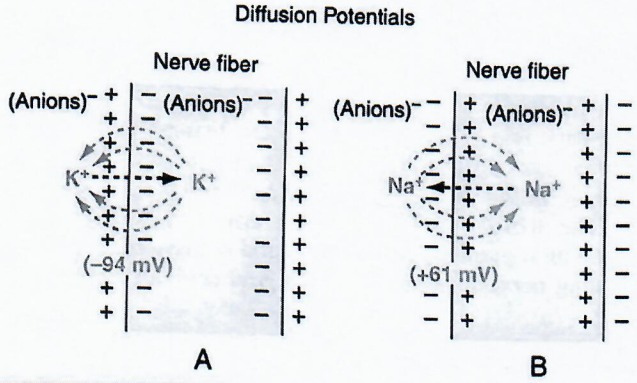

FIGURE 5–1

A, Establishment of a diffusion potential across a cell membrane, caused by diffusion of potassium ions from inside the cell to the outside through a membrane selectively permeable only to potassium. *B*, Establishment of a diffusion potential when the membrane is permeable only to sodium ions. Note that the internal membrane potential is negative when potassium ions diffuse and positive when sodium ions diffuse because of opposite concentration gradients of these two ions.

Calculation of the Diffusion Potential When the Membrane Is Permeable to Several Different Ions

When a membrane is permeable to several different ions, the diffusion potential that develops depends on three factors: (1) the polarity of the electrical charge of each ion, (2) the permeability of the membrane (*P*) to each ion, and (3) the concentrations (*C*) of the respective ions on the inside (*i*) and outside (*o*) of the membrane. Thus, the following formula, called the *Goldman equation*, or the *Goldman-Hodgkin-Katz equation*, gives the calculated membrane potential on the *inside* of the membrane when two univalent positive ions, sodium (Na^+) and potassium (K^+), and one univalent negative ion, chloride (Cl^-), are involved.

$$\text{EMF (millivolts)} = -61 \cdot \log \frac{C_{Na^+_i} P_{Na^+} + C_{K^-_i} P_{K^+} + C_{Cl^-_o} P_{Cl^-}}{C_{Na^+_o} P_{Na^-} + C_{K^-_o} P_{K^+} + C_{Cl^-_i} P_{Cl^-}}$$

Let us study the importance and the meaning of this equation. First, sodium, potassium, and chloride ions are the ions most importantly involved in the development of membrane potentials in nerve and muscle fibers, as well as in the neuronal cells in the nervous system. The concentration gradient of each of these ions across the membrane helps determine the voltage of the membrane potential.

Second, the degree of importance of each of the ions in determining the voltage is proportional to the membrane permeability for that particular ion. That is, if the membrane has zero permeability to both potassium and chloride ions, the membrane potential becomes entirely dominated by the concentration gradient of sodium ions alone, and the resulting potential will be equal to the Nernst potential for sodium. The same holds for each of the other two ions if the membrane should become selectively permeable for either one of them alone.

Third, a positive ion concentration gradient from *inside* the membrane *to the outside* causes electronegativity inside the membrane. The reason for this is that excess positive ions diffuse to the outside when their concentration is higher inside than outside. This carries positive charges to the outside but leaves the nondiffusible negative anions on the inside, thus creating electronegativity on the inside. The opposite effect occurs when there is a gradient for a negative ion. That is, a chloride ion gradient from the *outside to the inside* causes negativity inside the cell because excess negatively charged chloride ions then diffuse to the inside, while leaving the nondiffusible positive ions on the outside.

Fourth, we shall see later that the permeabilities of the sodium and potassium channels undergo rapid changes during transmission of the nerve impulse, whereas the permeability of the chloride channels does not change greatly during this process. Therefore, the changes in sodium and potassium permeabilities are primarily responsible for signal transmission in the nerves, which is the subject of most of the remainder of this chapter.

Measuring the Membrane Potential

The method for measuring the membrane potential is simple in theory but often difficult in practice because of the small sizes of most of the fibers. Figure 5–2 shows a small filled pipette containing an electrolyte solution that is impaled through the cell membrane to the interior of the fiber. Then another electrode, called the "indifferent electrode," is placed in the extracellular fluid, and the potential difference between the inside and outside of the fiber is measured using an appropriate voltmeter. This voltmeter is a highly sophisticated electronic apparatus that is capable of measuring very small voltages despite extremely high resistance to electrical flow through the tip of the micropipette, which has a lumen diameter usually less than 1 micrometer and a resistance often as great as 1 billion ohms. For recording rapid *changes* in the membrane potential during transmission of nerve impulses, the microelectrode is connected to an oscilloscope, as explained later in the chapter.

The lower part of Figure 5–3 shows the electrical potential that is measured at each point in or near the nerve fiber membrane, beginning at the left side of the figure and passing to the right. As long as the electrode is outside the nerve membrane, the potential that is recorded is zero, which is the potential of the extracellular fluid. Then, as the recording electrode passes through the voltage charge area at the cell membrane (called the *electrical dipole layer*), the potential decreases abruptly to −90 millivolts. Moving across the center

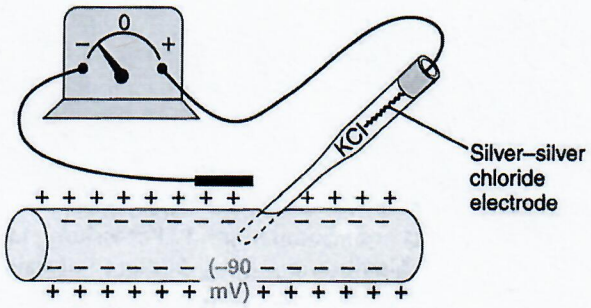

FIGURE 5–2

Measurement of the membrane potential of the nerve fiber using a microelectrode.

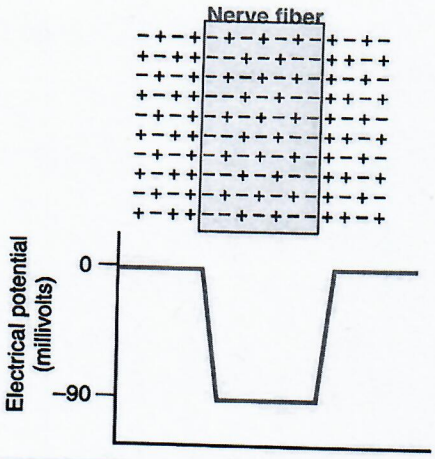

FIGURE 5–3

Distribution of positively and negatively charged ions in the extracellular fluid surrounding a nerve fiber and in the fluid inside the fiber; note the dipolar alignment of negative charges along the inside surface of the membrane and positive charges along the outside surface. In the lower panel are displayed the abrupt changes in membrane potential that occur at the membranes on the two sides of the fiber.

of the fiber, the potential remains at a steady −90-millivolt level but reverses back to zero the instant it passes through the membrane on the opposite side of the cell.

To create a negative potential inside the membrane, only enough positive ions must be transported outward to develop the electrical dipole layer at the membrane itself. All the remaining ions inside the nerve fiber still can be both positive and negative ions, as shown in the upper panel of Figure 5–3. Therefore, an incredibly small number of ions need to be transferred through the membrane to establish the normal potential of −90 millivolts inside the nerve fiber; this means that only about 1/3,000,000 to 1/100,000,000 of the total positive charges inside the fiber need be so transferred. Also, an equally small number of positive ions moving from outside to the inside of the fiber can reverse the potential from −90 millivolts to as much as +35 millivolts within as little as 1/10,000 of a second. Rapid shifting of ions in this manner causes the nerve signals that we discuss in subsequent sections of this chapter.

RESTING MEMBRANE POTENTIAL OF NERVES

The resting membrane potential of large nerve fibers when they are not transmitting nerve signals is about −90 millivolts. That is, the potential *inside the fiber* is 90 millivolts more negative than the potential in the extracellular fluid on the outside of the fiber. In the next few paragraphs, we explain all the factors that determine the level of this resting potential, but before doing so, we must describe the transport properties of the resting nerve membrane for sodium and potassium.

Active Transport of Sodium and Potassium Ions Through the Membrane—The Sodium-Potassium Pump. First, let us recall from the discussions of Chapter 4 that all cell membranes of the body have a powerful sodium-potassium pump that continually pumps sodium to the outside of the fiber and potassium to the inside, as illustrated on the left-hand side in Figure 5–4. Further, note that this is an *electrogenic pump* because more positive charges are pumped to the outside than to the inside (three Na^+ ions to the outside for each two K^+ ions to the inside), leaving a net deficit of positive ions on the inside; this causes a negative charge inside the cell membrane.

The sodium-potassium pump also causes large concentration gradients for sodium and potassium across the resting nerve membrane. These gradients are the following:

Na^+ (outside):	142 mEq/L
Na^+ (inside):	14 mEq/L
K^+ (outside):	4 mEq/L
K^+ (inside):	140 mEq/L

The ratios of these two respective ions from the inside to the outside are

$$Na^+_{inside}/Na^+_{outside} = 0.1$$

$$K^+_{inside}/K^+_{outside} = 35.0$$

Leakage of Potassium and Sodium Through the Nerve Membrane. To the right in Figure 5–4 is shown a channel protein in the cell membrane through which potassium and sodium ions can leak, called a *potassium-sodium "leak" channel.* The emphasis is on potassium leakage because, on average, the channels are far more permeable to potassium than to sodium, normally about 100 times as permeable. We see later that this differential in permeability is exceedingly important in determining the level of the normal resting membrane potential.

Origin of the Normal Resting Membrane Potential

Figure 5–5 shows the important factors in the establishment of the normal resting membrane potential of −90 millivolts. They are as follows.

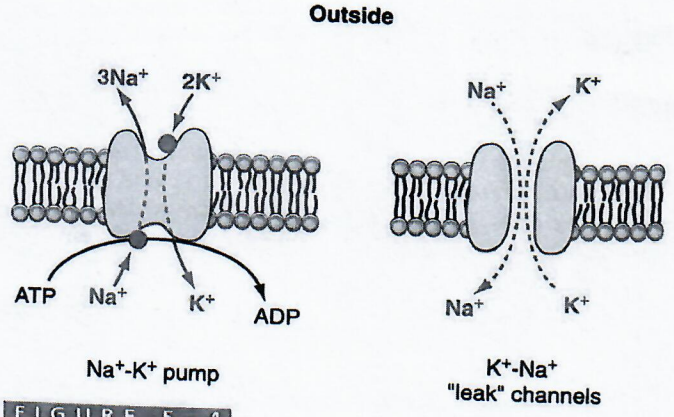

FIGURE 5–4

Functional characteristics of the Na^+-K^+ pump and of the potassium-sodium "leak" channels.

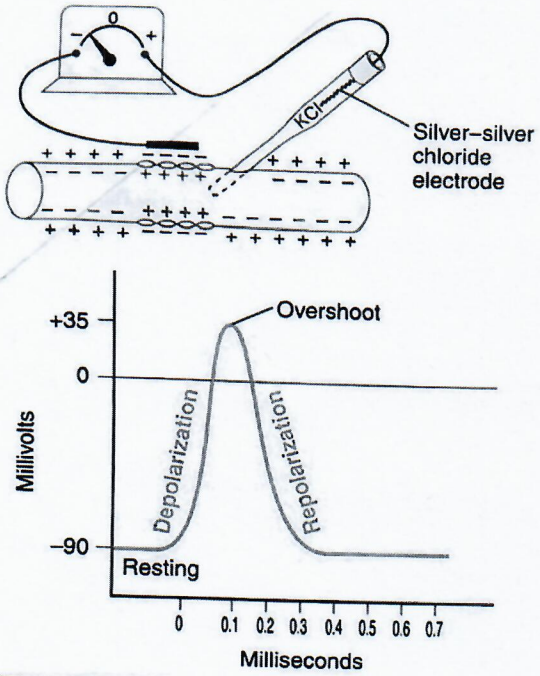

FIGURE 5–6

Typical action potential recorded by the method shown in the upper panel of the figure.

a second, illustrating the explosive onset of the action potential and the almost equally as rapid recovery.

The successive stages of the action potential are as follows.

Resting Stage. This is the resting membrane potential before the action potential begins. The membrane is said to be "polarized" during this stage because of the −90 millivolts negative membrane potential that is present.

Depolarization Stage. At this time, the membrane suddenly becomes very permeable to sodium ions, allowing tremendous numbers of positively charged sodium ions to flow to the interior of the axon. The normal "polarized" state of −90 millivolts is immediately neutralized by the inflowing sodium ions, with the potential rising rapidly in the positive direction. This is called *depolarization.* In large nerve fibers, the membrane potential actually "overshoots" beyond the zero level and becomes somewhat positive, but in some smaller fibers as well as many central nervous system neurons, the potential merely approaches the zero level and does not overshoot to the positive state.

Repolarization Stage. Within a few 10,000ths of a second after the membrane becomes highly permeable to sodium ions, the sodium channels begin to close and the potassium channels open more than they normally do. Then, rapid diffusion of potassium ions to the exterior re-establishes the normal negative resting membrane potential. This is called *repolarization* of the membrane.

To explain more fully the factors that cause both depolarization and repolarization, we need to describe the special characteristics of yet two other types of transport channels through the nerve membrane: the voltage-gated sodium and potassium channels.

Voltage-Gated Sodium and Potassium Channels

The necessary actor in causing both depolarization and repolarization of the nerve membrane during the action potential is the *voltage-gated sodium channel.* A *voltage-gated potassium channel* also plays an important role in increasing the rapidity of repolarization of the membrane. *These two voltage-gated channels are in addition to the Na^+-K^+ pump and the Na^+-K^+ leak channels.*

Voltage-Gated Sodium Channel—Activation and Inactivation of the Channel

The upper panel of Figure 5–7 shows the voltage-gated sodium channel in three separate states. This channel has two *gates,* one near the outside of the channel called the *activation gate* and another near the inside called the *inactivation gate.* To the left is shown the state of these two gates in the normal resting membrane when the membrane potential is −90 millivolts. In this state, the activation gate is closed, which prevents any entry of sodium ions to the interior of the fiber through these sodium channels.

Activation of the Sodium Channel. When the membrane potential becomes less negative than during the resting state, rising from −90 millivolts toward zero, it

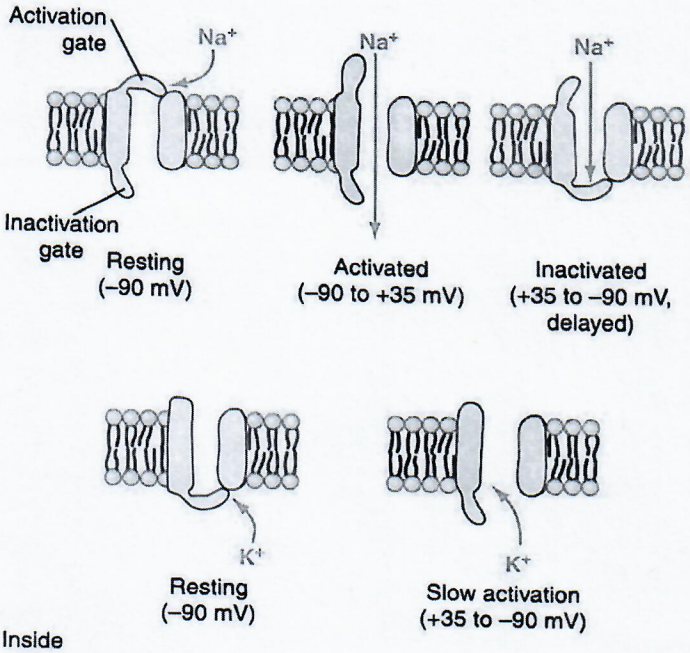

FIGURE 5–7

Characteristics of the voltage-gated sodium *(top)* and potassium *(bottom)* channels, showing both activation and inactivation of the sodium channels but activation alone of the potassium channels when the membrane potential is changed from the normal resting negative value to a positive value.

finally reaches a voltage, usually somewhere between −70 and −50 millivolts, that causes a sudden conformational change in the activation gate, flipping all the way to the open position. This is called the *activated state;* during this state, sodium ions literally can pour inward through the channel, increasing the sodium permeability of the membrane as much as 500- to 5000-fold.

Inactivation of the Sodium Channel. To the far right in the upper panel of Figure 5–7 is shown a third state of the sodium channel. The same increase in voltage that opens the activation gate also closes the inactivation gate. The inactivation gate, however, closes a few 10,000ths of a second after the activation gate opens. That is, the conformational change that flips the inactivation gate to the closed state is a slower process than the conformational change that opens the activation gate. Therefore, after the sodium channel has remained open for a few 10,000ths of a second, the inactivation gate closes and sodium ions no longer can pour to the inside of the membrane. At this point, the membrane potential begins to recover back toward the resting membrane state, which is the repolarization process.

Another important characteristic of the sodium channel inactivation process is that *the inactivation gate will not re-open until the membrane potential returns either to or nearly to the original resting membrane potential level.* Therefore, it usually is not possible for the sodium channels to open again without the nerve fiber's first repolarizing.

Voltage-Gated Potassium Channel and Its Activation

The lower panel of Figure 5–7 shows the voltage-gated potassium channel in two states: during the resting state and toward the end of the action potential. During the resting state, the gate of the potassium channel is closed, as illustrated to the left in the figure, and potassium ions are prevented from passing through this channel to the exterior. When the membrane potential rises from −90 millivolts toward zero, this voltage change causes a slow conformational opening of the gate and allows increased potassium diffusion outward through the channel. However, because of the slowness of opening of the potassium channels, they mainly open just at the same time that the sodium channels are beginning to close because of inactivation. Thus, the decrease in sodium entry to the cell and simultaneous increase in potassium exit from the cell combine to speed the repolarization process, leading to full recovery of the resting membrane potential within another few 10,000ths of a second.

Research Method for Measuring the Effect of Voltage on Opening and Closing of the Voltage-Gated Channels—The "Voltage Clamp." The original research that led to our quantitative understanding of the sodium and potassium channels was so ingenious that it led to Nobel prizes for the scientists responsible, Hodgkin and Huxley. The essence of these studies is shown in Figures 5–8 and 5–9.

Figure 5–8 shows an experimental apparatus called a *voltage clamp,* which is used to measure flow of ions through the different channels. In using this apparatus, two electrodes are inserted into the nerve fiber. One of these is for measuring the voltage of the membrane potential. The other is to conduct electrical current into or out of the nerve fiber. This apparatus is used in the following way: The investigator decides which voltage he or she wants to establish inside the nerve fiber. He or she then adjusts the electronic portion of the apparatus to the desired voltage, and this automatically injects either positive or negative electricity through the current electrode at whatever rate is required to hold the voltage, as measured by the voltage electrode, at the level set by the operator. For instance, when the membrane potential suddenly is increased by this voltage clamp from −90 millivolts to zero, the voltage-gated sodium and potassium channels open, and sodium and potassium ions begin to pour through the channels. To counterbalance the effect of these ion movements on the desired setting of the intracellular voltage, electrical current is injected automatically through the current electrode of the voltage clamp to maintain the intracellular voltage at the required steady zero level. To achieve this, the current injected must be equal to but of opposite polarity to the net current flow through the membrane channels. To measure how much current flow is occurring at each instant, the current electrode is connected to an oscilloscope that records the current flow, as demonstrated on the screen of the oscilloscope in Figure 5–8. Finally, the investigator adjusts the concentrations of the ions to other than normal levels both inside and outside the

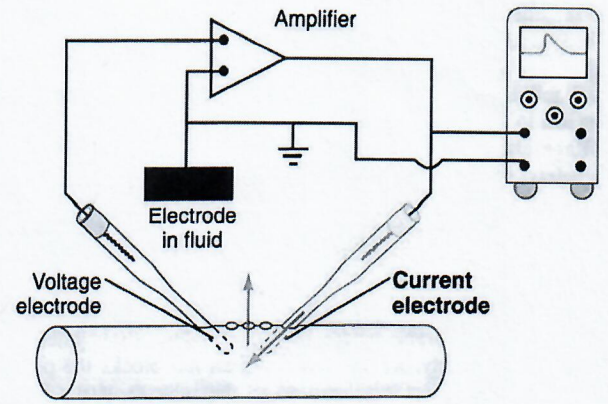

FIGURE 5–8

"Voltage clamp" method for studying flow of ions through specific channels.

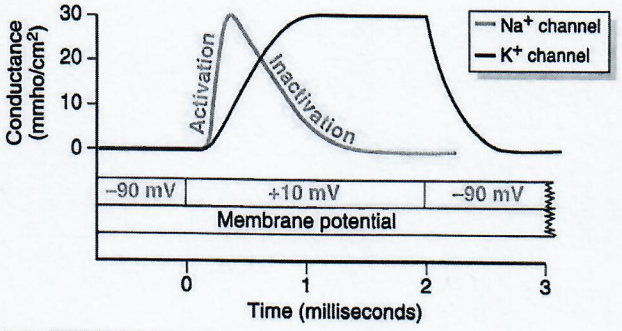

FIGURE 5–9

Typical changes in conductance of sodium and potassium ion channels when the membrane potential is suddenly increased from the normal resting value of −90 millivolts to a positive value of +10 millivolts for 2 milliseconds. This figure shows that the sodium channels open (activate) and then close (inactivate) before the end of the 2 milliseconds, whereas the potassium channels only open (activate), and the rate of opening is much slower than for sodium channels.