CHAPTER THREE

IONIC BASIS OF THE RESTING POTENTIAL

The electrical potential difference between the inside and the outside of a nerve cell membrane depends on the ionic concentration gradients across the cell membrane and the relative permeability of the membrane to the ions present. Using simple principles of physical chemistry, one can explain how resting membrane potentials arise in excitable cells. For a steady state to be maintained, the total distribution of ions on either side of a cell membrane must satisfy three major constraints: (1) The bulk solutions inside and outside the cell must each be electrically neutral; (2) the osmotic concentration of intracellular ions and molecules in solution must be equal to that in the extracellular fluid; and (3) there must be no net flux of any permeant ion across the membrane.

Each permeant ion species has quite different intracellular and extracellular concentrations and is subject to two separate gradients tending to drive it into or out of the cell: a concentration gradient and an electrical gradient. For example, potassium is more concentrated inside the cell than out, so outward movement of potassium ions along their concentration gradient would be expected. On the other hand, the inner surface of the membrane is negative with respect to the outside, tending to restrain the outward movement of positively charged ions. In normal resting cells, these concentration and electrical gradients are nearly in balance, so that the tendency of potassium ions to move out of the cell from high to low concentration is opposed almost exactly by the electrical gradient in the reverse direction. The membrane potential at which there is no net potassium flux is called the potassium equilibrium potential (E_K). The equilibrium potential for any ion, in terms of extracellular and intracellular ionic concentrations, is given by the Nernst equation.

Chloride concentration is higher outside the cell than inside, and this concentration gradient is again balanced by the membrane potential, the internal negativity tending to oppose inward movement of the negatively charged ion. The resting membrane potential is, however, determined mainly by the potassium concentration ratio because the internal chloride concentration, being low, can change to accommodate itself to changes in the resting potential.

Sodium is much more concentrated in the extracellular fluid than in the cell cytoplasm, so to oppose sodium entry the membrane would have to be positive on the inside; that is, the equilibrium potential for sodium (E_{Na}) is positive rather than negative. Thus, in a normal cell with a negative resting potential, the concentration gradient and membrane potential both favor inward movement of sodium. Although the resting membrane is only sparingly permeable to sodium, the inward sodium leak depolarizes the membrane slightly from the potassium equilibrium potential, so that there is an accompanying outward leak of potassium. To maintain a steady state in the face of these continual leaks, sodium is transported actively outward, and potassium inward, across the cell membrane.

The resting membrane potential depends on the relative permeabilities of the cell membrane to sodium and potassium. If the permeability is very much larger to potassium than to sodium, then the membrane potential will be very close to E_K. If the permeability to potassium is relatively smaller, then the membrane potential will be farther away from E_K and closer to E_{Na}. One way to express the dependence of membrane potential on cation concentrations and membrane permeabilities is the constant field equation. A more accurate description is provided by a steady-state equation that includes the contribution of the active transport processes for sodium and potassium.

These same conditions can be considered in terms of an electrical model of the membrane in which ionic equilibrium potentials are substituted for concentrations and membrane conductances for permeabilities.

The active-transport process for sodium and potassium involves a single protein, Na–K ATPase, that transports three sodium ions out of the cell and two potassium ions in for each molecule of ATP hydrolyzed. Other ions are also transported actively across the cell membrane, most of the transport processes being driven by the electrochemical gradient for sodium. In some cells chloride ions are transported outward and bicarbonate ions inward across the membrane by a process coupled to inward sodium movement. Other cells accumulate (rather than excrete) chloride, and at the same time accumulate potassium, in a similar way. Inward sodium movement is also coupled to proton excretion and to calcium extrusion. Calcium is also transported out of the cell by Ca–Mg ATPase.

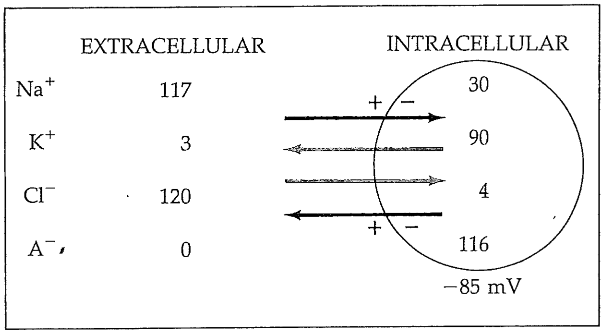
Electrical signals are generated in nerve cells and muscle fibers primarily by changes in the permeability of the cell membrane to ions such as sodium and potassium, allowing them to move inward or outward across the cell membrane along established electrochemical gradients. As we have seen in the previous chapter, such changes in permeability are associated with 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 such signals are generated, it is necessary to understand the nature of the established ionic gradients in the resting cell, and how such gradients come to exist.

It is useful to begin with the model cell shown in Figure 1. This cell contains potassium, sodium, chloride, and a large anion species and is bathed in a solution of sodium and potassium chloride. Other ions present in real cells, such as calcium or magnesium, are ignored for the moment, as their direct contributions to the membrane potential are negligible. The ionic concentrations inside and outside the model cell are similar to those found in frogs. In birds and mammals, ionic concentrations are somewhat higher; in marine invertebrates such as the squid, very much higher. In the model, the volume of the extracellular fluid is assumed to be infinitely large. Thus movements of ions and water into or out of the cell have no significant effect on extracellular concentrations. There are three major requirements for such a cell to exist:

1. The intracellular and extracellular solutions must each be electrically neutral. For example, a solution of chloride ions alone cannot exist; their charges must be balanced by an equal number of positive charges on cations such as sodium or potassium (otherwise electrical repulsion would literally blow the solution apart).
2. The cell must be in osmotic balance. If not, water will enter or leave the cell, causing it to swell or shrink, until osmotic balance is achieved. Osmotic balance is achieved when the total concentration of solute particles inside the cell is equal to that on the outside.
3. Finally, just as there can be no net movement of water, there must be no net movement of any particular ion into or out of the cell.

Another way of expressing the second and third conditions is to say that in the model cell, water and the permeating ions must be in EQUILIBRIUM. There is no equilibrium requirement for sodium and the internal anion, as both are impermeant. (We will see later that in real cells there is a small resting permeability to sodium and that, in general, none of the ions is in equilibrium.)

How are the equilibrium conditions satisfied for the permeant ions (potassium and chloride) and what electrical potential is developed across the cell membrane? Figure 1 shows that the two ions are distributed in reverse ratio; potassium is more concentrated on the inside of



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IONIC DISTRIBUTIONS IN A MODEL CELL. The cell membrane is impermeable to Na^+ and to the internal anion (A^-), and permeable to K^+ and Cl^-. The concentration gradient for K^+ tends to drive it out of the cell (blue arrow); the potential gradient tends to attract K^+ into the cell (black arrow). In a cell at rest the two forces are exactly in balance. Concentration and electrical gradients for Cl^- are in the reverse directions. Ionic concentrations are expressed in m*M*.

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 they cannot, because as they start to do so, a charge separation develops across the membrane; the resulting membrane potential then hinders further diffusion. As the ions leave the cell, positive charges accumulate on the outer surface of the membrane and an excess of negative charges is left on the inner surface. When the membrane potential becomes sufficiently large, further net efflux of potassium is stopped. The concentration gradient for potassium and the potential gradient across the membrane then balance one another exactly (arrows), and the potassium is said to be in electrochemical equilibrium. Individual ions may still enter and leave the cell, but no *net* movement occurs.

The conditions for potassium to be in equilibrium across the cell membrane are the same as those described in Chapter 2 for maintaining zero net flux through an individual channel in a membrane patch. There, a concentration gradient was balanced by a potential applied to the 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.

The Nernst equation

Exactly how large a membrane potential is required to balance a given potassium concentration difference across the membrane? This potential is called the POTASSIUM EQUILIBRIUM POTENTIAL (E_K). One guess might be that the potential would be proportional simply to the difference between the intracellular concentration $[K]_i$ and the extracellular concentration $[K]_o$; this is not quite right. It turns out instead that the required potential depends on the difference between the *logarithms* of the concentrations:

$$E_K = k(\ln[K]_o - \ln[K]_i)$$

The constant k is given by RT/zF, where R is the thermodynamic gas constant, T the absolute temperature, z the valence of the ion (in this case +1), and F the Faraday (the number of coulombs of electrical charge in one mole of monovalent ion). The answer, then, is

$$E_K = (RT/zF)(\ln[K]_o - \ln[K]_i)$$

which is the same as

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

This is the NERNST EQUATION for potassium. The expression RT/zF has the dimensions of volts and is equal to about 25 mV at room temperature (20°C). It is sometimes more convenient to use the logarithm to the base 10 (log) of the concentration ratio, rather than the natural logarithm. Then RT/zF must be multiplied by ln(10), or 2.306, which gives a value of 58 mV. At mammalian body temperature (37°C), this value increases to about 61 mV. For the cell shown in Figure 1, the concentration ratio for potassium is 1:30 and E_K is therefore 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 (E_{Cl}), in terms of the outside ($[Cl]_o$) and inside ($[Cl]_i$) concentrations, is given by

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

or (from the properties of logarithmic ratios):

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

In our model cell, $[Cl]_i/[Cl]_o$, like $[K]_o/[K]_i$, is 1/30 and E_{Cl} is therefore also −85 mV. As with potassium, this internal negativity balances exactly the tendency for chloride to move along its concentration gradient, in this case *into* the cell.

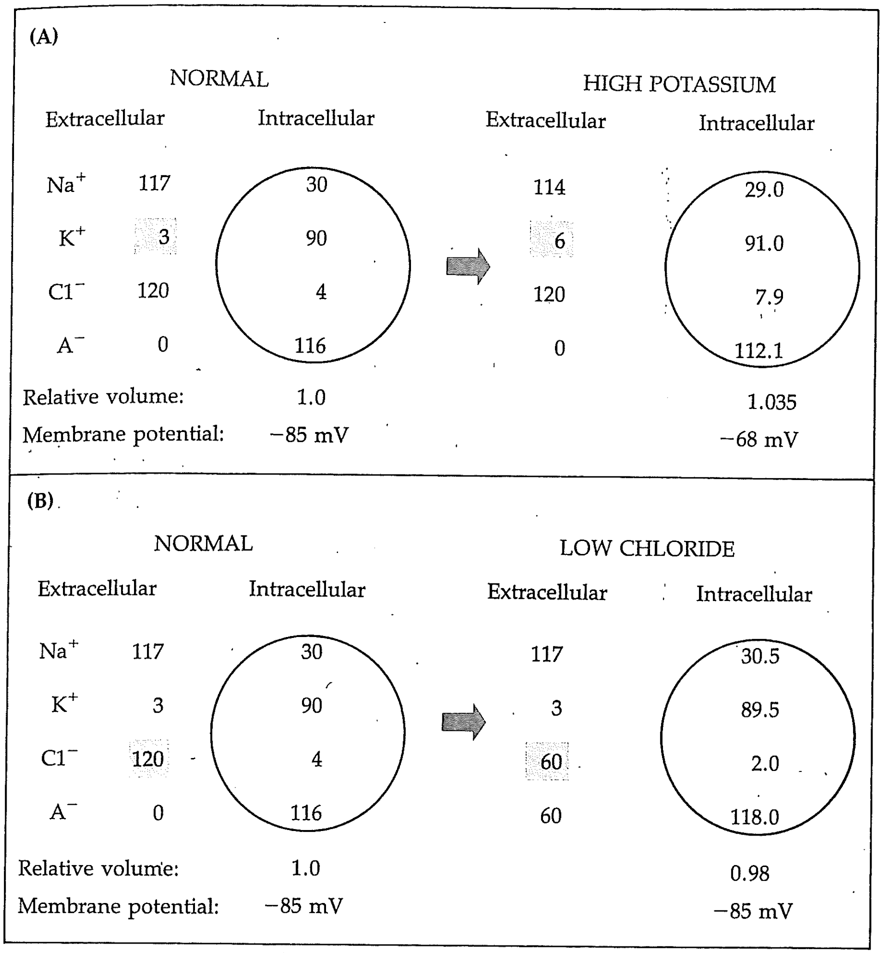
In summary, the tendency for potassium ions to leave the cell and for chloride ions to diffuse inward are both opposed by the membrane potential. Because the concentration ratios for the two ions are of exactly the same magnitude (1:30), their equilibrium potentials are exactly the same. As potassium 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 at the membrane means that there is an excess of anions at the inner surface and cations at the outer surface. This appears to violate the principle of electrical neutrality that we started out with, and indeed it does so in fact. Quantitatively, however, the charge separation produces differences in anionic and cationic concentrations so negligible that they could not possibly be measured. For example, if we consider our model cell to have a radius of 25 μm, then at a concentration of 120 m*M* there are 4×10^{12} cations and an equal number of anions inside the cell. At a resting membrane potential of −85 mV, we can calculate that there are approximately 4×10^7 excess negative charges on the inner surface of the membrane, which is 1/100,000 of the number of anions in free solution. Thus for every 100,000 cations inside the cell, there are 100,001 anions—a trivial difference.

The dependence of the resting potential on extracellular potassium

In neurons, and in many other cells, the membrane potential is sensitive to changes in extracellular potassium concentration but is relatively unaffected by changes in extracellular chloride concentration. To understand how this comes about it is useful to consider the consequences of such changes in the model cell. Figure 2A shows the changes in intracellular composition and membrane potential that result from increasing the extracellular potassium from 3 m*M* to 6 m*M*. This is done by replacing 3-m*M* NaCl with 3-m*M* KCl, thereby keeping the osmolarity unchanged with a total solute concentration of 240 m*M*. As a result of the increase in extracellular potassium concentration, the cell



2 EFFECTS OF CHANGING EXTRACELLULAR IONIC COMPOSITION on intracellular ionic concentrations and on membrane potential. (A) Extracellular K^+ is doubled, with a corresponding reduction in extracellular Na^+, to keep osmolarity constant. (B) Half the extracellular Cl^- is replaced by an impermeant anion, A^-. Ionic concentrations are in m*M*, 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.

is depolarized from −85 to −68 mV, the intracellular potassium concentration is increased slightly, and the intracellular chloride concentration almost doubled. How does this change come about? It is achieved by potassium and chloride entering the cell. First, when the external potassium concentration is increased, potassium is no longer in equilibrium. Consequently, potassium ions move inward. As positive charges accumulate on the inner surface of the membrane, chloride ions, being no longer in equilibrium, move in as well. This process of potassium and chloride entry continues until a new equilibrium is established, with both ions at a new concentration ratio, consistent with the new membrane potential.

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 (Figure 2A), intracellular potassium has increased in concentration from 90 to 91 m*M*, intracellular chloride from 4 to 7.9 m*M*, and the cell volume has increased by 3.5 percent. At first glance it seems that more chloride than potassium has entered the cell, but think what the concentrations would have been if the cell had *not* increased in volume: The concentrations of both ions would be 3.5 percent greater than the indicated values. Thus the intracellular chloride concentration would be about 8.2 m*M* (instead of 7.9 m*M* after the entry of water), and the intracellular potassium concentration would be about 94.2 m*M*, both 4.2 m*M* higher than in the original solution. In other words, we can think first of potassium and chloride entering in equal quantities, and then of water following to achieve the final concentrations shown in the figure.

Effect of changing extracellular chloride

Similar considerations apply to changes in the extracellular chloride concentration, but with a marked difference: No significant change occurs in membrane potential. The consequences of a 50 percent reduction in extracellular chloride concentration are shown in Figure 2B, in which 60 m*M* of chloride in the solution bathing the cell is replaced by an impermeant anion. Chloride leaves the cell because it is no longer in equilibrium and, as before, potassium and chloride move together, accompanied by water. Equal quantities of the ions leave the cell, and those remaining are concentrated slightly as the cell shrinks.

From Figure 2 we can arrive at some general conclusions: In the model cell, and in most real cells, changes in external potassium concentration result in changes in membrane potential, with the internal chloride concentration accommodating itself to the change; changes in external chloride concentration result in a similar accommodating change in internal chloride concentration without a major effect on membrane potential. This difference in the effects of a change in potassium concentration on the one hand, and a change in chloride concentration on the other, is due to the difference in their internal concentrations. When the external concentration of either is changed, the two ions move across the membrane in concert. Because the internal chloride concentration is low, relatively small ion fluxes are sufficient to adjust the chloride concentration ratio (and hence the chloride equilibrium potential) to the new conditions. In contrast, because of the high intracellular potassium concentration, small movements of KCl into or out of the cell have very little effect on the potassium concentration ratio. Thus potassium equilibrium can be restored only by an appropriate change in the membrane potential.

Membrane potentials in real cells

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 Bernstein in 1902.[1] He

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

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 3A). Further, 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 3B)! Thus their internal as well as external ionic composition can be controlled. A. L. Hodgkin, who together with A. F. Huxley initiated many experiments on squid axon (for which they later received the Nobel prize), has said:[3]

> It is arguable that the introduction of the squid giant nerve fiber by J. Z. Young in 1936 did more for axonology than any other single advance during the last forty years. Indeed a distinguished neurophysiologist remarked recently at a congress dinner (not, I thought, with the utmost tact), 'It's the squid that really ought to be given the Nobel Prize.'

The concentrations of some of the major ions in squid blood and in the axoplasm of the squid nerves are given in Table 1 (several ions, such as 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. If the membrane potential (V_m) were equal to the potassium equilibrium potential, it would be −93 mV. In fact, the measured membrane potential is considerably less negative (about −65 to −70 mV). On the other hand, the membrane potential is more negative than the chloride equilibrium potential, which is about −55 mV.

Bernstein's original hypothesis was tested not only by measuring the resting potential and comparing it with the potassium equilibrium potential, but also by examining how changes in extracellular potassium concentration affected the potential. (As with our model cell, such changes would be expected to produce no significant change in internal potassium concentration.) From the Nernst equation, changing the concentration ratio by a factor of 10 should change the membrane potential by 58 mV at room temperature. The results of such an experiment on squid axon, in which the external potassium concentration was changed, are shown in Figure 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

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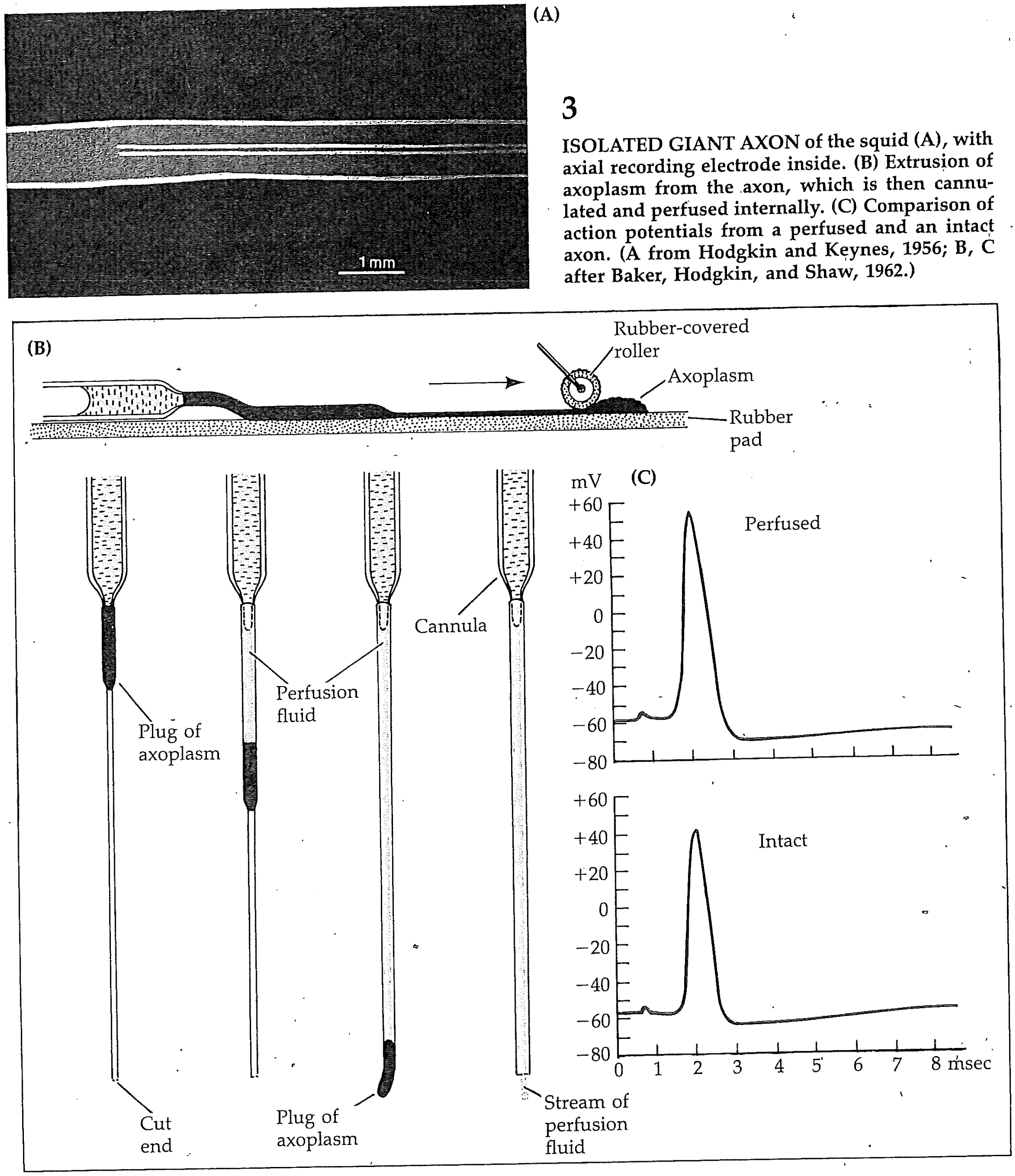
[2] Young, J. Z. 1936. *J. Microsc. Sci.* 78: 367–386.

[3] Hodgkin, A. L. 1973. *Proc. R. Soc. Lond. B* 183: 1–19.



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ISOLATED GIANT AXON of the squid (A), with axial recording electrode inside. (B) Extrusion of axoplasm from the axon, which is then cannulated and perfused internally. (C) Comparison of action potentials from a perfused and an intact axon. (A from Hodgkin and Keynes, 1956; B, C after Baker, Hodgkin, and Shaw, 1962.)

as the external potassium concentration is reduced. This result suggests that the potassium ion distribution is a major, but not the only, factor contributing to the membrane potential.

The effect of sodium permeability

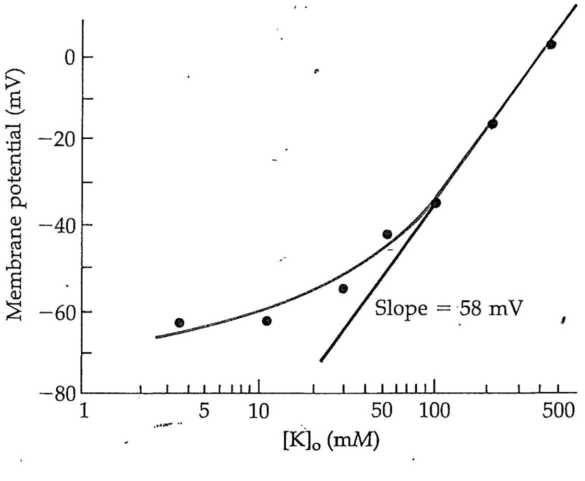
From the experiments on squid axon, then, we can conclude that the hypothesis made by Bernstein in 1902 was almost correct; the membrane potential is strongly but not exclusively dependent on the potas-

TABLE 1
CONCENTRATIONS OF IONS INSIDE AND OUTSIDE FRESHLY ISOLATED AXONS OF SQUID

	Concentration (mM)		
Ion	**Axoplasm**	**Blood**	**Seawater**
Potassium	400	20	10
Sodium	50	440	460
Chloride	60	560	540
Calcium	0.0001	10	10

Modified from Hodgkin (1964); ionized intracellular calcium from Baker, Hodgkin and Ridgeway (1971).

sium concentration ratio. How do we account for the deviation from the Nernst relation shown in Figure 4? Simply by abandoning the notion that the membrane is impermeable to sodium. Real cell membranes have, in fact, a permeability to sodium that ranges between 1 and 10 percent of their permeability to potassium. In the model, and in the squid axon, the concentration gradient and the membrane potential both tend to drive sodium into the cell. As sodium ions enter, the accumulation of positive charge depolarizes the membrane. As a result, potassium is no longer in equilibrium and potassium ions leave the cell. As we will discuss later, the intracellular sodium and potassium concentrations are maintained in the face of these constant leaks by a transport system that uses metabolic energy to pump sodium out of the cell and accumulate potassium. Unlike our original model, the cell is not in equilibrium; metabolic energy is used to maintain it in a STEADY STATE. For the moment we will ignore the transport system to consider the immediate question of how the membrane permeability to sodium affects the resting potential.



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MEMBRANE POTENTIAL AND EXTERNAL POTASSIUM concentration in squid axon, plotted on a semilogarithmic scale. The solid 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 (see text). (After Hodgkin and Keynes, 1955.)

The constant field equation

How to describe the membrane potential in a cell whose membrane is permeable to sodium, as well as to potassium and chloride, was considered originally by Goldman,[4] and later by Hodgkin and Katz.[5] The equation for the membrane potential is sometimes known, therefore, as the GHK EQUATION. The derivation of the equation is based on the idea that if the potential is constant, then the charge on the membrane must not change although ions leak across it. Thus currents carried by the inward leak of sodium, the outward leak of potassium, and any existing leak of chloride must add up to zero. Otherwise there would be a steady accumulation or loss of charge and hence a steady drift in membrane potential. Because the ionic currents depend on how readily the ions cross the membrane, the resulting equation includes the membrane permeability to each ion (p_K, p_{Na}, and p_{Cl}) as well as the ionic concentrations:

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

This equation is known also 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 equation looks like the Nernst equation, but with all the ions included instead of just one. This similarity extends to the fact that, unlike the cation concentrations, the internal chloride concentration appears in the numerator and the external concentration in the denominator. The equation differs from the Nernst equation in that it includes the ionic permeabilities in addition to concentrations.

If there is no net chloride current across the membrane, then the constant field equation can be written

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

where $b = p_{Na}/p_K$. It can be seen from the equation that if the permeability to sodium is very much smaller than that to potassium (i.e., if b is very small), then the membrane potential will be close to the potassium equilibrium potential. Conversely, if the permeability to sodium is relatively high (b is large), then the membrane potential will be near the sodium equilibrium potential. Thus the equation is consistent with what we might expect intuitively. It does not, however, provide a precise description of the resting membrane potential, because it fails to take into account the ion transport processes that maintain the intracellular sodium and potassium concentrations.

Active transport and the steady-state equation

Active transport of sodium and potassium across the cell membrane will be discussed shortly. For now it is sufficient to state that the major transport system for the two ions is the NA–K PUMP, which transports

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[4] Goldman, D. E. 1943. *J. Gen. Physiol.* 27: 37–60.

[5] Hodgkin, A. L. and Katz, B. 1949. *J. Physiol.* 108: 37–77.

three sodium ions out of the cell for each two potassium ions carried inward; in other words the Na:K coupling ratio of the pump is 3:2. Because the pump is not electrically neutral, it contributes directly to the membrane potential and is said to be ELECTROGENIC.

The relations between ionic permeabilities, ion transport, and membrane potential were considered in detail by Mullins and Noda,[6] who used intracellular microelectrodes to study the effects of ionic changes on membrane potential in muscle. They considered their experimental results in relation to a true steady-state condition, in which the net movement of *each* ion across the membrane is equal to zero. To use potassium as an example, its inside and outside concentrations determine E_K. The difference between the membrane potential and E_K, plus the permeability of the membrane to potassium, in turn determine the rate of outward potassium leak. In a steady state, this outward leak must be exactly equal in magnitude to the rate at which potassium is being transported into the cell. Accordingly, for a given concentration gradient, permeability, and transport rate, the resulting membrane potential must be positive to E_K by just the right amount, so that potassium ions leak out of the cell at the same rate as they are pumped in. The same type of argument applies to sodium.

Such considerations lead to a relatively straightforward expression for the membrane potential:

$$V_m = 58 \log \frac{r[\mathrm{K}]_o + b[\mathrm{Na}]_o}{r[\mathrm{K}]_i + b[\mathrm{Na}]_i}$$

where b, as before, is the ratio of sodium to potassium permeability (p_{Na}/p_K) and r is the coupling ratio of the transport system ($\mathrm{transport}_{Na}/\mathrm{transport}_K$). Because the net fluxes of the pertinent ions are zero, we can refer to the relation derived by Mullins and Noda as the STEADY-STATE EQUATION. Theoretically, it provides an exact description of the resting membrane potential, provided all the other permeant ions (e.g., chloride) are in a steady state.

Contribution of the transport system to membrane potential

How does the transport ratio for sodium relative to potassium (r) affect the steady-state membrane potential? If sodium and potassium are transported at the same rate ($r = 1$), the pump is not electrogenic and has no direct effect on resting potential; the membrane potential predicted by the steady-state equation is the same as that predicted by the constant field equation. If sodium is transported more rapidly than potassium ($r > 1$) the membrane is hyperpolarized; in the reverse situation ($r < 1$) the membrane is depolarized. The size of the pump contribution depends on a number of factors, particularly the relative ionic permeabilities. For a transport ratio of 3:2, steady-state contribution to the resting membrane potential is limited to about −11 mV.[7] If the transport process is stopped, the electrogenic contribution disap-

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[6] Mullins, L. J. and Noda, K. 1963. *J. Gen. Physiol.* 47: 117–132.

[7] Martin, A. R. and Levinson, S. R. 1985. *Muscle Nerve* 8: 359–362.

pears immediately, and the membrane potential then declines gradually as the cell gains sodium and loses potassium.

Under some conditions—for example, after accumulation of excess sodium inside a cell—the pump can be stimulated to transport sodium and potassium at rates that far exceed their passive leak rates. Such activity can produce a large net efflux of cations, resulting in a pronounced hyperpolarization that declines gradually as steady-state conditions are restored (see Figure 7).

Chloride distribution

How do the steady-state considerations apply to chloride? As we have already shown, 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. These are discussed in more detail later. In squid axon and in muscle, chloride is transported into the cells; in many nerve cells transport is outward. In either case, the intracellular chloride concentration is "pumped up" (or down) to a steady-state value such that the leak out of (or into) the cell matches the rate of active transport in the opposite direction.[8]

Predicted values of membrane potential

How do these considerations explain the relation between potassium concentration and membrane potential shown in Figure 4? This can be seen by using real numbers in the equations. In squid axon, the relative permeability constants for sodium and potassium are roughly in the ratio 0.04:1.0.[5] We can use this $p_{\mathrm{Na}}/p_{\mathrm{K}}$ ratio (b) together with the ionic concentrations given in Table 1 to calculate the resting membrane potential in seawater:

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

The constant field equation gives a smaller value:

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

The difference (6 mV) is the electrogenic contribution of the Na^+–K^+ transport system.

The numerical examples are useful in illustrating quantitatively why, when the extracellular potassium concentration is altered, the membrane potential does not follow the Nernst potential for potassium, as shown in Figure 4. If, in the numerator of the constant field equation, we look at the magnitude of the extracellular potassium concentration (10 m*M*) and the effective sodium concentration ($0.04 \times 460 = 18.4$ m*M*), we see that potassium contributes only about 35 percent 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 potential of changing the extracellular potassium concentration is less than would be expected if

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[8] Martin, A. R. 1979. Appendix to G. Matthews and W. O. Wickelgren. *J. Physiol.* 293: 393–414.

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potassium were the only permeant ion. When the external potassium concentration is raised to a high enough level, the potassium term becomes sufficiently dominant for the relation to approach the theoretical limit of 58 mV per tenfold change in concentration (Figure 4). This effect is further enhanced by a factor not yet discussed: The ionic permeabilities are not constant. In particular, when the membrane is depolarized, voltage-sensitive potassium channels are activated. Because of the increased permeability to potassium, the relative contribution of sodium to the membrane potential (represented in the equation by the permeability ratio b) is reduced.

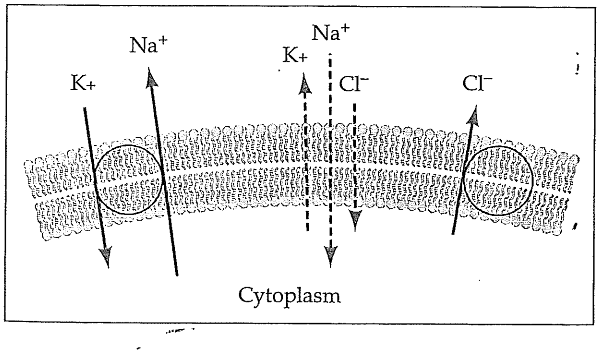
In summary, the membranes of real nerve cells are permeable to sodium, potassium, and chloride. Sodium and potassium concentrations inside the cell are kept constant by a Na–K transport mechanism that has a transport ratio of three sodium to two potassium and is therefore electrogenic. Chloride may be in equilibrium in some nerve cells, or be transported either inward or outward in others. These features are summarized in Figure 5, which shows the relative magnitudes and directions of the passive and active cation fluxes in a neuron at rest.

An electrical model of the resting membrane

So far we have discussed the resting membrane potential in terms of ionic concentrations and permeabilities. These same principles can be represented in a rather different way by an electrical model of the membrane, shown in Figure 6. Concentration ratios of the major ions are represented by their EQUILIBRIUM POTENTIALS (E_K, E_{Na}, and E_{Cl}) and ionic permeabilities are represented by CONDUCTANCES. The conductance of the membrane to a given ion (g_{Na}, g_K, or g_{Cl}) is simply the sum of the conductances of all the open channels permeable to that ion.

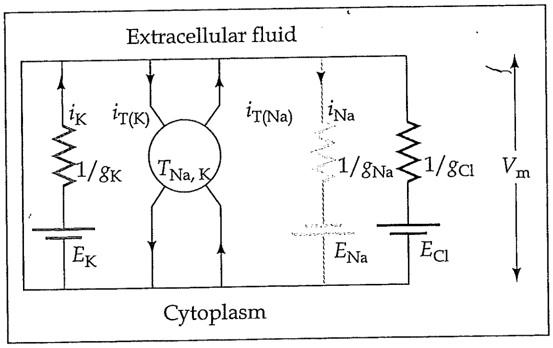
In the electrical model, the inward leak of sodium through the resting cell membrane is expressed as sodium current (i_{Na}), which is proportional to the net ionic flux through the sodium channels. This current depends on two factors: (1) the sodium conductance (g_{Na}) and (2) the driving potential, which is the difference between the membrane potential and the sodium equilibrium potential ($V_m - E_{Na}$). Thus:

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



5

IONIC LEAKS AND PUMPS IN A CELL IN A STEADY STATE. Net passive ion movements across the membrane are indicated by dashed arrows, 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 sodium ions is equal to rate of outward transport. Na:K transport is coupled with a ratio of 3:2.



6

ELECTRICAL MODEL OF THE STEADY STATE CELL MEMBRANE. E_K, E_{Na}, and E_{Cl} are the Nernst potentials for the individual ions. The individual ionic conductances are represented by resistors (having a resistance $1/g$ for each ion). The individual ionic currents i_K and i_{Na} are equal and opposite to the currents $i_{T(K)}$ and $i_{T(Na)}$ supplied by the active transport pump, $T_{Na,K}$, so that the net flux across the membrane of each ion is zero. The resulting membrane potential is V_m. For simplicity, it is assumed that $E_{Cl} = V_m$ so that $i_{Cl} = 0$.

Similarly, for potassium:

$$i_K = g_K\,(V_m - E_K)$$

and for chloride:

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

These currents are equivalent to the ionic fluxes across the cell membrane shown by the arrows in Figure 5. They are represented in the electrical model as currents produced by batteries and resistors (i.e., conductances) spanning the membrane. It is important to note that under normal resting conditions, i_{Na} is negative, which is the conventional sign for *inward* current across the membrane. Normally, i_K is *outward* and hence positive. As noted previously, chloride flux, and hence i_{Cl}, can be in either direction (or zero), depending upon the direction of transport.

To complete the electrical model in Figure 6, an active transport mechanism ($T_{Na,K}$ acting as a battery charger) is added. This corresponds to the sodium–potassium exchange pump in Figure 5. For simplicity, chloride transport is ignored. The Na–K pump produces ionic currents $i_{T(Na)}$ and $i_{T(K)}$. In the steady state, these must be equal and opposite to the corresponding leak currents. In other words, for sodium and potassium:

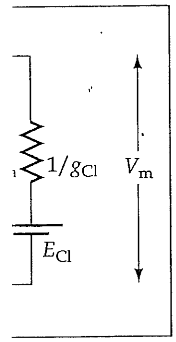
$$i_{T(Na)} = -g_{Na}(V_m - E_{Na})$$

$$i_{T(K)} = -g_K(V_m - E_K)$$

These two simple relations can be used to arrive at the membrane potential of the cell represented by the electrical model. Dividing one by the other, we write:

$$\frac{i_{T(Na)}}{i_{T(K)}} = \frac{-g_{Na}(V_m - E_{Na})}{-g_K(V_m - E_K)}$$

If we designate the sodium-to-potassium conductance ratio (g_{Na}/g_K) by b' (numerically different from the permeability ratio b) and the transport



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$$-r = b' \frac{(V_m - E_{Na})}{(V_m - E_K)}$$

Rearranging, we get:

$$V_m = \frac{rE_K + b'E_{Na}}{r + b'}$$

This is the electrical equivalent of the steady-state equation derived by Mullins and Noda, with equilibrium potentials rather than concentrations, and conductances rather than permeabilities. The electrical equivalent of the constant field equation can be obtained simply by assuming $r = 1$:

$$V_m = \frac{E_K + b'E_{Na}}{1 + b'}$$

What ion channels are associated with the resting membrane potential?

The resting conductances of membranes to sodium, potassium, and chloride have been determined in many nerve cells. It is a curious fact, however, that no precise identification of all the channels underlying these resting conductances has been made in any specific cell. A large number of potassium channel types have been identified (Chapter 4); many of them are activated by changes in membrane potential or by chemical ligands of one sort or another. Candidates for those active in the resting membrane vary from one cell to the next. Among the contributors to resting potassium conductance are channels activated by intracellular cations: sodium-activated and calcium-activated potassium channels. In addition, many nerve cells have **M CHANNELS** that are open at rest and closed by intracellular messengers. It is unlikely that a large fraction of voltage-activated potassium channels (**DELAYED RECTIFIER** and **A CHANNELS**) are open at rest; nevertheless only 0.1 to 1 percent of the total number would be required to account for a substantial fraction of the resting conductance.[9]

The specific source of the resting sodium conductance of nerve cells is also uncertain. Part of the conductance is due to movement of sodium through potassium channels, most of which have a sodium-to-potassium permeability ratio between 0.01 and 0.03.[10] In addition, both inward sodium and outward potassium leak may be through cation channels that show little selectivity for potassium over sodium.[11,12] Finally,

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[9] Edwards, C. 1982. *Neuroscience* 7: 1335–1366.

[10] Hille, B. 1992. *Ionic Channels of Excitable Membranes*, 2nd ed. Sinauer, Sunderland, MA. p. 352.

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tetrodotoxin has been shown to block a fraction of the resting sodium conductance, indicating a contribution by voltage-activated sodium channels.[9]

In central nervous system neurons, chloride channels may account for as much as 10 percent of the resting membrane conductance[13] and channels presumed to underlie this conductance have been described.[14]

ACTIVE TRANSPORT OF IONS

The Na–K Pump

The viability of nerve cells is maintained by the constant transport of sodium and potassium across the cell membranes against their electrochemical gradients. This perpetual task is carried out by the Na–K pump, the required energy being obtained from hydrolysis of adenosine triphosphate (ATP). Indeed, it has been shown that the phosphatase itself is an integral part of the ion transport system. The properties of the enzyme have been summarized succinctly in a review by Skou.[15] It consists of two molecular subunits: α, with an apparent molecular mass of about 100 kD, and β, about 38 kD. The active enzyme appears to exist in the membrane as a tetramer, $(\alpha\beta)_2$. The stoichiometry of the enzyme is as expected from the transport characteristics: An average of three sodium and two potassium ions are bound for each molecule of ATP hydrolyzed. The requirement for sodium is remarkably specific. It is the only substrate accepted for net outward transport; conversely it is the only monovalent cation *not* accepted for inward transport. Thus lithium, ammonium, rubidium, cesium, and thallium are all able to substitute for potassium in the external solution but not for sodium in the internal solution. The requirement for external potassium is not absolute. In its absence the pump will extrude sodium at about 10 percent of capacity in an "uncoupled" mode. The transport system is blocked specifically by the digitalis glycosides, particularly ouabain and strophanthidin.

Both the α and β subunits have been sequenced,[16,17] and various models have been proposed for their tertiary structure. The α subunit has six major hydrophobic regions capable of forming transmembrane helices; the β subunit has only one such region. Various schemes for the transport mechanism have been proposed. All involve alternate exposure of sodium and potassium binding sites (presumably within a channel-like structure) to the extracellular and intracellular solutions. The cyclic conformational changes are driven by phosphorylation and dephosphorylation of the protein and are accompanied by changes in binding affinity for the two ions. Thus sodium is bound during intracellular exposure of the sites and subsequently released to the extracel-

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[13] Gold, M. R. and Martin, A. R. 1983. *J. Physiol.* 342: 99–117.

[14] Krouse, M. E., Schneider, G. T. and Gage, P. W. 1986. *Nature* 319: 58–60.

[15] Skou, J. C. 1988. *Methods Enzymol.* 156: 1–25.

[16] Kawakami et al. 1985. *Nature* 316: 733–736.

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lular solution; potassium is bound during extracellular exposure and released to the cytoplasm.

Experimental evidence that the pump is electrogenic

Transport of sodium and potassium was studied in squid axon by Hodgkin and Keynes and their colleagues[18,19] and in snail neurons by Thomas.[20,21] To examine the relations among internal sodium concentration, pump current, and membrane potential, Thomas used two intracellular pipettes to deposit ions in the cell, one filled with sodium acetate and the other with lithium acetate (Figure 7A). A third intracellular pipette was used as an electrode to record membrane potential. A fourth pipette was used as a current electrode for voltage clamp experiments (Chapter 4), and a fifth, made of sodium-sensitive glass, to monitor the intracellular sodium concentration. To inject sodium, the sodium-filled pipette was made positive with respect to the lithium pipette. Thus, current flow in the injection system was between the two pipettes, with none of the injected current flowing through the cell membrane. The result of such a sodium injection is shown in Figure 7B. After a brief injection the cell became hyperpolarized by about 20 mV and gradually recovered over several minutes. Injection of lithium (by making the lithium pipette positive) produced no hyperpolarization.

Several lines of evidence showed that the potential change after sodium injection was due to the action of a sodium pump and not to changes in membrane permeability. For example, the input resistance of the cell did not decrease, as might be expected if hyperpolarization were the result of an increased permeability to potassium or chloride. The hyperpolarization could, however, be greatly reduced or abolished by addition of the transport inhibitor ouabain to the bathing solution (Figure 7C), as would be expected if it were due to pump activity. Similarly, sodium injection had little effect on potential when potassium was absent from the external solution; reintroduction of potassium after injection, however, resulted in immediate hyperpolarization (Figure 7D).

Quantitative estimates of the pump rate and the exchange ratio were obtained by voltage clamp experiments in which membrane current was measured while the membrane potential was being held constant (clamped). At the same time, intracellular sodium concentration was monitored. Sodium injection gave rise to an outward surge of current whose amplitude and duration followed the intracellular sodium concentration (Figure 7E). The total charge carried out of the cell, measured by integrating the total membrane current, was only about one-third of the charge injected in the form of sodium ions. This evidence was consistent with the idea that for every three sodium ions pumped out of the cell, two potassium ions were carried inward.

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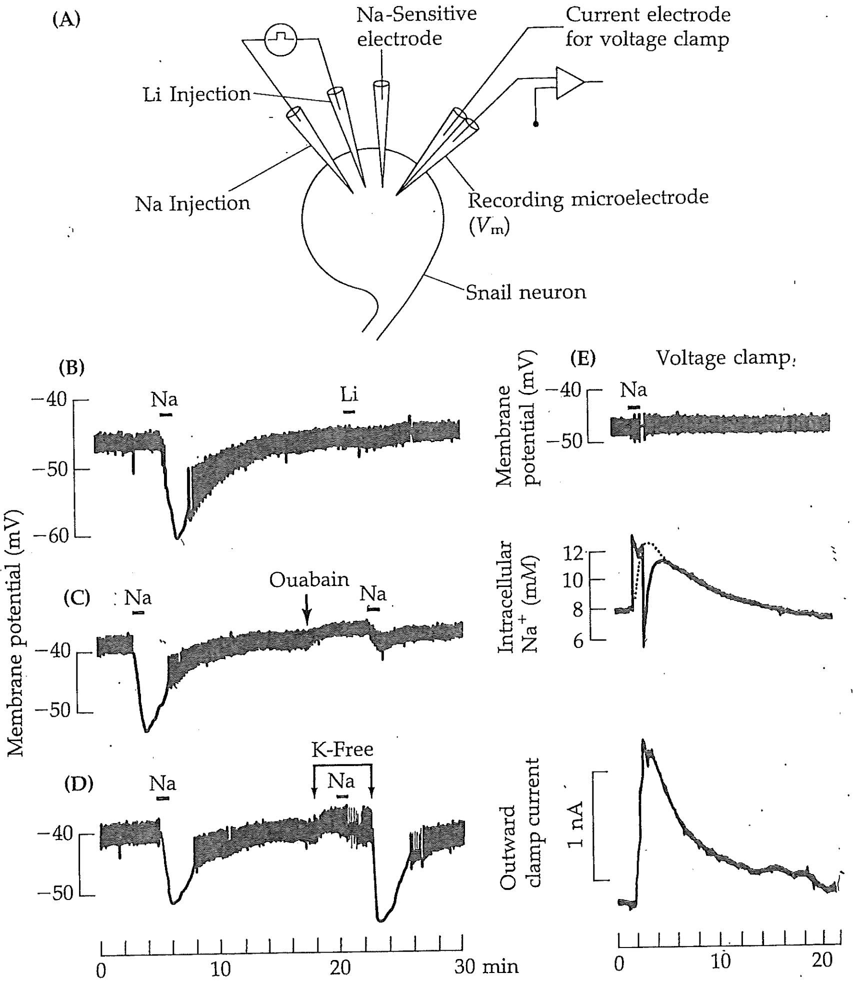
[18] Hodgkin, A. L. and Keynes, R. D. 1955. *J. Physiol.* 128: 28–60

[19] Baker, P. F. et al. 1969. *J. Physiol.* 200: 459–496.

[20] Thomas, R. C. 1969. *J. Physiol.* 201: 495–514.

[21] Thomas, R. C. 1972. *J.Physiol.* 220: 55–71.



7 EFFECT OF SODIUM INJECTION. Changes in intracellular sodium concentration, membrane potential, and membrane current following injection of sodium into snail neurons. (A) Sodium is injected by passing current between two electrodes filled with sodium acetate and lithium acetate (see text). A sodium-sensitive electrode measures $[Na]_i$; two other electrodes measure membrane potential and pass current through the cell membrane to obtain the voltage clamp records in (E). (B) Hyperpolarization of the membrane following intracellular injection of sodium. (The small rapid deflections are spontaneously occurring action potentials, reduced in size because of the poor frequency response of the pen recorder.) Injection of lithium does not produce hyperpolarization. (C) After application of ouabain (20 μg/ml), which blocks the sodium pump, hyperpolarization by sodium injection is greatly reduced. (D) Removal of potassium from the extracellular solution blocks the pump, so that sodium injection produces no hyperpolarization until potassium is restored. (E) Voltage clamp records. Sodium injection results in increased intracellular sodium concentration and in outward current across the cell membrane. The sharp deflections on the sodium concentration record are artifacts from the injection system. The time course of the concentration change is indicated by dashed lines. (After Thomas, 1969.)

Active transport of chloride and bicarbonate

It has already been noted that chloride may be transported into or out of nerve cells. Such transport is accompanied by movement of other ions. In neurons in which chloride is transported out of the cell, the outward movement is associated with inward transport of bicarbonate. Such a chloride–bicarbonate exchange mechanism is found in a number of cell types and has been studied by Thomas in relation to intracellular pH regulation in snail neurons.[22] Recovery from acidification of the cytoplasm (by exposure to CO_2 or intracellular injection of HCl) was prolonged when extracellular bicarbonate concentration was reduced or when intracellular chloride was depleted. Furthermore, recovery was virtually abolished when sodium was removed from the extracellular bathing solution. The recovery, then, appeared to involve inward movement of sodium and bicarbonate in exchange for chloride. Such an exchange mechanism exists in a variety of cells types; it is inhibited by 4-aceto-4′-isothiocyanostilbene-2,2′-disulfonic acid (SITS) and a related compound, DIDS. Although both anions are carried against their electrochemical gradients, the exchange mechanism is not an ATPase. Instead, the energy required appears to be obtained through passive inward movement of sodium down its electrochemical gradient.

An outward transport mechanism for chloride that is SITS-insensitive has been reported in mammalian cortical neurons.[23] The system appears to involve co-transport of chloride and potassium out of the cells. Whether or not the effluxes are coupled to inward sodium movement is not clear.

Secondary active transport

In describing the chloride–bicarbonate exchange system, we have introduced a new idea into our consideration of the properties of cell membranes, namely SECONDARY ACTIVE TRANSPORT, in which the electrochemical gradient for sodium is used to transport other ions across the membrane against their individual electrochemical gradients. Although part of the continual movement of sodium into the cell is simply through passive channels, some sodium entry is through such coupled transport mechanisms. The mechanisms require the sodium gradient for their operation and are, therefore, ultimately dependent upon maintenance of that gradient by Na–K ATPase. As a general rule, such mechanisms tend to be electrically neutral; that is, there is no net transfer of charge across the membrane. Such neutrality is achieved by the appropriate stoichiometry; for example, transport of one sodium and two bicarbonate ions into the cell for every chloride transported outward. A simpler example is the 1:1 sodium–hydrogen exchange mechanism, which also contributes to the maintenance of intracellular pH.[24] Protons are carried out of the cell against their electrochemical gradient in exchange for inward movement of sodium.

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[24] Moody, W. J. 1981. *J. Physiol.* 316: 293–308.

Inward chloride transport

In cells such as muscle fibers and squid axon, chloride is actively accumulated. It has been shown by Russell that in squid axon inward chloride transport requires both sodium and potassium in the extracellular bathing medium.[25] It appears that for the entry of every two sodium ions, three chloride ions and one potassium ion are carried into the cell; that is, there is a Na:K:Cl ratio of 2:1:3,[26] with the sodium influx again supplying the necessary energy for transport of the other ions against their electrochemical gradients. This chloride transport system is insensitive to DIDS but is blocked by furosemide and bumetamide, substances known to block chloride transport in other tissues such as renal tubular cells.

Regulation of intracellular calcium

As we will see later, changes in intracellular calcium concentrations play an important role in many neuronal functions such as action potential generation, release of neurotransmitters at synapses, initiation of postsynaptic conductance changes, and photoreceptor responses. In addition, calcium plays a primary role in initiation of muscle contraction. Intracellular concentrations of free calcium have been measured by injecting molecules such as aequorin[27] or FURA2[28] that emit or absorb light in the presence of ionized calcium. The absorption or fluorescence, which is dependent on calcium concentration, is then monitored with highly sensitive optical techniques. In squid axon and in various other neurons, the resting intracellular concentration of free calcium ranges from 10 to 100 n*M*. The extracellular calcium concentration is about 10 m*M* in squid blood (Table 1) and 2 to 5 m*M* in vertebrate interstitial fluid. Maintenance of the low intracellular concentrations requires that calcium be transported out of the cells against a very large electrochemical gradient.

Two major transport systems are responsible for extrusion of calcium from the cytoplasm across the plasma membrane.[29,30] The first is an ATPase, activated by calcium; magnesium is a necessary cofactor for ATP binding. The enzyme, then, is known as Ca–Mg ATPase. It has a high affinity for calcium ($K_{m(Ca)} < 300$ n*M*) and transports one calcium ion out of the cell for every ATP hydrolyzed. The distribution of the enzyme in the plasma membrane is generally sparse, however, so that the transport capacity is low. Nevertheless, it serves to maintain the low cytoplasmic calcium concentration in resting cells.

The second means of extruding calcium across the plasma membrane is sodium–calcium exchange, driven by the electrochemical gradient for sodium. The system has a lower affinity for calcium ($K_{m(Ca)} \leq 1.0\ \mu M$), but about 50 times the transport capacity. In most cells, one calcium ion

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[25] Russell, J. M. 1983. *J. Gen. Physiol.* 81: 909–925.

[26] Altamirano, A. A. and Russell, J. M. 1987. *J. Gen. Physiol.* 89: 669–686.

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[28] Tsien, R. Y. 1988. *Trends Neurosci.* 11: 419–424.

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is transported outward for each three sodium ions entering the cell.[31] Consequently the exchange is electrogenic, one positive ionic charge entering on each cycle. The exchange system is called into play in excitable cells when calcium influx due to electrical activity overwhelms the transport ability of the ATPase.

Reversal of sodium–calcium exchange

In general, ion exchange mechanisms can be made to run backward by altering or reversing one or more of the ionic gradients involved in the exchange. An interesting feature of sodium–calcium exchange is that such reversal can occur readily under physiological conditions, in which case calcium *enters* through the system and sodium is extruded. The direction of transport is determined simply by whether the energy provided by the entry of three sodium ions is greater than or less than the energy required to extrude one calcium ion. One factor determining this energy balance is the membrane potential of the cell. The energy dissipated by sodium entry (or required for extrusion) is simply the amount of charge moved across the membrane times the driving potential for such movement or, in other words, the charge times the difference between the sodium equilibrium potential (E_{Na}) and the membrane potential (V_m). For three sodium ions this is $3(E_{Na} - V_m)$. Similarly, for a single (divalent) calcium ion the energy is $2(E_{Ca} - V_m)$. There is no exchange when the energies are exactly equal—that is, when

$$3(E_{Na} - V_m) = 2(E_{Ca} - V_m)$$

or (by rearrangement) when

$$V_m = 3E_{Na} - 2E_{Ca}$$

Now suppose a nerve cell has internal sodium and calcium concentrations of 15 m*M* and 100 n*M*, respectively, and is bathed in a solution containing 150 m*M* sodium and 2 m*M* calcium. These are reasonable physiological values for mammalian cells. The equilibrium potential for sodium is +58 mV and for calcium +124 mV. Ion movement through the exchanger will be zero when $V_m = -74$ mV. At more negative membrane potentials, sodium will enter through the system and calcium will be extruded; at less negative potentials calcium will enter, extruding sodium. (The reader who cares to do the same calculation with the ionic distributions given in Table 1 for squid axon in seawater will find that calcium extrusion will occur only when the membrane potential is more negative than −121 mV!) The value of −74 mV is in the range of resting membrane potentials for many cells so that, in any given cell, ion movements through the exchanger may be in one direction or the other, depending on membrane potential or on whether there has been previous sodium or calcium accumulation.

In heart muscle, it appears that entry of calcium through the sodium–calcium exchange system during the action potential may contribute to

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[31] Caputo, C., Bezanilla, F. and DiPolo, R. 1989. *Biochim. Biophys. Acta* 986: 250–256.

activation of the contractile process,[32] and that calcium extrusion through the same system after repolarization may be important for relaxation.[33] These theoretical and experimental observations indicate that the role of the sodium–calcium exchange system extends beyond merely transporting calcium out of the cell, and that the bidirectional nature of the system may have significant physiological consequences.

It is useful to keep in mind that although the cytoplasmic concentration of free calcium is very low, neurons contain substantial quantities of calcium buffered by intracellular organelles, principally the endoplasmic reticulum. In squid axon, for example, the concentration of bound calcium is about 50 μM, or about 500 times the free concentration (Table 1). A number of neuronal functions are mediated by release of calcium from these intracellular stores (Chapter 8).

In summary, intracellular ionic concentrations are governed by several different transport mechanisms. Sodium and potassium concentrations are maintained by Na–K ATPase. Similarly, Ca–Mg ATPase contributes to the maintenance of low intracellular calcium concentrations. The rest of the transport systems are driven by the electrochemical gradient for sodium rather than by direct hydrolysis of ATP. Thus they are all dependent, in the long run, upon Na–K ATPase. In the secondary transport systems, inward sodium movement is accompanied variously by chloride–bicarbonate exchange, by inward co-transport of chloride and potassium, by outward transport of protons, or by outward transport of calcium. Of these, only the sodium–calcium exchange system is electrogenic; however, the ion fluxes involved in sodium–calcium exchange are so small that their contributions to the resting membrane potential are trivial.

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