CHAPTER 5

Ions and Excitation

An important property of living tissues is electrical excitability. Both the discovery that tissues produce electric currents and the origin of electrochemical theory can be traced to observations made late in the eighteenth century by Luigi Galvani, a professor of anatomy at Bologna, Italy. Working with a nerve–muscle preparation from a frog leg, Galvani noted that muscles contract when dissimilar metals in contact with each other are brought into contact with the tissue, one metal touching the muscle and the other touching the nerve. Galvani and his nephew Giovanni Aldini, a physicist, ascribed this response to a discharge of "animal electricity" delivered by the nerves and stored in the muscle. They postulated that an "electric fluid" passed from the muscle through the metal and back into the nerve, and that the discharge of electricity from the muscle triggered the contraction. In retrospect this interpretation, which was published in 1791, is seen to have been largely incorrect; nevertheless, this work stimulated many inquisitive amateur and professional scientists of that revolutionary age to investigate two new and important areas of science, the physiology of excitation in nerve and muscle and the chemical origin of electricity.

Alessandro Volta, a physicist at Pavia, Italy, quickly took up Galvani's experiments and in 1792 proposed that the electric stimulus leading to contraction in Galvani's experiments did not come from a discharge of currents from the tissue, as claimed by Galvani and Aldini, but was in fact generated outside the tissue by the contact of dissimilar metals with the saline fluids of the tissue. It took several years for Volta to demonstrate unequivocally the electrolytic origin of electric current from dissimilar metals, for there was no physical instrument available at that time sufficiently sensitive to detect weak currents. Indeed, the nerve–muscle preparation from the frog leg was probably the most sensitive indicator of electric current in use at that time.

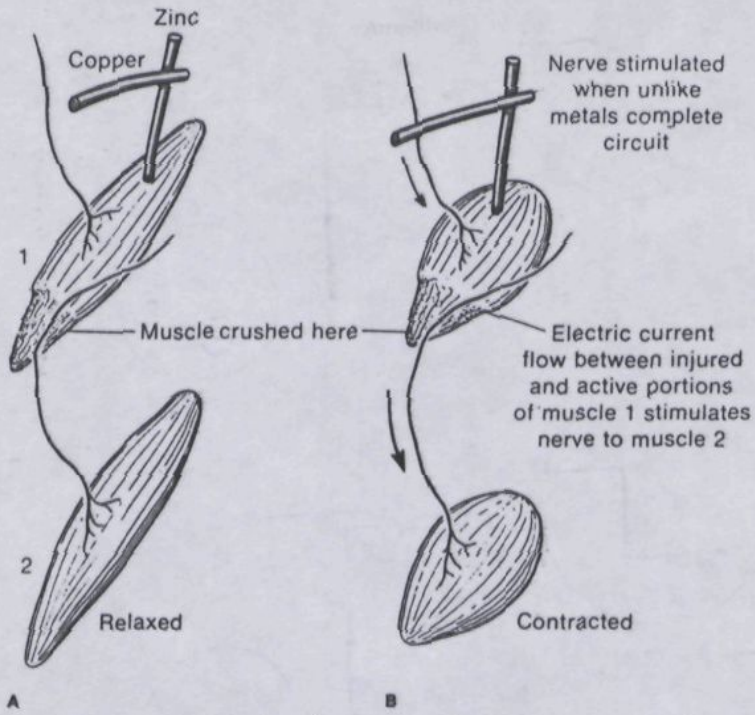
In his search for a means of producing stronger sources of electricity, Volta found that he could increase the electricity produced electrolytically by placing metal–saline cells in series. The fruit of his labor was the so-called voltaic pile—a stack of alternating silver and zinc plates separated by saline-soaked papers. This first "wet-cell" battery produced proportionately higher voltages than can be produced by a single silver–zinc cell.

Although Galvani's original experiments did not really prove the existence of "animal electricity," they did demonstrate the sensitivity of excitable tissues to minute electric currents. In 1840 Carlo Matteucci used the action current of a contracting muscle to stimulate another nerve–muscle preparation (Figure 5-1). His experiment was the first recorded demonstration that excitable tissue produces electric current. Since the nineteenth century it has become evident that the production of signals in the nervous system and other excitable tissues depends on the electrical properties of cell membranes. In this chapter we will consider the physical and molecular mechanisms that form the basis for electrical phenomena in excitable membranes.

The Concept of Membrane Excitation

We will begin with a brief consideration of some general properties of electrically excitable membranes, such as those found in nerve and muscle cells.* Electrical phenomena in living tissues can be detected by placing two electrodes in the tissue to measure the potential field set up by electric currents flowing through the extracellular fluids. Since these currents originate across cell membranes, a more direct and more quantitative approach is to measure electrical events as they take place across the membranes of single cells. Such measurements are carried out by comparing the electric potential (voltage) of the bulk fluid on one side of the membrane with that of the fluid on the other side of the membrane. Subtracting the one from the other gives

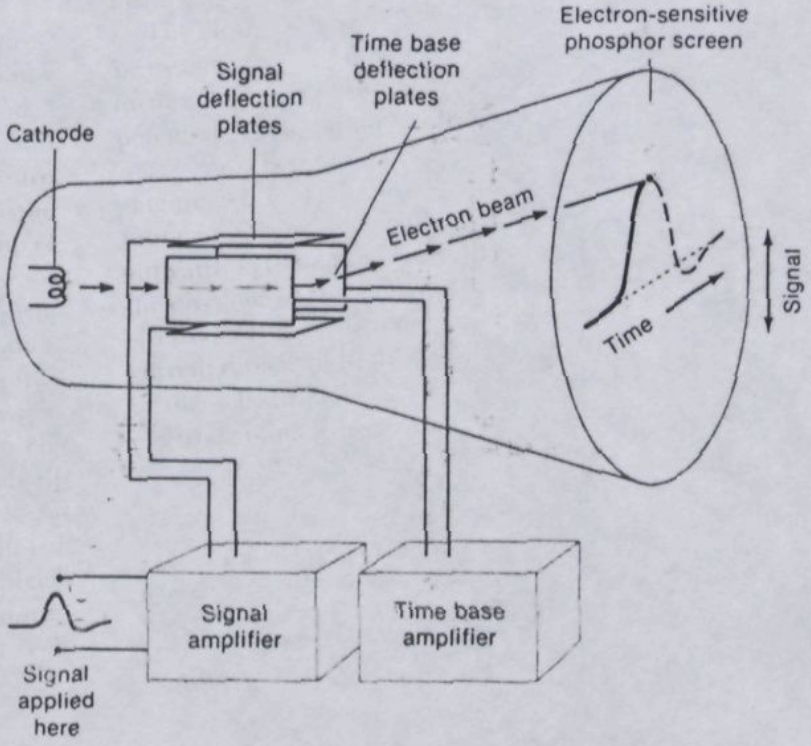
*A review of the electrical definitions and conventions given in Box 2-1 may be useful for this chapter.



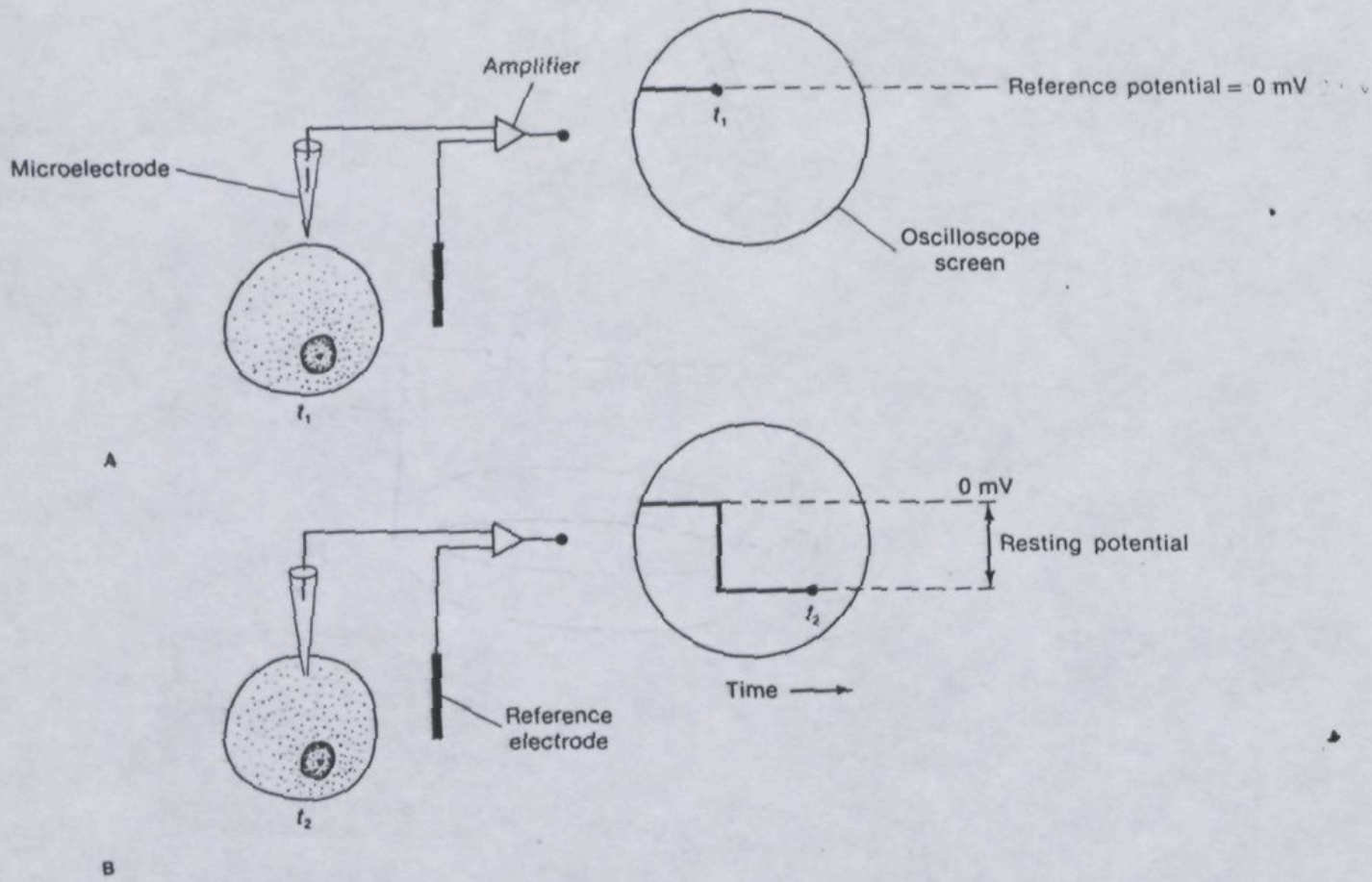
5-1 Experiment performed by Matteucci in which the current produced in the upper muscle during a twitch stimulated the nerve to the lower muscle, causing the latter to twitch also. In part **B**, the upper muscle was activated by stimulating its motor nerve directly with current produced by electrolysis between dissimilar metals.

the *potential difference* (*p.d.*), which is commonly called the *membrane potential* and is denoted by V_m. One sensing electrode is placed in electrical continuity with the fluid on one side of the membrane, and another in electrical continuity with the fluid on the other side of the membrane, and the p.d. is electronically amplified for display against time on a recording instrument such as an oscilloscope (Figure 5-2), which is a kind of voltmeter. This procedure depends on the use of a glass *capillary pipette microelectrode* (Figure 5-3A), invented by Gilbert Ling and Ralph W. Gerard (1949). Because of their minute tip diameter, microelectrodes can be inserted into medium and large cells with negligible damage.

The lumen of the hollow glass microelectrode is filled with an electrolyte solution (e.g., 3 M KCl) connected by a silver wire to the input of an amplifier. Insertion of the electrode tip through the plasma membrane into the cell brings the cell interior into continuity with the voltage-recording amplifier. By convention, the membrane potential is always taken as the intracellular po-



5-2 The oscilloscope is an instrument that plots an electrical signal vertically against a horizontal time axis. A beam of electrons "writes" on the phosphor screen while driven from left to right by a time base generator. An input signal applied to the oscilloscope is amplified and fed to the signal deflection plates as a changing voltage, thus plotting the input signal against time.



5-3 *Reference and resting potentials.* (**A**) *No potential difference is recorded between the reference electrode and the microelectrode tip outside the cell in the bath.* (**B**) *On penetrating the membrane, the electrode records an abrupt negative-going shift, the resting potential, which is shown as a downward deflection of the oscilloscope screen.*

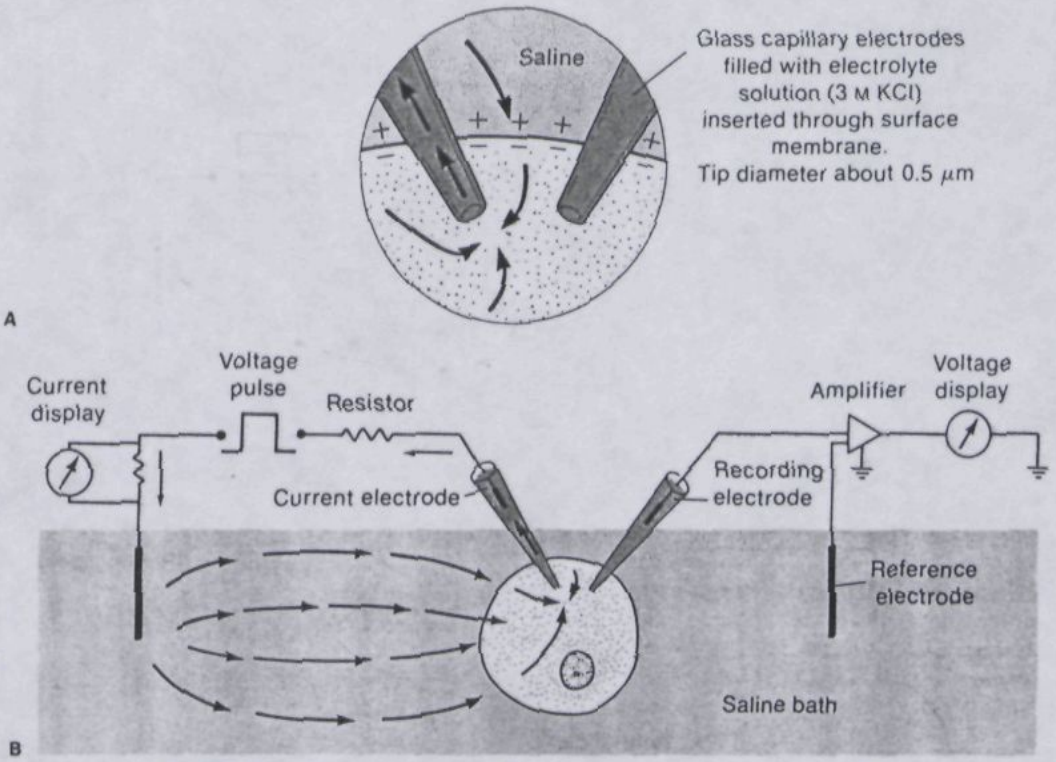
tential (recorded by the microelectrode) relative to the extracellular potential (recorded by a silver wire in the bath). In other words, the extracellular potential is arbitrarily defined as zero. In practice the amplifier subtracts the extracellular potential from the intracellular potential to give the p.d.

A simple recording arrangement is shown in Figures 5-3 and 5-4. A cell is immersed in a physiological saline solution that is in contact with a reference electrode. Before the tip of the *recording microelectrode* enters the cell, the microelectrode and the reference electrode are at the same potential, and thus the p.d. between the two electrodes is then zero (Figure 5-3A). As the tip of the microelectrode is advanced, a negative voltage, or potential, at some point suddenly appears as a downward shift of the voltage trace, indicating penetration of the cell membrane (Figure 5-3B). In electrophysiological convention, negative potentials are shown as downward displacements of the oscilloscope trace. The steady negative potential recorded by the electrode tip following its entry into the cytoplasm is the *resting potential*, V_{rest}, of the cell membrane and is given in *millivolts* (mV, thousandths of a volt). Virtually all cells that have been investigated have a negative resting potential. The size of this potential in various cells ranges up to -100 mV.

The potential sensed by the intracellular electrode does not change as the tip is advanced farther into the cell. Thus, the entire p.d. between the cell interior and cell exterior exists across the surface membrane and the regions immediately adjacent to the inner and outer membrane surfaces.

The electrical properties of the cell membrane can be examined by passing a pulse of current through the membrane to produce a perturbation of the membrane potential. To do this, a second microelectrode, the *current electrode*, is inserted into the cell to deliver a current (Figure 5-4) that can be made to flow across the membrane in either the inward (bath to cytoplasm) or the outward (cytoplasm to bath) direction,* depending on the direction of the electric current delivered from the electrode. If the electrode is made positive, it will send current directly into the cell. This current will flow out of the cell through its membrane. Conversely, if the electrode is made negative, it will draw positive charge

*As was noted in Chapter 2, all the current carried in solution and through the membrane is in the form of migrating ions. By convention, the flow of ionic current is from a region of relative positivity to one of relative negativity and corresponds to the direction of cation migration.



5-4 (**A**) Glass capillary microelectrodes inserted through the membrane of a cell. The electrode at the left is used to pass current into or out of the cell. Current passed into the cell through the electrode depolarizes the cell as it passes out of the cell across the surface membrane. (**B**) Current flows in a circuit through the wires, bath, resistor, electrodes, and cell membrane. The resistor is selected to have a far greater resistance than the other elements of the stimulating circuit to maintain the constancy of the stimulating current. The recording amplifier has a very high input resistance, preventing any appreciable current from leaving the cell through the recording electrode.

out of the cell and cause current flow into the cell through the membrane.

When current pulses are passed so that positive charge is removed from inside the cell through the current electrode (i.e., by making the inside of the electrode negative), the negative interior of the cell becomes still more negative and the cell is said to *hyperpolarize*. As an example, the intracellular potential may increase from a resting potential of −60 mV to a new hyperpolarized potential of −70 mV. The membrane generally responds passively to hyperpolarization, producing no response of its own other than the simple ohmic potential change due to the applied current (Figure 5-5).

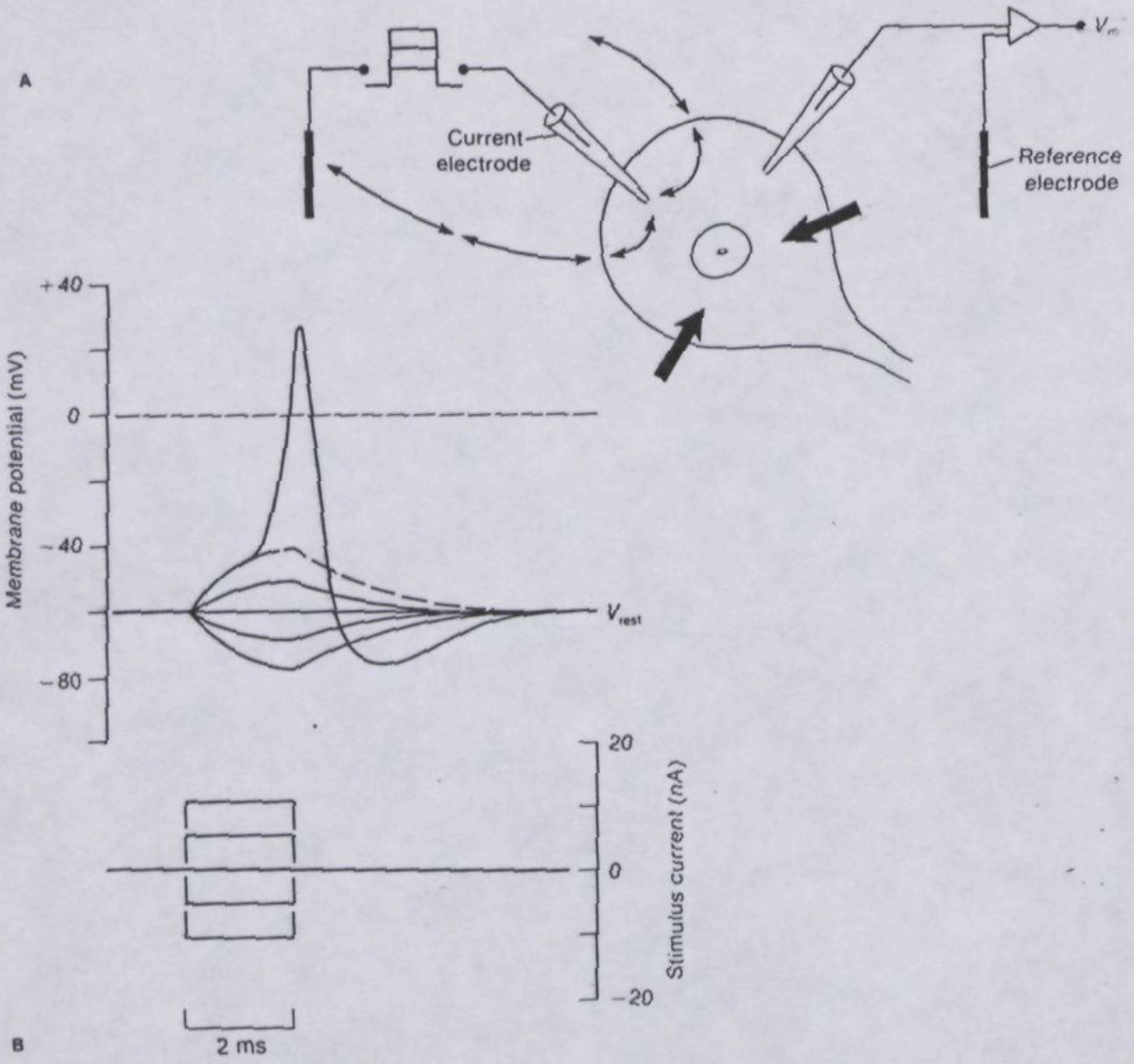
If positive charge is added to the inner surface of the cell membrane by passing current from the electrode into the cell, the p.d. across the membrane will be diminished, and the cell then is said to *depolarize*. That is, the intracellular potential becomes less negative (e.g., shifting from −60 to −50 mV). If the strength of the outward current pulse is increased, the degree of depolarization will also increase.

The membranes of excitable cells, such as many nerve, muscle, and receptor cells, have a *threshold potential* beyond which the membrane will produce a strong active response, the *action potential*, or AP (Figure 5-5). The AP is caused by the activation of membrane channels permeable to sodium. These channels have the property of being *activated* (i.e., caused to open) by the reduction in p.d. between the two sides of the cell membrane. The opening of the sodium channels in response to depolarization and the resulting flow of sodium ions into the cell provide an example of *membrane excitation*. The mechanisms underlying the AP and other instances of membrane excitation are considered in more detail later.

As just illustrated, cell membranes respond to electrical stimuli with two quite different classes of electrical behavior—passive and active responses:

1. *A passive electrical response* is a shift in membrane potential produced when an electric current arising elsewhere flows across the cell membrane. The voltage response occurs independent of any molecular changes such as opening or closing of membrane channels. This distinguishes it from an active electrical response. The ionic current that produces passive electrical responses flows primarily through inexcitable (i.e., nonreactive) channels that selectively pass K ions. These, the *resting potassium channels* (Table 5-1), are open in the resting membrane.

2. An *active electrical response*, found in excitable tissues such as nerve, muscle, and sensory receptors, depends on the *gating* (opening or closing) of numerous



5-5 Passive and active membrane responses in a neuron soma. *Stimulus current (black arrows in part* **A***) produces passive shifts* in membrane potential (black traces in part **B**). With sufficient depolarization, an additional current enters the cell (colored arrows in A), which results in a sudden active electrical response, an action potential (colored part of B). *Note that the passive responses are more or less proportional to the stimulus current,* whereas the active response is strongly disproportionate because of the effect of the Na^+ current that results from the opening of sodium channels.

minute ion channels in response to a stimulus. The opening (or closing) of a population of ion channels controls the flow of ionic current driven from one side of the membrane to the other by the electrochemical gradient of the ionic species permeating the channels.

Some ion channels are gated by changes in the membrane potential; others are gated by the binding of transmitter or messenger molecules to receptor sites; still others, found in sensory receptor cells, are activated by specific stimulus energies such as light (photoreceptors) or mechanical strain (mechanoreceptors). Most types of excitable channels exhibit some degree of *ion selectivity*, that is, they allow one or a few species of ions to pass much more readily than all other ions. Excitable channels are often named for the ionic species that normally move through them; for example, the *sodium channel* (Table 5-1) allows certain ions other than sodium to pass (e.g., lithium ions), but sodium is the ion that normally moves through this channel during nerve impulses. When such a channel opens, a small current can be carried across the membrane through the channel by the permeating ion species. The simultaneous opening of many such channels produces a current large enough to produce a measurable voltage signal across the membrane. The gating of ion channels, as we will see later, is the immediate cause for nearly all electrical signals in living tissue.

Passive Electrical Properties of Cell Membranes

Before we can go on to gain an understanding of active electrical phenomena in excitable cells, it is essential that we consider the passive electrical properties of the cell membrane. Two types of structural elements present in the membrane give rise to two corresponding electrical properties:

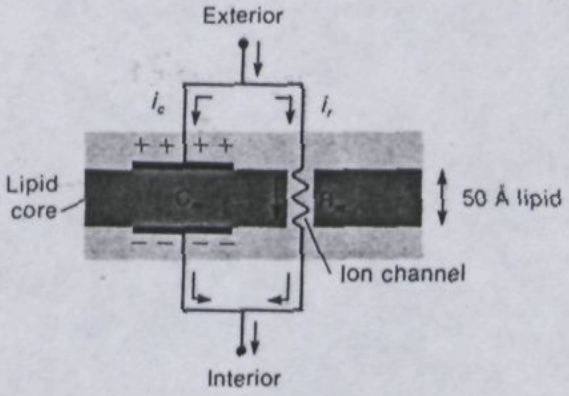
TABLE 5-1 Some nonsynaptic ion channels found in excitable cells.

Channel	Current	Characteristics	Blocked by	Function
Potassium channel, resting	$I_{K(leak)}$	Responsible for potassium leakiness of resting cell	Partially by tetraethylammonium (TEA)	Largely responsible for resting potential
Sodium channel	I_{Na}	Rapid activation by depolarization, followed by voltage-dependent inactivation	Tetrodotoxin (TTX)	Carries current for upstroke of AP; impulse conduction
Calcium channel	I_{Ca}	Slower activation by depolarization; and lower single-channel conductance than sodium channel; inactivates as function of [Ca], and/or membrane potential	Verapamil, D600, Co^{2+}, Cd^{2+}, Mn^{2+}, Ni^{2+}, La^{3+}	Slow depolarizations; calcium acts as "messenger molecule" to cell interior
Potassium channel, delayed rectifying	$I_{K(V)}$	Delayed activation by depolarization; inactivates slowly and incompletely under steady depolarization	Intra- and extracellular TEA; amino pyridines	Carries current for rapid repolarization to terminate action potential
Potassium channel, calcium activated	$I_{K(Ca)}$	Activation by $[Ca^{2+}]_i$. Remains activated until [Ca], is lowered. Activation by calcium is enhanced by depolarization	Extracellular TEA	Carries current for repolarization of sodium and/or calcium AP; production of outward current to balance inward calcium current, thereby limiting depolarization due to I_{Ca}

1. A large proportion of the membrane consists of a bimolecular lipid leaflet (p. 69), and this lipid bilayer is impermeant to ions and thus can separate charges present in the form of ions. The bilayer of lipid thus conveys the property of membrane *capacitance* to the membrane.

2. Ion channels, as noted above, inserted in the lipid bilayer provide a pathway for inorganic ions to carry electric charges across the membrane. These channels thus covey the property of *conductance* to the cell membrane. These two properties, membrane capacitance and membrane conductance, account for the passive electrical behavior of cell membranes.

It is helpful to conceptualize the properties of conductance and capacitance in the form of an *equivalent circuit* (Figure 5-6) in which an electrical capacitor is depicted as wired in parallel with a resistor. The resistor, R_m, represents the conductance conferred on the membrane by its ion channels. The capacitor, C_m, represents the lipid bilayer, which is essentially impermeable to ions.



5-6 Simplest equivalent circuit for a cell membrane, showing membrane capacitance, C_m, and resistance, R_m. Arrows indicate flow of capacitive current, i_c, and resistive current, i_r.

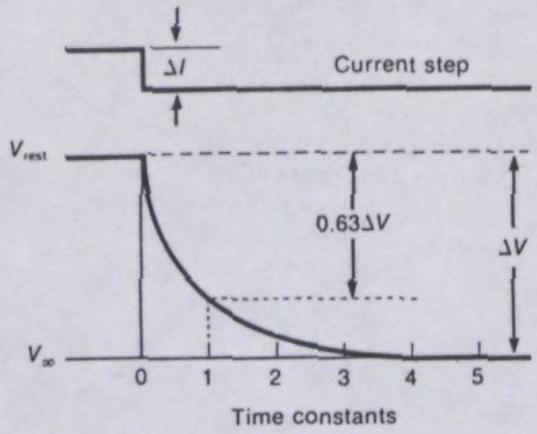
Membrane Conductance

The conductance of a membrane is a measure of its permeability to ions. The greater the conductance, the more ionic charges will cross the membrane via ion channels per unit time under a given electrical force—that is, potential difference. When a steplike pulse of steady current is applied across the membrane from an external source, the membrane potential shifts with a delay toward a steady-state level along an exponential time course (Figure 5-7). The displacement of the membrane potential from the resting value to the asymptotic value, ΔV_m, is a function of the magnitude of both the applied current (ΔI) and the input conductance (G_{input}) that the current encounters as it passes across the membrane of a cell. The relationship between applied current, conductance, and recorded steady-state voltage is described by Ohm's law, which states that *the voltage drop produced across a membrane by a current passed across the membrane is proportional to the current and inversely proportional to the conductance of the membrane.* Thus,

$$\Delta V_m = \frac{\Delta I}{G_{input}} \qquad (5\text{-}1a)$$

or

$$G_{input} = \frac{\Delta I}{\Delta V_m} \qquad (5\text{-}1b)$$



5-7 Electrical response of a cell membrane to an applied step of inward-going current, ΔI, plotted against time. The time required for the voltage to reach 63% of its asymptotic value is proportional to the product of the resistance and the capacitance of the membrane. This product is termed the time constant, τ, of the membrane.

It will be recalled that the reciprocal of conductance (in units of siemens) is resistance (R, in units of ohms):

$$R = \frac{1}{G} \tag{5-1c}$$

Consider two spherical cells, one small, the other large, both with membranes having the same *specific resistance*, R_m, to electric current (i.e., the same resistance to current flowing across a square centimeter of membrane). For a given increment of current, ΔI, the large cell will show a smaller increment of voltage, ΔV_m, because the same current will flow through a larger area of membrane; the current density will therefore be smaller across the membrane of the large cell than across the membrane of the smaller cell, since, if all else is equal, a large cell will have a lower electrical resistance across its membrane than a small cell (Figure 5-8). This stems from the larger number of ion channels available in the larger membrane area of the larger cell. This principle is illustrated by the fact that a current passing through two equal parallel resistors produces half the p.d. produced by the same total amount of current passing through only one of these resistors. Each ion channel can be thought of as a tiny electrical conductor (or resistor) carrying current (in the form of ion flow) across the surface membrane. Because the *input resistance*, R, of a cell (i.e., the total resistance encountered by current flowing into or out of the cell) is a function of both membrane area, A, and specific resistance, R_m, of the membrane, it is useful when comparing membranes of different cells to correct for the effect of membrane area on the current density. Thus, the specific resistance is calculated as

$$R_m = RA \tag{5-2a}$$

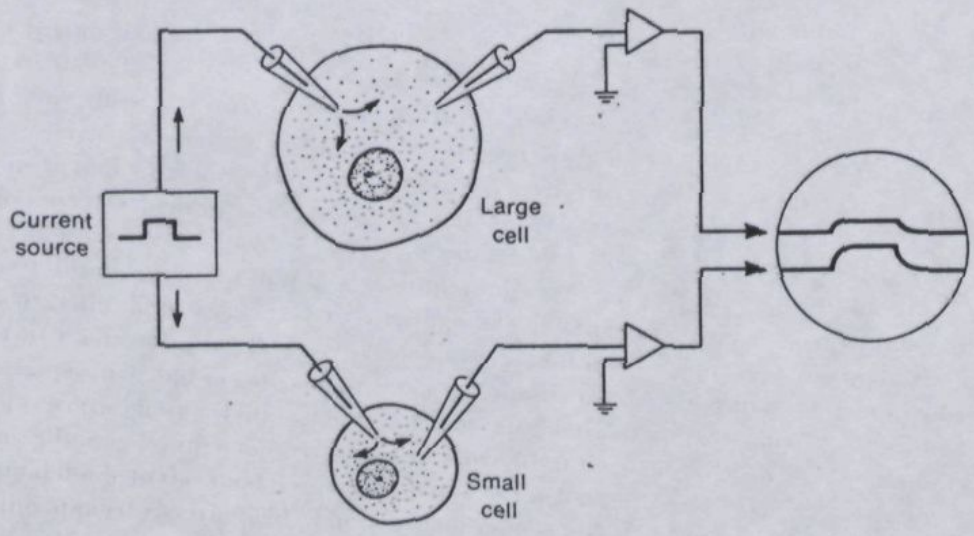
Since

$$R = \frac{\Delta V_m}{\Delta I} \tag{5-2b}$$

then

$$R_m = \frac{\Delta V_m A}{\Delta I} \tag{5-2c}$$

Since $\Delta V_m/\Delta I$ has the units of ohms, and area is in square centimeters, R_m is in units of ohms times centimeters squared. Note that membrane area and input resistance, R, are reciprocally related. The specific resistance, R_m, of the membrane is, of course, a property of the population of membrane ion channels carrying



5-8 Effect of cell size on voltage response to a given current. The input resistance of the larger cell is lower than that of the smaller cell. Thus, passage of the same amount of current into both cells results in a larger potential change in the smaller cell, as predicted by Ohm's law.

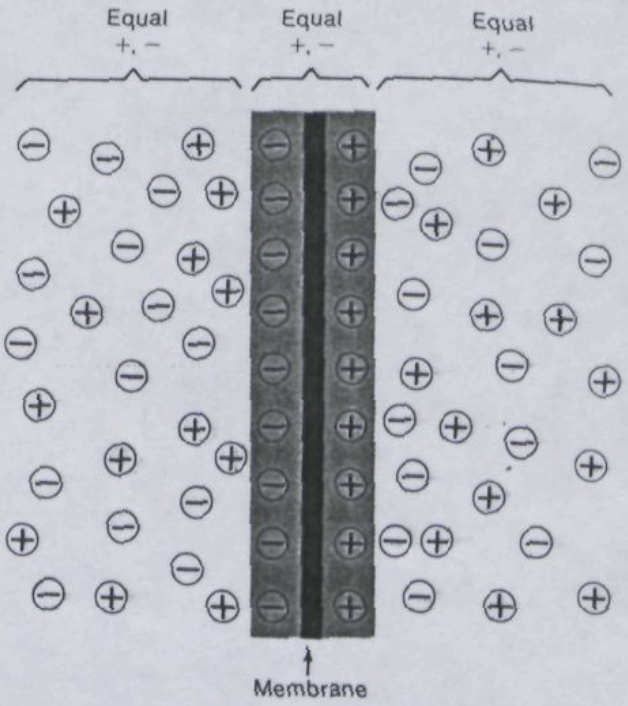
the current. Specific resistances of various cell membranes range from hundreds to tens of thousands of ohms times centimeters squared.

The reciprocal of the specific resistance of a membrane is the specific *membrane conductance*, G_m (in units of siemens per square centimeter). Conductance is related to the ionic permeability of the membrane, but conductance and permeability are not synonymous. Conductance to a given species of ion is defined by Ohm's law as the current carried by that species of ion divided by the electrical force acting on that species. Thus, membrane conductance for species X is defined as

$$g_X = \frac{I_X}{\text{emf}_X} \qquad (5\text{-}3)$$

in which g_X is the membrane conductance for ion species X, I_X is the current carried by that species, and emf_X is the electromotive force (in volts) acting on that species. Although emf_X varies with membrane potential, it is not identical with membrane potential, as noted later.

Even though a membrane may be permeable to ion X, the conductance, g_X, depends on the presence and concentration of this species in the solution; unless it is present, it cannot carry current. It is also evident that permeability of nonelectrolytes does not contribute any conductance, since a nonelectrolyte does not carry a charge and hence cannot carry current. Thus, the terms conductance and permeability are not synonymous.



5-9 The cell membrane is able to separate charges because it acts as a capacitor. The cations and anions form a diffuse layer on the two sides of the membrane and interact electrostatically across the thin barrier. Because of this interaction, the charge differences that occur between the two sides of the membrane are largely confined to the regions immediately adjacent to the two membrane surfaces. Except for these few excess cations and anions, the bulk phases conform to the principle of electroneutrality.

Membrane Capacitance

The rate at which ions traverse the lipid bilayer of a membrane is less that 10^{-8} times the average rate at which they diffuse across an equivalent distance (5–10 nm) of aqueous solution, as in cytoplasm or extracellular fluid. Since electric current in an aqueous solution is carried by ions, the very low mobility of ions through the lipid bilayer results in a high electrical resistance of the bilayer, and thus very few ions pass directly through the lipid bilayer itself. Nearly all the ions that cross the membrane do so via ion channels or carrier molecules.

Although the lipid bilayer is virtually impermeable to ions, there is a way in which electric charge can *in effect* move transiently across the lipid bilayer without ions actually crossing the membrane. This effective charge movement is purely transient, and comes about because the electric fields of ions can extend over the short distance across a thin layer of insulating material such as the lipid bilayer, and can therefore interact across the membrane. Because of such interaction between positive and negative charges, a membrane can store electric charge. Thus, when a p.d. is applied across a membrane, positive ions will tend to move from the cathodal side to the anodal side in response to the force of the applied electric field. The ions will not be able to get through the lipid bilayer, however, but tend to pile up at the surface of the membrane, the cations on the cathodal side and the anions on the anodal side. For a while, more ions will accumulate at the membrane surface, but eventually the mutual repulsion of the cations on the cathodal side will balance the force due to the applied voltage, and no more cations will accumulate on that side, the same being true of the anions on the anodal side (Figure 5-9), leaving a net excess of oppositely charged ions on opposite sides of the membrane. The result of this process is that for some time after the voltage step is first applied, ions move up to the membrane on the one side and move away from the other side. This movement of charge constitutes a transient *capacitive current*, even though no charges actually physically cross the membrane in this process.

The oppositely charged ions that have accumulated on opposite sides of the membrane can interact electrostatically with one another because of the short distance across the membrane. The ability of the bilayer to separate or store charges in this way is called *capacitance*, and it is measured in units of *coulombs per volt*, or *farads* (F). The amount of charge that can be separated by a layer of insulating material depends on its thickness and its *dielectric constant*. Cell membranes are very thin (less than 10 nm) and are virtually impermeable to ions

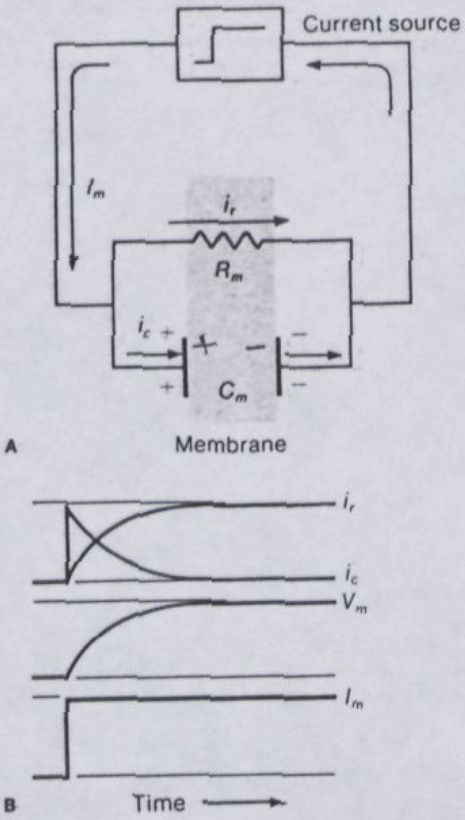
over most of their surface areas. Knowing the thickness of cell membranes, and the dielectric constant (a property that reflects the ability of an insulator to store charge) of the lipids most commonly found in the membranes of cells, we can calculate an expected value for the capacitance of nerve cell membranes. Most membranes appear to contain a layer of lipid about 5 nm thick. Thus, if we assume that the lipid has a dielectric constant of 3, which is about that of an 18-carbon fatty acid, the membrane capacitance can be calculated to be about 1 microfarad (1 μF = 10^{-6} F) per square centimeter. Measured values for biological membranes are generally close to 1 μF/cm^2.

Electrotonic Potentials

The properties of membrane conductance and capacitance as illustrated by the equivalent ciruit in Figure 5-10A can be demonstrated with an experimental setup like that shown in Figure 5-4. Consider a current of constant intensity, I (amperes), passed across the membrane from the reference electrode in the bath to the current electrode that has its tip inserted into the cell. This current is applied as a steplike pulse having an abrupt onset. All the current must pass through the membrane to complete the circuit. While crossing the membrane, the current must distribute itself between the conductance and capacitance that occur in parallel across the membrane (Figure 5-10A). In doing so the current produces a passive potential change across the membrane, as shown in Figure 5-10B (trace V_m). This passive potential change, termed an *electrotonic potential*, is produced by the applied current flowing through the capacitive and resistive pathways across the membrane. When the steplike pulse of current, I_m, is forced across a membrane, the current divides itself between the membrane capacitance and resistance in a manner that changes with time, so that initially most of the current flows through the membrane capacitance. (Recall, however, that capacitive current passing "through" a capacitor does not mean the physical passage of charges across the capacitor, but merely the electrostatically determined displacement of charge onto or off the opposite sides of the lipid bilayer.) As the transient current flows "across" the capacitor, the resulting accumulation of charges causes the p.d. across the capacitor to increase (or decrease in the case of a depolarization). This p.d. across the capacitor repels new charges, causing the rate of charging to exhibit a decay. Hence, the capacitive current, i_c (Figure 5-10B), *drops* along an exponential time course as the p.d. *rises* with the same time course. At the same time, the current, i_r, passing through the membrane conductance (i.e., ion channels) undergoes complementary exponential increase (Figure 5-10B).

The relationship between potential and time during the charging of the capacitance is given by the equation

$$V_t = V_\infty(1 - e^{-t/RC}) \qquad (5\text{-}4)$$



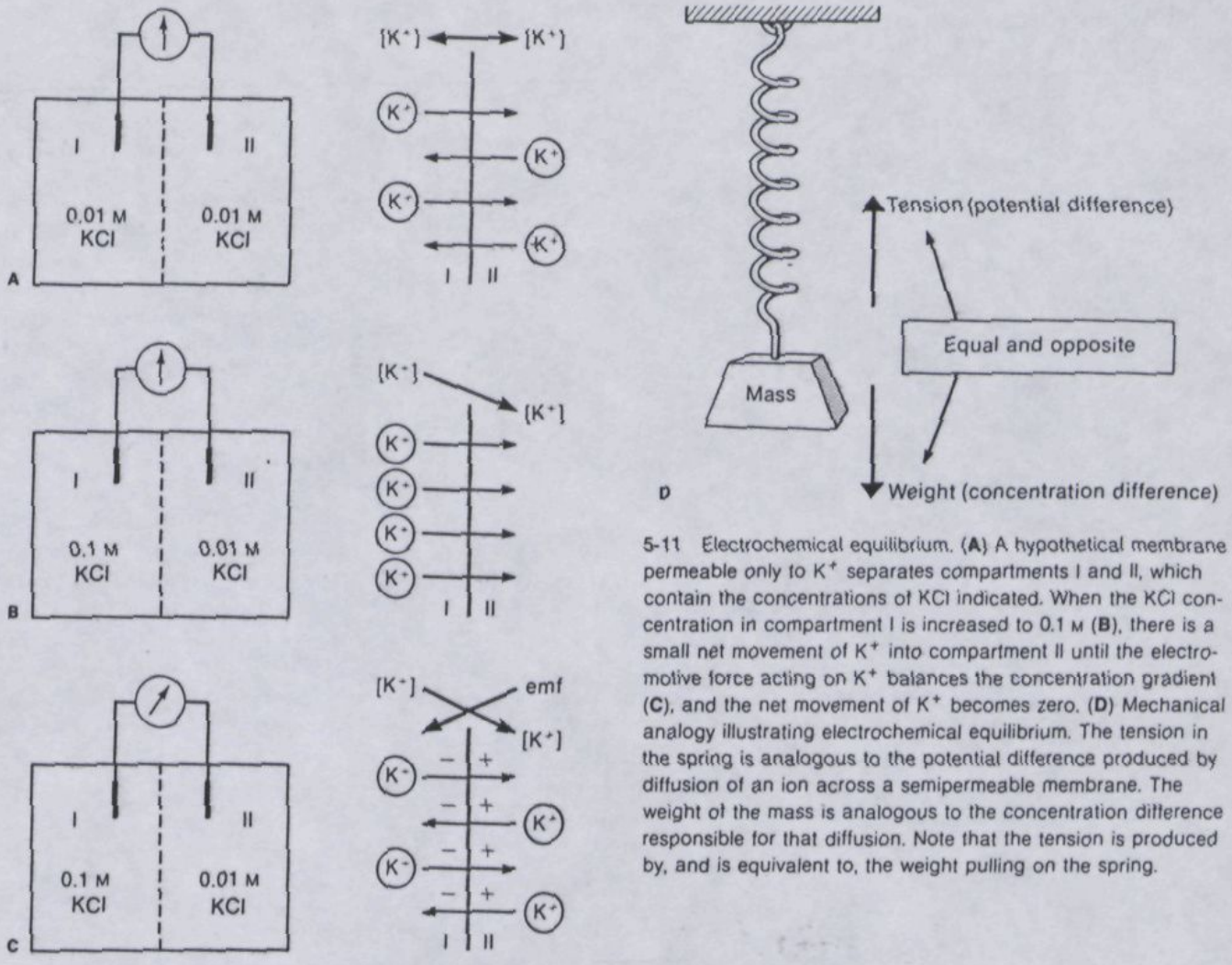
5-10 (A) The equivalent circuit for a cell membrane across which an abrupt pulse of constant current is passed. The high resistance is used to supply an unvarying current. (B) Time courses of resistive current, i_r, capacitive current, i_c, membrane potential, V_m (i.e., the potential across both the membrane resistance and capacitance), and the total membrane current, I_m.

where V_∞ is the potential across the capacitor at $t = \infty$ produced by a constant current applied to the network, t is the time in seconds after the beginning of the current pulse, R is the resistance of the circuit in ohms, C is the capacitance of the circuit in farads, and V_t is the potential across the capacitor at time t.

When t is equal to the product RC, then $V_t = V_\infty(1 - 1/e) = 0.63V_\infty$. The value of t (in seconds) that equals RC is termed the *time constant* (τ) of the process. Note that it is independent of both V_∞ and current strength, and is the time required for the voltage across a charging capacitor to reach 63% of the asymptotic value, V_∞ (Figure 5-7).

Electrochemical Potentials

Now that we have considered the two basic passive electrical properties of cell membranes, capacitance and conductance, we can go on to consider the origin of *electrochemical potentials*, which energize active electrical responses and underlie the resting potential. Indeed, electrochemical potentials are directly responsible for nearly all the electrical phenomena that occur in the animal body. These potentials originate, as we will see, from two features found in all eukaryotic cells: (1) Metabolically sustained asymmetrical distributions of ions between the intracellular and the extracellular



5-11 Electrochemical equilibrium. (**A**) A hypothetical membrane permeable only to K^+ separates compartments I and II, which contain the concentrations of KCl indicated. When the KCl concentration in compartment I is increased to 0.1 M (**B**), there is a small net movement of K^+ into compartment II until the electromotive force acting on K^+ balances the concentration gradient (**C**), and the net movement of K^+ becomes zero. (**D**) Mechanical analogy illustrating electrochemical equilibrium. The tension in the spring is analogous to the potential difference produced by diffusion of an ion across a semipermeable membrane. The weight of the mass is analogous to the concentration difference responsible for that diffusion. Note that the tension is produced by, and is equivalent to, the weight pulling on the spring.

compartments, and (2) the selective permeability of ion channels found in the surface membrane.

To begin with, we will consider the thought experiment shown in Figure 5-11. A chamber is divided into two compartments by a hypothetical selectively permeable membrane, one that is permeable only to the potassium ions present. At the start of the thought experiment both compartments contain 0.01 M KCl. Under those conditions, electrodes inserted one each into the two compartments record no voltage across the membrane. Since this hypothetical membrane is permeable to K^+ but *not* to Cl^-, K^+ diffuses across the membrane without its counterion. On the average, for each potassium ion that passes in one direction through the membrane, another will pass in the opposite direction, since the concentrations in the two compartments are equal, and thus the net K^+ flux is zero. The p.d. between the two sides of the membrane thus also remains zero (Figure 5-11A). We then place additional KCl in compartment I to instantaneously increase its concentration to 0.1 M (10 times that of compartment II, Figure 5-11B). Because of the increased concentration in compartment I, K^+ now exhibits net diffusion from compartment I to compartment II, producing an increase in positive charge in the latter. An increased positive potential thus quickly develops in compartment II and the voltmeter will indicate the p.d. (Figure 5-11C). This p.d. will reach a given value that will be maintained indefinitely, provided there is no leakage of Cl^- across the membrane.

How do we explain the steadily maintained p.d.? After we increase the KCl concentration in compartment I, for every K^+ that is statistically available for diffusion through K channels from compartment II to I, 10 potassium ions in I are available to pass through the membrane to compartment II. That is, the difference in K^+ concentration gradients represents a chemical gradient, or *chemical p.d.*, that causes an initial net diffusion through the membrane from I to II (Figure 5-11B). Each additional K^+ that diffuses from I to II adds its positive charge to that side, since Cl^- cannot accompany K^+ across this hypothetical membrane. As potassium ions accumulate in compartment II, the p.d. across the membrane quickly rises, since the membrane then separates an excess of positive charges on one side from an excess of negative charges on the other (Figure 5-9). As K^+ leaks into compartment II and builds up positive potential in that compartment, further movement of K^+ becomes more difficult because of electrostatic repulsion by the increasing positive charge in

Box 5-1 *Charge Separation by Membranes*

Very few ions actually diffuse across 1 cm^2 of the membrane in Figure 5-11 before the membrane potential equals E_K. The actual number of excess ions that cross the membrane is easy to calculate for a system with a single diffusible ion. The number of excess potassium ions accumulated in compartment II (and excess chloride ions left behind in compartment I) depends on two factors: (1) the potassium equilibrium potential and (2) the capacitance of the membrane. The charge, Q (in coulombs, C), accumulated across a capacitor is proportional to both the capacitance, C, of the capacitor (given in farads) and the voltage, V, developed across the capacitor (given in volts). Biological membranes typically have capacitances of about 1 μF (10^{-6} F) per square centimeter. We can calculate the coulombs of charge that diffuse across 1 cm^2 of membrane when the membrane separates a 10-fold difference in the concentrations of a diffusible monovalent cation (i.e., a potential difference of 58 mV after equilibrium is achieved):

$$\begin{aligned} Q &= CV \\ &= (10^{-6}\ \text{F/cm}^2)(5.8 \times 10^{-2}\ \text{V}) \\ &= 5.8 \times 10^{-8}\ \text{C/cm}^2 \end{aligned}$$

There is one faraday of charge (1 $\mathcal{F}$ = 96,500 C) in 1 g·equiv wt, or 1 mol, of a monovalent ion. Thus, the amount of K^+ in moles required to transfer 5.8×10^{-8} C across 1 cm^2 of membrane is calculated by dividing the amount of charge transferred by the number of coulombs in 1 $\mathcal{F}$:

$$\frac{5.8 \times 10^{-8}\ \text{C/cm}^2}{9.65 \times 10^{4}\ \text{C/mol K}^+} = 6 \times 10^{-13}\ \text{mol K}^+/\text{cm}^2$$

The number of potassium ions accumulated in compartment II of the membrane in Figure 5-11 is found by multiplying the number of moles by Avogadro's number (6×10^{23} molecules/mol):

$$(6 \times 10^{-13})(6 \times 10^{23}) = 3.6 \times 10^{11}\ \text{K}^+/\text{cm}^2$$

An equal number of chloride ions remains in excess in compartment I of the membrane. This number is more than 10,000,000 times smaller than the number of potassium ions in a cubic centimeter of solution II (6×10^{18} potassium ions). Thus, the concentrations in compartments I and II are virtually unchanged as a result of the charge separation across the membrane. Even though there is a slight separation of anions from cations across the membrane, the segregation exists only on a microscopic scale, separated by about the thickness of the membrane (Figure 5-6). The rule of electroneutrality—that positive charges must equal negative charges—remains essentially unviolated on the macroscopic scale.

compartment II. Thus, each K^+ now entering the membrane via a potassium channel has two forces acting on it—a chemical p.d. favoring net K^+ flux from I to II, and an *electrical p.d.* favoring net K^+ flux from II to I (Figure 5-11C). After a certain p.d. is built up across the membrane as a result of excess K^+ accumulating in compartment II, the opposing forces come into equilibrium and remain balanced, with the electrostatic force of the p.d. across the membrane precisely offsetting the tendency for K^+ to diffuse down its concentration gradient. The potassuim ion is then said to be in *electrochemical equilibrium;* the p.d. across the membrane established in this way is termed the *equilibrium potential* for the ion in question (in this case, it is the potassium equilibrium potential, E_K).

To illustrate the equilibrium state between ionic concentration gradient and the resulting electrical p.d., a simple analogy is given in Figure 5-11D. A mass is gently lowered from a spring. As gravity pulls the mass down, tension develops in the spring as the mass stretches the spring. This tension holds the mass up with a force equal and opposite to the force of gravity acting to pull down the mass; the system is therefore in equilibrium, with the mass suspended on the stretched spring. The gravity pulling on the mass is analogous to the chemical gradient, and the tension developed in the spring is analogous to the potential developed across the membrane. The gravity acting on the mass produces the tension in the spring by stretching it, and the tension develops until it just balances the pull of gravity and keeps the mass suspended. Likewise, movement of charge from compartment I to II produces the electrical "tension" (p.d.); the p.d., in turn, prevents further net movement of charge and thereby balances the unequal ionic concentrations in a state of equilibrium.

When an ion is in electrochemical equilibrium, it undergoes no further net flux across the membrane, even if the membrane is freely permeable to that ion. Conversely, if an ion present in the system is not permeant (i.e., cannot diffuse across the membrane) its presence does not influence the equilibrium state. Thus, in our hypothetical system, Cl^-, even though it is far out of electrochemical equilibrium (it would tend to enter compartment II from compartment I), contributes nothing to the membrane potential because it is unable to cross the membrane.

It is important to note that the process of establishing the equilibrium state involves the diffusion of only a very small number of ions (relative to those present in solution) across the membrane from the one compartment to the other. Thus, virtually no change takes place in the concentrations of KCl in the two compartments during this process, since the number of K^+ crossing into compartment II is insignificant compared to the numbers originally present in the solution. This concept receives further attention in Box 5-1.

The Nernst Relation

It seems right, intuitively, that the equilibrium potential of an ion should increase in value with an increase in its concentration gradient across the membrane, just as the tension developed in a spring (Figure 5-11) increases with the mass of the suspended body. In other words, a greater chemical gradient across the membrane requires a greater electrical p.d. across the membrane to offset the greater tendency for the ions to diffuse down their concentration gradient. The equilibrium potential, in fact, is proportional to the *logarithm* of the ratio of the concentrations in the two compartments. The relation between concentration ratios and membrane potential was derived in the latter part of the nineteenth century by Walther Nernst from the gas laws (Box 5-2). The equilibrium potential depends on the absolute temperature, the valence of the diffusible ion, and, of course, the ratio of concentrations on the two sides of the membrane:

$$E_X = \frac{RT}{Fz} \ln \frac{[X]_I}{[X]_{II}} \qquad (5\text{-}5)$$

Here R is the gas constant, T is the absolute (Kelvin) temperature; F is the Faraday constant (96,500 C/g·equiv charge); z is the valence of ion X; $[X]_I$ and $[X]_{II}$ are the concentrations (more accurately, activities) of ion X on sides I and II of the membrane; and E_X is the equilibrium potential for ion X (potential of side II minus side I). At a temperature of 18°C and with a monovalent ion, and converting from ln to log, the Nernst equation can be reduced to

$$E_X = \frac{0.058}{z} \log \frac{[X]_I}{[X]_{II}} \qquad (5\text{-}6)$$

where E_X is expressed in volts.* Note that E_X will be positive if X is a cation and the ratio of $[X]_I$ to $[X]_{II}$ is greater than unity. The sign will become negative if the ratio is less than 1. Likewise, the sign will be reversed if X is an anion rather than a cation, because z will be negative. The Nernst relation predicts a rise in p.d. of 58 mV every time the concentration ratio of the permeant ion is increased by a factor of 10. When the potential is plotted as a function of $\log [K^+]_I/[K^+]_{II}$, the relation has a slope of 58 mV per 10-fold increase in the ratio (Figure 5-12).

Recall the convention that the electrical potential inside the living cell, V_i, is described in reference to the potential outside the cell, V_o. That is, the membrane potential, V_m, is given as $V_i - V_o$, so that the potential of the cell exterior is arbitrarily defined as zero. For this reason, when determining the equilibrium potential, we place the extracellular concentration of the ion in question in the numerator and the intracellular concentration in the denominator of the concentration ratio. Applying the Nernst relation (Equation 5-5), we can calculate the potential at which potassium will be in equilibrium, E_K, in a hypothetical cell in which $[K]_o = 0.01$ M and $[K]_i = 0.1$ M:

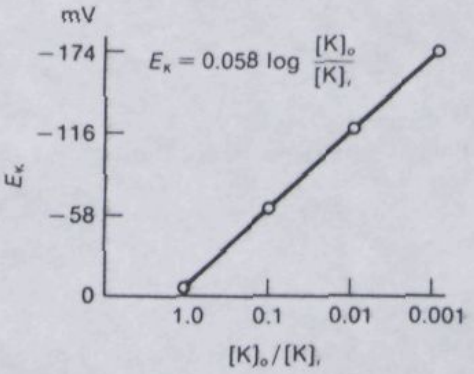
$$E_K = 0.058 \log \frac{[K]_o}{[K]_i}$$

$$= 0.058 \log \frac{0.01}{0.1}$$

$$= 0.058(-1)$$

$$= -0.058 \text{ V} = -58 \text{ mV}$$

Note that E_K has a negative sign, since intracellular negativity will result when a minute amount of K^+ leaks out of the cell owing to the high intracellular and low extracellular concentrations of K^+. It should also be apparent from Equation 5-6 that if the ion in question is a divalent cation (i.e., $z = +2$), the slope of the relation becomes 29 mV per 10-fold increase in concentration ratio.



5-12 Calculated relationship between the equilibrium potential of a monovalent ion, such as K^+, and the ratio of concentrations of that ion on the two sides of a membrane.

The Resting Potential

Each cell has a p.d. across its surface membrane characteristic of its "resting" (i.e., nonexcited) state. This resting potential typically lies between −30 and −100 mV, depending on the kind of cell and on the ionic environment, although some cell types have a smaller p.d. Two factors play a role in the origin of the resting potential. The first is the unequal distribution of inorganic ions between the cell interior and cell exterior, caused by active transport by membrane pumps (p. 84) and Donnan distribution (p. 76). The second factor is the presence of open ion channels in the cell membrane that are permeable to some of the ionic species present. As we have seen, the unequal distribution of ions provides the chemical driving force for the establishment of an equilibrium potential. This, as we will see, provides the basic principle underlying the resting potential. The principle of an equilibrium potential was illustrated in the preceding section with a

*At 38°C, which is approximately the body temperature of many mammals, the multiplication factor is $0.061/z$.

Box 5-2 Derivation of the Nernst Equation

The Nernst equation is one of the most widely used mathematical relations in physiology, and is essential for an understanding of bioelectric phenomena. Its derivation is based on the concept of a thermodynamic equilibrium between the osmotic work that is required to move a given number of ions across a membrane in one direction and the electrical work required to move an equivalent number of charges back across the membrane in the opposite direction. The osmotic work required to transfer 1 mol·equiv of an ion, X, from a concentration $[X]_I$ to a concentration 10 times higher, $[X]_{II}$, can be derived from the gas laws as

$$W = RT \ln \frac{[X]_I}{[X]_{II}} \qquad (1)$$

in which W is mechanical (or osmotic) work (equals force times distance).

This, then, represents the thermodynamic work that would be required to establish an e-fold difference in the concentration across a membrane barrier of 1 mol of ion X, starting with equal concentrations on both sides of the membrane. If the membrane is permeable to this species, these ions will tend to diffuse back toward the low concentration, until the resulting equilibrium potential just balances this tendency. Note that we can relate osmotic work to electrical work through the equality

$$W = EFz \qquad (2)$$

in which the potential difference, E, is multiplied by the Faraday constant (F), namely, the charge per mole of univalent ion. The valence, z, of the ion corrects for multivalent species. Substituting Equation 2 in Equation 1, we obtain

$$EFz = RT \ln \frac{[X]_I}{[X]_{II}} \qquad (3)$$

or

$$E = \frac{RT}{Fz} \ln \frac{[X]_I}{[X]_{II}} \qquad (4)$$

which is the general form of the Nernst equation.

simplified, ideal system in which only one ionic species was diffusible. This principle will now be applied to biological membranes, which, unlike the imaginary membrane, are permeable in varying degrees to all of the inorganic ions present, and hence require a somewhat more complex treatment.

The Role of Ion Gradients and Channels

It is evident that the electrochemical gradient of a given ion species has no effect on the membrane potential if the membrane is impermeable to that species. After all, nonpermeant ions cannot carry charge from one side of the membrane to the other. It follows that an ion to which the membrane is only slightly permeable should have a smaller effect on the membrane potential than another species that can diffuse across the membrane more freely. It is, in fact, the relative ease with which different ions can cross the membrane that determines their relative contributions to the potential they produce in diffusing across the membrane. On this basis, and by making the simplifying (and somewhat incorrect) assumption that the potential drop across the membrane exists with a uniform gradient through the membrane from one side to the other side, David E. Goldman (1943) derived an equation that is related to the Nernst equation and that takes into consideration the relative permeability of each species of ion:

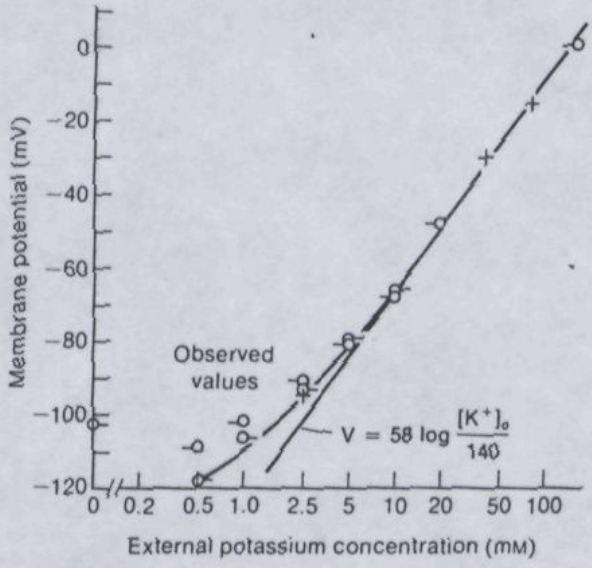
$$V_m = \frac{RT}{F} \ln \frac{P_K[K^+]_o + P_{Na}[Na^+]_o + P_{Cl}[Cl^-]_i}{P_K[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_o} \qquad (5\text{-}7)$$

in which P_K, P_{Na}, and P_{Cl} are respective permeability constants of the major ion species in the intra- and extracellular compartments.

Thus, the probability that the ions of one species will cross the membrane is predicted to be proportional to the product of their concentration (more accurately, their thermodynamic *activity*) on that side and the permeability of the membrane to that species. As a result, the contribution of an ion species to the membrane potential should diminish as its concentration is reduced. This point is illustrated in Figure 5-13, in which the membrane potential of a living cell is plotted as a function of the concentration of extracellular K^+, the ion most important in determining the resting potential. At the higher K^+ concentrations the slope of the plot is about 58 mV per 10-fold increase in potassium concentration. At lower K^+ concentrations the curve deviates from this theoretical slope for E_K because Na^+ now becomes a more important contributor to the potential, in spite of its low permeability, as the product $P_{Na}[Na^+]_o$ approaches the product $P_K[K^+]_o$.

Richard D. Keynes (1954) determined the permeability constants for the major ions in frog muscle with the use of radioisotopes. The permeability of sodium was found to be about 0.01 times that of potassium. Thus, for muscle cell membranes* the Goldman equation can be simplified to

*Unlike Na^+ and K^+, the chloride ion is not actively transported across the muscle cell membrane; thus, it merely distributes itself passively according to the membrane potential, so that $E_{Cl} = V_m$. Since it merely follows rather than contributes to the membrane potential, it can be ignored in this calculation. In other cells chloride is not in electrochemical equilibrium and may contribute to the membrane potential.



5-13 Resting potential of a frog muscle cell plotted against the extracellular K^+ concentration. The theoretical 58 mV change for every 10-fold increase in the ratio of $[K^+]_o$ to $[K^+]_i$, as predicted by the Nernst equation, is shown as a straight line. The measured values are shown by the plotted points. Curved line was calculated from Equation 5-7, using $P_{Na} = 0.01\ P_K$. $[K^+]_i$ was taken as 140 mM. [Hodgkin and Horowicz, 1960.]

$$V_m = \frac{RT}{F} \ln \frac{[K^+]_o + 0.01[Na^+]_o}{[K^+]_i + 0.01[Na^+]_i}$$

$$= 0.058 \log \frac{2.5 + (0.01)(120)}{140 + (0.01)(10)}$$

$$= -0.092\ V = -92\ mV$$

Microelectrode measurements of the resting potential in frog skeletal muscle cells range from −90 to −100 mV. The closeness of match between the theoretical and measured values supports the idea that the resting potential arises in large part from simple diffusion potentials of inorganic ions.

Resting potentials of muscle, nerve, and most other cells are far more sensitive to changes in the extracellular potassium level than to changes in the concentrations of other cations. This is consistent with the relatively high permeability of cell membranes to K^+ as compared to other cations, due to the preponderence of potassium-selective channels open in the resting membrane. Large changes in extracellular Na^+, for example, have little effect on the resting potential, because of the low permeability of the cell membrane to sodium ions.

The Role of Active Transport

An idealized membrane such as the one in Figure 5-11, permeable to only one ionic species, will maintain a constant membrane potential indefinitely (if that ionic species is unequally distributed on the two sides of the membrane) without expenditure of energy, because such a system is in a state of thermodynamic equilibrium. Real membranes, however, are leaky to varying degrees to all inorganic ions; thus cells must have a way of maintaining their inorganic ions at appropriate concentrations. This is done by the active transport of certain ions so as to counteract the downhill movement of those ions.

Take the case of Na^+. The concentrations of extracellular and intracellular Na^+ in frog muscle (Figure 4-17) are about 120 and 10 mM, respectively. From these values we can calculate the sodium equilibrium potential as follows:

$$E_{Na} = 0.058 \log \frac{120}{10} = 0.063\ V + 63\ mV$$

Since V_m in frog muscle ranges from −90 to −100 mV, the sodium ions are more than −150 mV (i.e., $V_m - E_{Na}$) out of equilibrium. Even with only a small resting membrane permeability to Na^+, there will be a steady influx of Na^+ driven by the large potential acting on that ion. If it were not removed from the cell interior at the same rate at which it leaks in, Na^+ would gradually accumulate in the cell, displacing internal K^+, which would leak out in response to intracellular sodium accumulation. The high internal $[K^+]$ and low internal $[Na^+]$ result from a continuous metabolically energized transport of Na^+ out of the cell. During the active transport of Na^+ out of the cell there is an obligatory uptake of K^+, typically two potassium for three sodium ions. Since the back leak of Na^+ is slow, because of the low Na permeability of the resting membrane, the effect of the Na–K pump is to maintain the intracellular Na^+ concentration low, about an order of magnitude lower in concentration than the extracellular Na^+ concentration. The resting permeability to K^+ is high, allowing this ion to diffuse readily across the membrane. The high intracellular K^+ is maintained, of course, by the membrane p.d. produced by the small deficit of intracellular positive charge that results from the initial free movement of K^+ out of the cell.

When metabolically driven sodium transport is eliminated by an inhibitor of oxidative metabolism, such as cyanide or azide, or by a specific inhibitor of sodium transport, such as ouabain, Na^+ exhibits a net influx, internal K^+ is gradually displaced, and the resting potential shows a corresponding *slow* decay as the ratio of $[K^+]_i$ to $[K^+]_o$ gradually decreases. Thus, over the long term, it is the metabolically energized extrusion of Na^+ that keeps the Na^+ and K^+ concentration gradients from running downhill to equilibrium. By continual maintenance of the potassium concentration gradient, the sodium pump plays an important *indirect* role in determining the resting potential (Figure 5-14).

Active transport has also been shown to contribute *directly* to the resting potential of some cells. This situation occurs because the amount of Na^+ extruded from the cell per unit time exceeds (by 3:2) the rate of K^+ uptake by the pump (Figure 5-14). The Na–K exchange pump is said to be *electrogenic* because it is