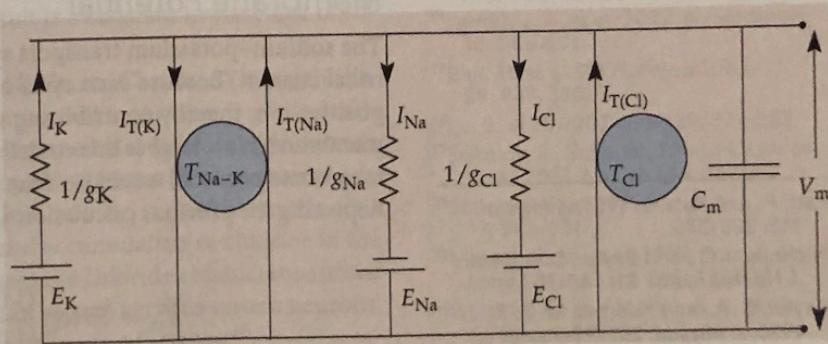
An Electrical Model of the Membrane

The characteristics of the nerve cell membrane endow it with the electrical properties illustrated in Figure 6.6. First, because the membrane is an insulating layer separating electrical charges on its inner and outer surfaces, it has the properties of a capacitor. In parallel with the capacitor are conductance pathways, represented by resistors that allow ion fluxes into and out of the cell. The

⁵Mullins, L. J., and Noda, K. 1963. *J. Gen. Physiol.* 47: 117-132.

⁶Martin, A. R. 1979. Appendix to Matthews, G., and Wickelgren, W. O. *J. Physiol.* 293: 393-414.

FIGURE 6.6 Electrical Model of the Steady-State Cell shown in Figure 6.2. E_K , E_{Na} , and E_{Cl} are the Nernst potentials for the individual ions. The individual ion conductances are represented by resistors, with a resistance of $1/g$ for each ion. The individual ion currents I_K , I_{Na} , and I_{Cl} are equal and opposite to the currents $I_{T(K)}$, $I_{T(Na)}$, and $I_{T(Cl)}$ supplied by the sodium-potassium exchange pump (T_{Na-K}) and the chloride pump (T_{Cl}), so that the net flux of each ion across the membrane is zero. The resulting membrane potential (V_m) determines the amount of charge stored on the membrane capacitor (C_m).



electrical resistance in each pathway is inversely related to the conductance for the ion in question: The greater the ion conductance, the lower the resistance to current flow. Passive ion currents through the resistors are driven by batteries that represent the equilibrium potentials for each of the ions. The passive currents are equal and opposite to the corresponding currents generated by the pumps, so that the net current across the membrane for each ion is zero.

Predicted Values of Membrane Potential

How do these considerations explain the relation between potassium concentration and membrane potential shown in Figure 6.4? This can be seen by using real numbers in the equations. In squid axon, the permeability constants for potassium and sodium are roughly in the ratio 1.0:0.04.⁷ We can use these relative values, together with the ion concentrations given in Table 6.1, to calculate the resting membrane potential in seawater:

$$V_m = 58 \log \frac{(1.5)(10) + (0.04)(460)}{(1.5)(400) + (0.04)(50)} = -73 \text{ mV}$$

Now we can see quantitatively why, when extracellular potassium is altered, the membrane potential fails to follow the Nernst potential for potassium. If, in the numerator of the equation, we look at the magnitude of the term involving extracellular potassium concentration ($1.5 \times 10 = 15 \text{ mM}$) and the term that involves extracellular sodium concentration ($0.04 \times 460 = 18.4 \text{ mM}$), we see that potassium contributes only about 45% of the total. Because of this, doubling the external potassium concentration does not double the numerator (as would happen in the Nernst equation), and as a consequence, the effect on the potential of changing extracellular potassium concentration is less than would be expected if potassium were the only permeant ion. When the external potassium concentration is raised to a high enough level (100 mM in Figure 6.4), the potassium term becomes sufficiently dominant for the current-voltage relation to approach the theoretical limit of 58 mV per tenfold change in concentration. This effect is enhanced by the fact that many potassium channels are voltage-activated (see Chapter 7). When the membrane is depolarized by increasing the extracellular potassium concentration, the voltage-activated channels open, thereby increasing the potassium permeability. As a result, the relative contribution of potassium to the membrane potential is increased still further.

In general, nerve cells have resting potentials of the order of -70 mV. In some cells, such as vertebrate skeletal muscle,⁸ the resting potential can be -90 mV or larger, reflecting a low ratio of sodium-to-potassium permeability. Glial cells in particular have a very low resting permeability to sodium, so their membrane potential is nearly identical to the potassium equilibrium potential (see Chapter 10). Other cells, such as leech ganglion cells⁹ and photoreceptors in the vertebrate retina,⁹ have relatively high membrane permeability to sodium and resting membrane potentials as small as -40 mV.

Contribution of the Sodium-Potassium Pump to the Membrane Potential

The sodium-potassium transport system is **electrogenic** (capable of generating an electrical current) because each cycle of the pump results in the net outward transfer of one positive ion, thereby contributing to the excess negative charge on the inner face of the membrane. How large is this contribution? An easy way to find out is to calculate what the membrane potential would be if the pump were *not* electrogenic or, in other words, if $r = 1$. Repeating the previous calculation with this condition gives the following:

$$V_m = 58 \log \frac{(1.0)(10) + (0.04)(460)}{(1.0)(400) + (0.04)(50)} = -67 \text{ mV}$$

⁷Fatt, P., and Katz, B. 1951. *J. Physiol.* 115: 320-370.

⁸Nicholls, J. G., and Baylor, D. A. 1968. *J. Neurophysiol.* 31: 740-757.

⁹Baylor, D. A., and Fuortes, M. G. F. 1970. *J. Physiol.* 207: 77-92.

The result is 6 mV less than the previous value, so the pump contributes -6 mV to the resting potential. In general, the size of the pump contribution depends on several factors, particularly the relative ion permeabilities. For a transport ratio of 3:2, the steady-state contribution to the resting membrane potential is limited to a maximum of about -11 mV.¹⁰ If the transport process is stopped, the electrogenic contribution disappears immediately and the membrane potential then declines gradually as the cell gains sodium and loses potassium.

It is interesting that the *rate* of transport does not appear in the equation, apart from the implicit requirement that it must match the passive ion fluxes. Theoretical calculations indicate that once that requirement is met, any further increase in sodium-potassium pump activity can be expected to have very little effect on the steady-state resting membrane potential.¹¹ This is largely because the transport system is dependent on intracellular sodium concentration (see Chapter 9). Any increase in pump rate results in hyperpolarization and depletion of intracellular sodium. As the sodium concentration falls, the pump rate and the membrane potential return toward their previous value.

What Ion Channels Are Associated with the Resting Potential?

The resting permeabilities of membranes to sodium, potassium, and chloride have been determined in many nerve cells. Underlying these is a wide variety of membrane channels that permit the passage of anions and cations into and out of the cell. However, the precise identification of the channels underlying these ionic **leak currents** in any specific cells is difficult. A significant fraction of the resting potassium current is likely to be through 2P potassium channels (see Chapter 5), which tend to be open at the resting membrane potential.^{12,13} In addition, many nerve cells have M-type potassium channels that are open at rest and closed by intracellular messengers (see Chapter 19). M-currents, together with accompanying h-currents, are responsible for most of the potassium leak in sympathetic ganglion cells.¹⁴ H-currents are carried by HCN (hyperpolarization-activated cyclic nucleotide-gated) channels that are activated by hyperpolarization—and some of which are open at normal resting potentials.¹⁵ HCN channels are cation channels with a sodium-to-potassium permeability ratio ($p_{\text{Na}}/p_{\text{K}}$) of about 0.25 and thus are responsible for a fraction of the sodium leak current as well. Other contributors to resting potassium permeability include channels activated by intracellular cations, namely sodium-activated and calcium-activated potassium channels. Finally, a few voltage-sensitive potassium channels associated with the action potential may be open at rest.

Apart from HCN channels, the major source of resting sodium permeability is the so-called NALCN (sodium leak channel, nonselective) channel that is open at rest.¹⁶ NALCN channels are virtually nonselective for monovalent cations ($p_{\text{Na}}/p_{\text{K}} \approx 1.1$), so that at normal resting potentials the main ion movement through the channels is inward sodium flux. An additional sodium influx occurs not through channels, but rather through sodium-dependent secondary active transport systems (see Chapter 9). Finally, tetrodotoxin has been shown to block a small fraction of the resting sodium conductance,⁸ indicating a contribution by voltage-activated sodium channels.

An unusual pathological leak current in skeletal muscle cells is found in a neuromuscular disease known as hypokalemic periodic paralysis. The current is unusual in that it does not involve ion movements through open channels. Instead, it is associated with cation fluxes through a normally occluded protein pore around the sodium channel S4 helix.¹⁷ This so-called omega current (see Chapter 7) is associated with a mutation in which charge-carrying arginine residues on the helix are replaced by smaller neutral amino acids, thereby allowing cations to permeate the pore. The result is a constant inward leak of sodium into the muscle cell.

The family of chloride channels of the CLC family (CLCs; see Chapter 5) is widely distributed in nerve and muscle. The presence of CLCs is important in that they serve to stabilize the membrane potential (see the next section). The channels also interact with chloride transport systems to determine intracellular chloride concentrations.^{18,19} For example, in embryonic hippocampal neurons, CLC expression is low, and E_{Cl} is positive to the resting membrane potential because of inward transport and accumulation of chloride in the cytoplasm. In adult neurons, expression of CLCs increases the chloride conductance of the membrane so that excess accumulation does not occur. In central nervous system neurons, CLCs can account for as much as 10% of the resting membrane conductance.²⁰

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- ¹⁰Martin, A. R., and Levinson, S. R. 1985. *Muscle Nerve* 8: 354-362.
- ¹¹Fraser, J. A., and Huang, C. L.-H. 2004. *J. Physiol.* 559: 459-478.
- ¹²Brown, D. A. 2000. *Curr. Biol.* 10: R456-459.
- ¹³Goldstein, S. A. N. et al. 2001. *Nat. Rev. Neurosci.* 2: 175-184.
- ¹⁴Lamas, J. A. et al. 2002. *Neuroreport* 13: 585-591.
- ¹⁵Biel, M. et al. 2009. *Physiol. Rev.* 89: 847-885.
- ¹⁶Lu, B. et al. 2007. *Cell* 129: 371-383.
- ¹⁷Sokolov, S., Scheuer, T., and Catterall, W. A. 2007. *Nature* 446: 76-78.
- ¹⁸Staley, K. et al. 1997. *Neuron* 17: 543-551.
- ¹⁹Meladinić, M. et al. 1999. *Proc. R. Soc. Lond., B, Biol. Sci.* 266: 1207-1213.
- ²⁰Gold, M. R., and Martin, A. R. 1983. *J. Physiol.* 342: 99-117.

Changes in Membrane Potential

It is important to keep in mind that this discussion of resting membrane potential is always in reference to *steady-state* conditions. For example, we have said that changing extracellular chloride concentration has little effect on membrane potential because the intracellular chloride concentration accommodates to the change. That is true in the long run, but the intracellular adjustment takes time, and while it is occurring, there is indeed a transient effect.

The steady-state potential is the baseline on which all changes in membrane potential are superimposed. How are such changes in potential produced? In general, transient changes, such as those that mediate signaling between cells in the nervous system, are the result of transient changes in membrane permeability. As we already know from the constant field equation, an increase in sodium permeability (or a decrease in potassium permeability) will move the membrane potential toward the sodium equilibrium potential, producing depolarization. Conversely, an increase in potassium permeability will produce hyperpolarization. Another ion of importance in signaling is calcium. Intracellular calcium concentration is very low, and in most cells E_{Ca} is greater than +150 mV. Thus, an increase in calcium permeability results in calcium influx and depolarization.

The role of chloride permeability in the control of membrane potential is of particular interest. As we have noted, chloride makes little contribution to the resting membrane potential. Instead, intracellular chloride concentration adjusts to the potential and is modified by whatever chloride transport mechanisms are operating in the cell membrane. The effect of a transient increase in chloride permeability can be either hyperpolarizing or depolarizing, depending on whether the chloride equilibrium potential is negative or positive to the resting potential. The equilibrium potential, in turn, depends on whether intracellular chloride is depleted or concentrated by the transport system. In either case, the change in potential is usually relatively small. Even so, an increase in chloride permeability can be important for the regulation of signaling because it tends to hold the membrane potential near the chloride equilibrium potential and thus attenuates changes in potential produced by other influences.

Stabilization of the membrane potential in this way is important for controlling the excitability of many cells, such as skeletal muscle fibers, that have a relatively high chloride permeability at rest. In such cells, a transient influx of positive ions causes less depolarization than would otherwise be the case, because it is countered by an influx of chloride through already open channels. This mechanism is of some significance, as illustrated by the fact that chloride channel mutations that reduce chloride conductance are responsible for several muscle diseases. The diseased muscles are hyperexcitable (myotonic) due to loss of the normal stabilizing influence of a high chloride conductance.^{21,22}

²¹Barchi, R. L. 1997. *Neurobiol. Dis.* 4: 254-264.

²²Cannon, S. C. 1997. *Trends. Neurosci.* 19: 3-10.

SUMMARY

- Nerve cells have high intracellular concentrations of potassium and low intracellular concentrations of sodium and chloride, so potassium tends to diffuse out of the cell and sodium and chloride tend to diffuse in. The tendency for potassium and chloride to diffuse down their concentration gradients is opposed by the electrical potential across the cell membrane.
- In a model cell that is permeable only to potassium and chloride, the concentration gradients can be balanced exactly by the membrane potential, so that there is no net flux of either ion across the membrane. The membrane potential is then equal to the equilibrium potential for both potassium and chloride.
- Changing the extracellular potassium concentration changes the potassium equilibrium potential and, hence, the membrane potential. Changing extracellular chloride concentration, by contrast, leads ultimately to a change in intracellular chloride, so that the chloride equilibrium potential and the membrane potential differ from their original values only transiently.
- In addition to being permeable to potassium and chloride, the cell membrane of real cells is permeable to sodium. As a result, there is a constant influx of sodium into the cell and an efflux of potassium. These fluxes are balanced exactly by active transport of the ions in opposite directions, in the ratio of 3 Na to 2 K. Under these circumstances, the membrane potential depends on the sodium equilibrium potential, the potassium equilibrium potential, the relative conductance of the membrane to the two ions, and the sodium-potassium exchange pump ratio.
- Because the sodium-potassium exchange pump transports more positive ions outward than inward across the membrane, it can make a direct contribution of several millivolts to the membrane potential.
- The chloride equilibrium potential may be positive or negative to the resting membrane potential, depending on chloride transport processes. Although the chloride distribution plays little role in determining the resting membrane potential, high chloride permeability is important for electrical stability.

Suggested Reading

- Hodgkin, A. L., and Katz, B. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol.* 108: 37–77. (The constant field equation is derived in Appendix A of this paper.)
- Mullins, L. J., and Noda, K. 1963. The influence of sodium-free solutions on membrane potential of frog muscle fibers. *J. Gen. Physiol.* 47: 117–132.