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Chapter **1**

INTRODUCTION

Ionic channels are pores

Ionic channels are macromolecular pores in cell membranes. When they evolved and what role they may have played in the earliest forms of life we do not know, but today ionic channels are most obvious as the fundamental excitable elements in the membranes of excitable cells. These channels bear the same relation to electrical signaling in nerve, muscle, and synapse as enzymes bear to metabolism. Although their diversity is less broad than that of enzymes, there are many types of channels working in concert, opening and closing to shape the signals and responses of the nervous system. As sensitive but potent amplifiers, these ionic channels detect the sounds of chamber music, guide the artist's paintbrush, or generate the violent electric discharges of the electric eel or the electric ray. They tell the *Paramecium* to swim backward after a gentle collision, and they propagate the leaf-closing response of the *Mimosa* plant.

More than three billion years ago, primitive replicating forms became enveloped in a lipid film, a bimolecular diffusion barrier that separated the living cell from its environment. Although a lipid membrane had the advantage of retaining vital cell components, it would also prevent access to necessary ionized substrates and the loss of ionized waste products. Thus new transport mechanisms had to be developed hand in hand with the appearance of the membrane. One general solution would have been to make pores big enough to pass all small metabolites and small enough to retain macromolecules. Indeed, the *outer* membranes of gram-negative bacteria and of mitochondria are built on this plan. However, the cytoplasmic membranes of all contemporary organisms follow a more elaborate design, with many, more-selective transport devices handling different jobs, often under separate physiological control.

How do these devices work? Most of what we know about them comes from physiological flux measurements. Physiologists traditionally divided transport mechanisms into two classes, carriers and pores, largely on the basis of kinetic criteria. For example, the early literature tried to distinguish carrier from pore on the basis of molecular selectivity, saturating concentration dependence of fluxes, or stoichiometric coupling of the number of molecules transported. A carrier was viewed as a ferryboat diffusing back and forth across the membrane while carrying small molecules that could bind to stereospecific binding sites, and a pore was viewed as a narrow, water-filled tunnel, permeable to the few ions and molecules small enough to fit through the hole. The moving-ferryboat view of a

carrier is now no longer considered valid because the numerous carrier devices that have been purified from membranes are large proteins—too large to diffuse or spin around at the rate needed to account for the fluxes they catalyze. Furthermore, their amino acid sequences show that the peptide chains of the transport protein are already stably threaded back and forth in a large number of transmembrane segments. The newer view of carrier transport is that much smaller motions within the protein leave the macromolecule fixed in the membrane while exposing the transport binding site(s) alternately to the intracellular and extracellular media. It is not difficult to imagine various ways to do this, but we must develop new experimental insights before such ideas can be tested. Thus the specific mechanism of transport by such important carrier devices as the Na^+-K^+ pump, the Ca^{2+} pump, Na^+-Ca^{2+} exchange, Cl^--HCO_3^- exchange, glucose transport, the Na^+-coupled co- and countertransporters, and so on, remains unknown.

On the other hand, the water-filled pore view for the other class of transport mechanisms has now been firmly established for ionic channels of excitable membranes. In the period between 1965 and 1980, a valuable interplay between studies of excitable membrane and studies on model pores, such as the gramicidin channel in lipid bilayers, accelerated the pace of research and greatly sharpened our understanding of the transport mechanism. The biggest technical advance of this period was the development of methods to resolve the activity of single, channel molecules. As we consider much more extensively in later chapters, this led to the discovery that the rate of passage of ions through one open channel—often more than 10^6 ions per second—is far too high for any mechanism other than a pore. The criteria of selectivity, saturation, and stoichiometry are no longer the best for distinguishing pore and carrier.

Channels and ions are needed for excitation

Physiologists have long known that ions play a central role in the excitability of nerve and muscle. In an important series of papers from 1881 to 1887, Sidney Ringer showed that the solution perfusing a frog heart must contain salts of sodium, potassium, and calcium mixed in a definite proportion if the heart is to continue beating long. Nernst's (1888) work with electrical potentials arising from the diffusion of electrolytes in solution inspired numerous speculations of an ionic origin of bioelectric potentials. For example, some suggested that the cell is more negative than the surrounding medium because metabolizing tissue makes acids, and the resulting protons (positive charge) can diffuse away from the cell more easily than the larger organic anions. Soon, Julius Bernstein (1902, 1912) correctly proposed that excitable cells are surrounded by a membrane selectively permeable to K^+ ions at rest and that during excitation the membrane permeability to other ions increases. His "membrane hypothesis" explained the resting potential of nerve and muscle as a diffusion potential set up by the tendency of positively charged ions to diffuse from their high concentration in cytoplasm to their low concentration in the extracellular solution. During excita-

tion the internal negativity would be lost transiently as other ions are allowed to diffuse across the membrane, effectively short circuiting the K^+ diffusion potential. In the English-language literature, the words "membrane breakdown" were used to describe Bernstein's view of excitation.

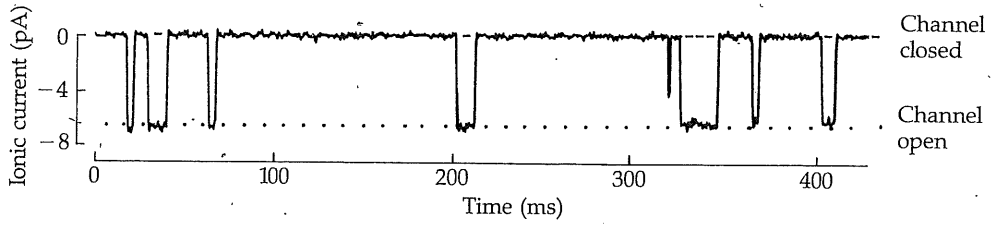
During the twentieth century, major cellular roles have been discovered for each of the cations of Ringer's solution: Na^+, K^+, Ca^{2+}; as well as for most of the other inorganic ions of body fluids: H^+, Mg^{2+}, Cl^-, HCO_3^-, and PO_4^{2-}. The rate of discovery of new roles for ions in cell physiology has been accelerating rather than slowing, so the list of ions and their uses will continue to lengthen. Evidently, no major ion has been overlooked in evolution. Each has been assigned at least one special regulatory, transport, or metabolic task. None is purely passively distributed across the cell membrane. Each has at least one carrier-like transport device coupling its movement to the movement of another ion. Both Na^+ and H^+ ions have transport devices coupling their "downhill" movements to the "uphill" movements of organic molecules. Na^+, K^+, H^+, and Ca^{2+} ions are pumped uphill by ATP-driven pumps. Protons are pumped across some membranes by electron transport chains, and their subsequent downhill flow can drive the phosphorylation of ADP to make ATP. Proton movements, through their effects on intracellular pH, will also influence the relative rates of virtually every enzymatic reaction. All of the ionic movements listed above are considered to be mediated by the carrier class of transport devices, and although they establish the ionic gradients needed for excitation, they are not themselves part of the excitation process. Readers interested in the details of ion pumps or coupled cotransport and exchange devices can consult other books on cell physiology.

Excitation and electrical signaling in the nervous system involve the movement of ions through ionic channels. The Na^+, K^+, Ca^{2+}, and Cl^- ions seem to be responsible for almost all of the action. Each channel may be regarded as an excitable molecule, as it is specifically responsive to some stimulus: a membrane potential change, a neurotransmitter or other chemical stimulus, a mechanical deformation, and so on. The channel's response, called GATING, is apparently a simple opening or closing of the pore. The open pore has the important property of SELECTIVE PERMEABILITY, allowing some restricted class of small ions to flow passively down their electrochemical activity gradients at a rate that is very high ($>10^6$ ions per second) when considered from a molecular viewpoint. We consider the high throughput rate as a diagnostic feature distinguishing ionic channel mechanisms from those of other ion transport devices such as the Na^+-K^+ pump. An additional major feature is a restriction to downhill fluxes not coupled stoichiometrically to the immediate injection of metabolic energy.

These concepts can be illustrated using the neurotransmitter-sensitive channels of muscle fibers. At the neuromuscular junction or endplate region of vertebrate skeletal muscle, the nerve axon has the job of instructing the muscle fiber when it is time to contract. Pulse-like electrical messages called ACTION POTENTIALS are sent down the motor nerve from the central nervous system. When they reach the nerve terminal, action potentials evoke the release of a

chemical signal, the neurotransmitter acetylcholine, which in turn diffuses to the nearby muscle surface and causes acetylcholine-sensitive channels to open there. Figure 1 shows an electrical recording from a tiny patch of muscle membrane. The cell is actually an embryonic muscle in tissue culture without nerves, but it still has neurotransmitter-sensitive channels that can be opened by applying a low concentration of acetylcholine. In this experiment, ionic fluxes in the channels are detected as electric current flow in the recording circuit, and since the recording sensitivity is very high, the opening and closing of one channel appear as clear step changes in the record. Each elementary current step corresponds to over 10^7 ions flowing per second in the open channel. Gating keeps the channel open for a few milliseconds. Other experiments with substitutions of ions in the bathing medium show that this type of channel readily passes monovalent cations with diameters up to 6.5 Å (0.65 nm) but does not pass anions.

How do gated ionic fluxes through pores make a useful signal for the nervous system? For the electrophysiologist the answer is clear. Ionic fluxes are electric currents across the membrane and therefore they have an immediate effect on membrane potential. Other voltage-gated channels in the membrane detect the change in membrane potential, and they in turn become excited. In this way the electric response is made regenerative and self-propagating. This explanation does describe how most signals are propagated, but it is circular. Is the ultimate purpose of excitation to make electricity so that other channels will be excited and make electricity? Clearly not, except in the case of an electric organ. Electricity is the means to carry the signal to the point where a nonelectrical response is generated. As far as is known, this final transduction always starts through a single common pathway: A membrane potential change opens or closes a Ca^{2+}-permeable channel, either on the surface membrane or on an



1 OPEN–SHUT GATING OF AN IONIC CHANNEL

Ionic current flowing across a tiny patch of excitable membrane showing eight brief openings (downward current deflections) of single ionic channels. The membrane patch has been excised from a cultured rat myotube and is bathed on both sides by Na salt solutions. Approximately 300 nM of the neurotransmitter, acetylcholine, applied to the extracellular membrane face is causing channels to open occasionally. At the −140 mV applied membrane potential, one open channel passes −6.6 pA, corresponding to a prodigious flow of 4.1×10^7 ions per second through a single pore. $T = 23°C$. [From Sánchez et al., 1986.]

internal membrane, and a Ca^{2+} flux into the cytoplasm is altered, causing a change in the internal free Ca^{2+} concentration. The ultimate response is then triggered by the internal Ca^{2+} ions. This is how the nervous system controls the contraction of a muscle fiber or the secretion of neurotransmitters, neurohormones, digestive enzymes, and so on. Internal free Ca^{2+} also controls the gating of some channels and the activities of many enzymes.

Ionic channels are undoubtedly found in the membranes of all cells. Their known functions include establishing a resting membrane potential, shaping electrical signals, gating the flow of messenger Ca^{2+} ions, controlling cell volume, and regulating the net flow of ions across epithelial cells of secretory and resorptive tissues. The emphasis in this book is on well-known channels underlying the action potentials and synaptic potentials of nerve and muscle cells. These have long been the focus of traditional membrane biophysics. As the biophysical methods eventually were applied to study fertilization of eggs, swimming of protozoa, glucose-controlled secretion of insulin by pancreatic beta cells, or acetylcholine-induced secretion of epinephrine from chromaffin cells, similar channels were found to play central roles. We must now consider that nerve, muscle, endocrine and secretory glands, white blood cells, platelets, gametes, and protists all share common membrane mechanisms in their responsiveness to stimuli. Similarly, as biophysical methods were applied to transporting epithelia, ionic channels were found. They too are ion-selective, gated pores, controlled by hormonal influences.

Nomenclature of channels

The naming of ionic channels has not been systematic. In most cases, the biophysicist first attempts to distinguish different components of membrane permeability by their kinetics, pharmacology, and response to ionic substitution. Then a kinetic model is often made expressing each of the apparent components mathematically. Finally, it is tacitly assumed that each component of the model corresponds to a type of channel, and the putative channels are given the same names as the permeability components in the original analysis. Thus in their classical analysis of ionic currents in the squid giant axon, Hodgkin and Huxley (1952d) recognized three different components of current, which they called sodium, potassium, and leakage. Today the names NA CHANNEL and K CHANNEL are universally accepted for the corresponding ionic channels in axons. The name LEAKAGE CHANNEL is also used, although there is no experimental evidence regarding the ions or transport mechanism involved.

Naming a channel after the most important permeant ion seems rational but fails when the ions involved are not adequately known, or when no ion is the *major* ion, or when numerous different kinetic components are all clearly carried by one type of ion. Such problems have led to such "names" as A, B, C, and so on, for permeability components in molluscan ganglion cells (Adams, Smith, and Thompson, 1980) or qr, si, and x_1 in cardiac Purkinje fibers (McAllister et al., 1975). Other approaches are simply descriptive: Channels have been named

after anatomical regions, as in the endplate channel; after inhibitors, as in the amiloride-sensitive Na channel; or after neurotransmitters, as in glutamate channels of crustacean muscle. Finally, a surprising number of molecular subtypes of major channels are being recognized by molecular genetic methods. These amino acid sequence differences have led to names like brain-type-I (-II or -III) Na channels. Eventually, all this loose nomenclature will be confusing, and perhaps a systematic approach analogous to that taken by the Enzyme Commission will be needed. However, such a revision ought to wait until the diversity of channels is better understood. By that time some clear structural and evolutionary relationships may form the basis for a natural classification.

Channels have families

Biophysicists long recognized that voltage-gated Na, K, and Ca channels have some functional similarities. Likewise synaptic channels gated by acetylcholine, glutamate, glycine, and γ-aminobutyric acid seemed similar. One of the great advances of the 1980s has been the sequencing by methods of molecular genetics of messenger RNAs, and even genes, that code for ionic channels. The predicted amino acid sequences reveal strong structural similarities among groups of channels that now allow us to talk about families of homologous channel proteins that would have evolved by processes of successive gene duplication, mutation, and selection from common ancestral channels. An unexpected discovery is the large size of these gene families. As has also been found for enzymes and other proteins, none of the channels we have mentioned is a single structural entity. They all come in various isoforms coded by different genes that may be selectively expressed in certain cell types and in certain periods of development and growth of the organism. Thus we suppose that there are hundreds of genes coding for channels in any individual.

Ohm's law is central

In the study of ionic channels, we see—more than in most areas of biology—how much can be learned by applying simple laws of physics. Much of what we know about ionic channels was deduced from electrical measurements. Therefore, it is essential to remember rules of electricity before discussing experiments. The remainder of this chapter is a digression on the necessary rules of physics. To do biophysical experiments well, one must often make sophisticated use of electrical ideas; however, as this book is concerned with channels and not with techniques of measurement, the essential principles are few. The most important is Ohm's law, a relation between current, voltage, and conductance, which we now review.

All matter is made up of charged particles. They are normally present in equal numbers, so most bodies are electrically neutral. A mole of hydrogen atoms contains Avogadro's number ($N = 6.02 \times 10^{23}$) of protons and the same number of electrons. Quantity of charge is measured in coulombs (abbreviated

TABLE 1. PHYSICAL CONSTANTS

Avogadro's number	$N = 6.022 \times 10^{23}$ mol^{-1}
Elementary charge	$e = 1.602 \times 10^{-19}$ C
Faraday's constant	$F = 9.648 \times 10^{4}$ C mol^{-1}
Absolute temperature	T(K) = 273.16 + T (°Celsius)
Boltzmann's constant (in electrical units)	$k = 1.381 \times 10^{-23}$ V C K^{-1}
Gas constant (in energy units)	$R = 1.987$ cal K^{-1} mol^{-1} = 8.315 J K^{-1} mol^{-1}
Polarizability of free space	$\varepsilon_0 = 8.854 \times 10^{-12}$ C V^{-1} m^{-1}
One joule	1 J = 1 kg m^2 s^{-2} = 1 V C = 1 W s = 0.2389 cal

C), where the charge of a proton is $e = 1.6 \times 10^{-19}$ C. Avogadro's number of elementary charges is called the FARADAY CONSTANT: $F = Ne = 6 \times 10^{23} \times 1.6 \times 10^{-19} \approx 10^5$ C/mol. This is thus the charge on a mole of protons or on a mole of Na^+, K^+, or any other monovalent cation. The charge on a mole of Ca^{2+}, Mg^{2+}, or on other divalents cations is $2F$ and the charge on a mole of Cl^- ions or other monovalent anions is $-F$.

Electrical phenomena arise whenever charges of opposite sign are separated or can move independently. Any net flow of charges is called a CURRENT. Current is measured in amperes (abbreviated A), where one ampere corresponds to a steady flow of one coulomb per second. By the convention of Benjamin Franklin, positive current flows in the direction of movement of positive charges. Hence if positive and negative electrodes are placed in Ringer's solution, Na^+, K^+, and Ca^{2+} ions will start to move toward the negative pole, Cl^- ions will move toward the positive pole, and an electric current is said to flow through the solution from positive to negative pole. Michael Faraday named the positive electrode the ANODE and the negative, the CATHODE. In his terminology, anions flow to the anode, cations to the cathode, and current from anode to cathode. The size of the current will be determined by two factors: the potential difference between the electrodes and the electrical conductance of the solution between them. POTENTIAL DIFFERENCE is measured in volts (abbreviated V) and is defined as the work needed to move a unit test charge in a frictionless manner from one point to another. To move a coulomb of charge across a 1-V difference requires a joule of work. In common usage the words "potential," "voltage," and "voltage difference" are used interchangeably to mean potential difference, especially when referring to a membrane.

ELECTRICAL CONDUCTANCE is a measure of the ease of flow of current between two points. The conductance between two electrodes in salt water can be

increased by adding more salt or by bringing the electrodes closer together, and it can be decreased by placing a nonconducting obstruction between the electrodes, by moving them farther apart, or by making the solution between them more viscous. Conductance is measured in siemens (abbreviated S and formerly called mho) and is defined by Ohm's law in simple conductors:

$$I = gE \tag{1-1a}$$

which says that current (I) equals the product of conductance (g) and voltage difference (E) across the conductor. The reciprocal of conductance is called RESISTANCE (symbolized R) and is measured in ohms (abbreviated Ω). Ohm's law may also be written in terms of resistance:

$$E = IR \tag{1-1b}$$

One can draw an analogy between Ohm's law for electric current flow and the rule for flow of liquids in narrow tubes. In tubes the flow (analog of current) is proportional to the pressure difference (analog of voltage difference) divided by the frictional resistance.

Homogeneous conducting materials may be characterized by a bulk property called the RESISTIVITY, abbreviated ρ. It is the resistance measured by two 1-cm^2 electrodes applied to opposite sides of a 1-cm cube of the material and has the dimensions ohm · centimeter (Ω · cm). Resistivity is useful for calculating resistance of arbitrary shapes of materials. For example, for a right cylindrical block of length l and cross-sectional area A with electrodes of area A on the end faces, the resistance is

$$R = \frac{\rho l}{A} \tag{1-2}$$

Later in the book we will use this formula to estimate the resistance in a cylindrical pore. Resistivity decreases as salts are added to a solution. Consider the following approximate examples at 20°C: frog Ringer's solution 80 Ω · cm, mammalian saline 60 Ω · cm, and seawater 20 Ω · cm. Indeed, in sufficiently dilute solutions each added ion gives a known increment to the overall solution conductance, and the resistivity of electrolyte solutions can be predicted by calculations from tables of single-ion equivalent conductivities, like those in Robinson and Stokes (1965). In saline solutions the resistivity of pure phospholipid bilayers is as high as 10^{15} Ω · cm, because although the physiological ions can move in lipid, they far prefer an aqueous environment over a hydrophobic one. The electrical conductivity of biological membranes comes not from the lipid, but from the ionic channels embedded in the lipid.

To summarize what we have said so far, when one volt is applied across a 1 Ω resistor or 1-S conductor, a current of one ampere flows; every second, 1/F moles of charge (10.4 μmol) move and one joule of heat is produced. Ohm's law plays a central role in membrane biophysics because each ionic channel is an elementary conductor spanning the insulating lipid membrane. The total electrical conductance of a membrane is the sum of all these elementary conductances in parallel.

It is a measure of how many ionic channels are open, how many ions are available to go through them, and how easily the ions pass.

The membrane as a capacitor

In addition to containing many conducting channels, the lipid bilayer of biological membranes separates internal and external conducting solutions by an extremely thin insulating layer. Such a narrow gap between two conductors forms, of necessity, a significant electrical capacitor.

To create a potential difference between objects requires only a separation of charge. CAPACITANCE (symbolized C) is a measure of how much charge (Q) needs to be transferred from one conductor to another to set up a given potential and is defined by

$$C = \frac{Q}{E} \tag{1-3}$$

The unit of capacitance is the farad (abbreviated F). A 1-F capacitor will be charged to 1 V when +1.0 C of charge is on one conductor and −1.0 C on the other. In an ideal capacitor the passage of current simply removes charge from one conductor and stores it on another in a fully reversible manner and without evolving heat. The rate of change of the potential under a current I_C is obtained by differentiating Equation 1-3.

$$\frac{dE}{dt} = \frac{I_C}{C} \tag{1-4}$$

The capacity to store charges arises from their mutual attraction across the gap and by the polarization they develop in the insulating medium. The capacitance depends on the dielectric constant of that medium and on the geometry of the conductors. In a simple capacitor formed by two parallel plates of area A and separated by an insulator of dielectric constant ϵ and thickness d, the capacitance is

$$C = \frac{\varepsilon\varepsilon_0 A}{d} \tag{1-5}$$

where ε_0, called the polarizability of free space, is $8.85 \times 10^{-12}\ \mathrm{CV^{-1}m^{-1}}$. Cell membranes are parallel-plate capacitors with specific capacitances[1] near 1.0 μF/cm^2, just slightly higher than that of a pure lipid bilayer, 0.8 μF/cm^2 (see Cole, 1968; Almers, 1978). According to Equation 1-5, this means that the thickness d of the insulating bilayer is only 23 Å (2.3 nm), assuming that the dielectric constant of hydrocarbon chains is 2.1. Hence the high electrical capacitance of biological membranes is a direct consequence of their molecular dimensions.

The high capacitance gives a lower limit to how many ions (charges) must move (Equation 1-3) and how rapidly they must move (Equation 1-4) to make a

[1] In describing cell membranes, the phrases "specific capacitance," "specific resistance," and "specific conductance" refer to electrical properties of a 1-cm^2 area of membrane. They are useful for comparing the properties of different membranes.

a given electrical signal. In general, capacitance slows down the voltage response to any current by a characteristic time τ that depends on the product RC of the capacitance and any effective parallel resistance. For example, suppose that a capacitor is charged up to 1.0 V and then allowed to discharge through a resistor R as in Figure 2. From Ohm's law the current in the resistor is $I = E/R$, which discharges the capacitor at a rate (Equation 1-4)

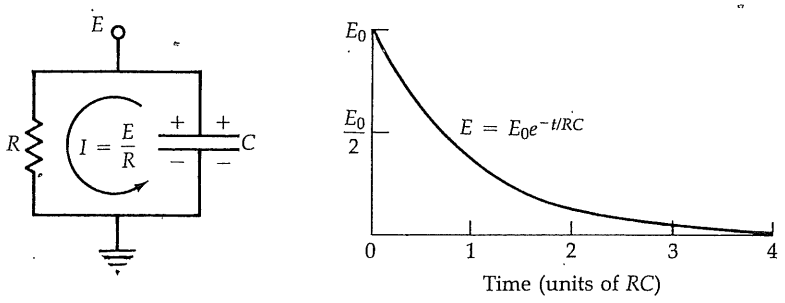
$$\frac{dE}{dt} = \frac{I_C}{C} = -\frac{E}{RC} \tag{1-4a}$$

The solution of this first-order differential equation has an exponentially decaying time course

$$E = E_0 \exp\left(-\frac{t}{RC}\right) = E_0 \exp\left(-\frac{t}{\tau}\right) \tag{1-6}$$

where E_0 is the starting voltage, t is time in seconds, and exp is the exponential function (power of e, the base of natural logarithms).

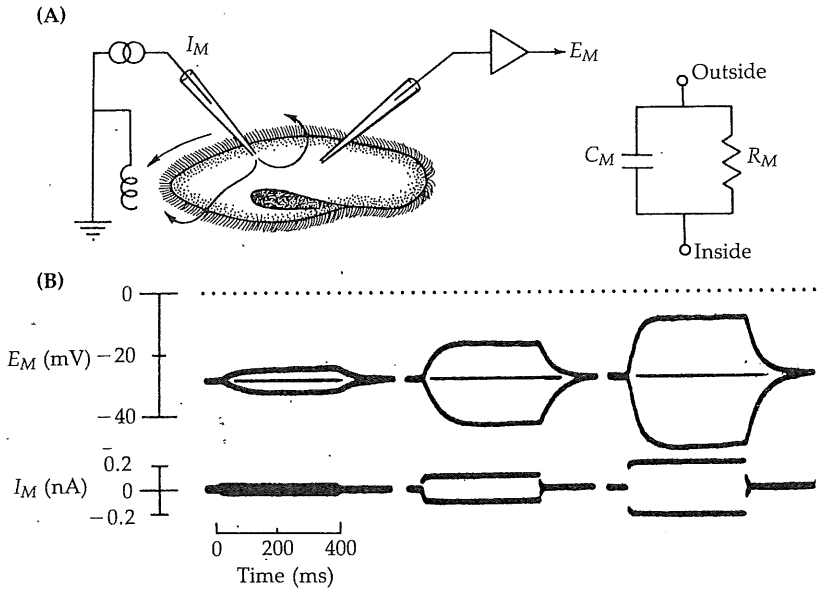
For biological membranes the product, $R_M C_M$, of membrane resistance and capacitance is often called the membrane time constant, τ_M. It can be determined, using equations like Equation 1-6, from measurements of the time course of membrane potential changes as small steps of current are applied across the membrane. For example, in Figure 3 steps of current are applied from an intracellular microelectrode across the cell membrane of a *Paramecium*. The time course of the membrane potential changes corresponds to a membrane time constant of 60 ms. Since C_M is approximately 1 μF/cm^2 in all biological membranes, the measured τ_M gives a convenient first estimate of specific membrane resistance. For the *Paramecium* in the figure, R_M is τ_M/C_M or 60,000 $\Omega \cdot$ cm^2. In



(A) CIRCUIT **(B)** TIME COURSE OF DISCHARGE

2 DISCHARGE OF AN *RC* CIRCUIT

The circuit has a resistor and a capacitor connected in parallel, and the voltage across the capacitor is measured from the two terminals. At zero time the capacitor has been charged up to a voltage of E_0 and begins to discharge through the resistor. Charge and voltage decay exponentially so that in every RC seconds they fall to $1/e$ or 0.367... of their previous value.



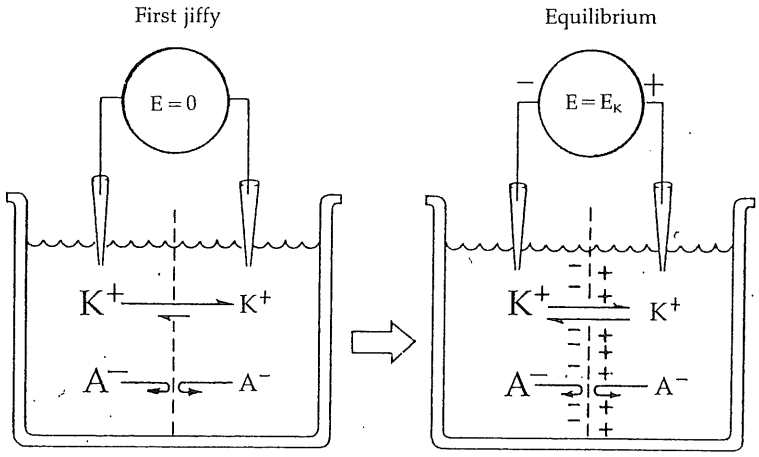
3 THE CELL MEMBRANE AS AN *RC* CIRCUIT

An experiment to study membrane electrical properties of a *Paramecium*. The cell is impaled with two intracellular electrodes. One of them passes steps of current I_M across the membrane to an electrode in the bath. The other records the changes of membrane potential E_M with an amplifier, symbolized as a triangle. On the right, a current of 0.23 nA makes a voltage deflection of 23 mV, corresponding from Ohm's law to a membrane resistance of 100 MΩ (10^8 Ω). The exponential time constant τ_M of the rise and fall of the voltage response is approximately 60 ms. This *Paramecium* contains a genetic mutation of the normal excitability mechanism, so its responses to current steps are simpler than for the genetic wild-type *Paramecium*. [From Hille, 1989a; after Kung and Eckert, 1972.]

different resting cell membranes, τ_M ranges from 10 μs to 1 s, corresponding to resting R_M values of 10 to 10^6 Ω · cm^2. This broad range of specific resistances shows that the number of ionic channels open at rest differs vastly from cell to cell.

Equilibrium potentials and the Nernst equation

The final physical topic concerns equilibrium. All systems are moving toward EQUILIBRIUM, a state where the tendency for further change vanishes. At equilibrium, thermal forces balance the other existing forces and forward and backward fluxes in every microscopic transport mechanism and chemical reaction are equal. We want to consider the problem illustrated in Figure 4. Two compartments of a bath are separated by a membrane containing pores permeable only to K^+ ions. A high concentration of a salt KA (A for anion) is introduced into the



4 DIFFUSION POTENTIALS IN PORES

A membrane with perfectly K^+-selective pores separates solutions with different concentrations of a potassium salt, KA. A voltmeter records the potential across the porous membrane. At the moment when the salt solutions are poured in, there is no membrane potential, $E = 0$. However, as a few K^+ ions diffuse from side 1 to side 2, a potential develops with side 2 becoming positive. Eventually the membrane potential reaches the Nernst potential for K^+ ions, $E = E_K$.

left side and a low concentration into the right side. A voltmeter measures the membrane potential. In the first jiffy, the voltmeter reads 0 mV, as both sides are neutral. However, K^+ ions immediately start diffusing down their concentration gradient into the right-hand side, giving that side an excess positive charge and building up an electrical potential difference across the membrane. The anion cannot cross the membrane, so the charge separation persists. However, the thermal "forces" causing net diffusion of K^+ to the right are now countered by a growing electrical force tending to oppose the flow of K^+. The potential builds up until it finally reaches an equilibrium value, E_K, where the electrical force balances the diffusional force and the system no longer changes. The problem is to find a formula for E_K, the "equilibrium potential for K^+ ions." This is called an equilibrium problem even though parts of the system, such as the anions A^- and the water molecules (osmotic pressure), are not allowed to equilibrate. We may focus on K^+ ions alone and discuss their equilibrium. As we shall see, equilibrium potentials are the starting point in any description of biological membrane potentials.

A physicist would begin the problem with the BOLTZMANN EQUATION of statistical mechanics, which gives the relative probabilities at equilibrium of finding a particle in state 1 or in state 2 if the energy difference between these states is $u_2 - u_1$:

$$\frac{p_2}{p_1} = \exp\left(-\frac{u_2 - u_1}{kT}\right) \tag{1-7}$$

Here k is Boltzmann's constant and T is absolute temperature on the Kelvin scale. This equation conveniently describes the equilibrium distribution of particles in force fields. Qualitatively it says that a particle spends less time in states of higher energy than in states of lower energy. For example, the molecules in the Earth's atmosphere are attracted by the Earth's gravitational field, and Equation 1-7 correctly predicts that the probability of finding O_2 molecules at the top of Mt. Everest is only ⅓ of that of finding them at sea level.

For our purposes, Equation 1-7 can be recast into in a slightly more chemical form by changing from probabilities p to concentrations c and from single-particle energies u to molar energies U

$$\frac{c_2}{c_1} = \exp\left(-\frac{U_2 - U_1}{RT}\right) \tag{1-8}$$

where R is the gas constant ($R = kN$). Finally, taking natural logarithms of both sides and rearranging gives

$$U_1 - U_2 = RT \ln \frac{c_2}{c_1} \tag{1-9}$$

Now we have a useful equilibrium relation between concentration ratios and energy differences. In our problem $U_1 - U_2$ is the molar electrical energy difference of the permeable ion due to the membrane potential difference $E_1 - E_2$. If we consider a mole of an arbitrary ion S with charge z_S, then $U_1 - U_2$ becomes $z_S F(E_1 - E_2)$. Substituting into Equation 1-9 gives the equilibrium potential E_S as a function of the concentration ratio and the valence:

$$E_S = E_1 - E_2 = \frac{RT}{z_S F} \ln \frac{[S]_2}{[S]_1} \tag{1-10}$$

This well-known relationship is called the NERNST EQUATION (Nernst, 1888).

Before discussing the meaning of the equation, let us note as an aside that the equilibrium potential E_S can be derived in other, equivalent ways. A chemist would probably think in terms of the thermodynamics, using the principle of J.W. Gibbs that the electrochemical potential of ion S is the same on both sides at equilibrium, or equivalently that the work of transfer of a tiny quantity of S from side 2 to side 1 has to be zero. This work comprises two terms: the work of concentrating the ions as they cross, $-RT \ln (c_2/c_1)$, plus all other energy changes, $U_1 - U_2$, which in this case is only the electrical term. These considerations lead at once to Equations 1-9 and 1-10. Thermodynamics would also point out that because all solutions are at least slightly nonideal (unlike ideal gases), one should use activities rather than concentrations (see, e.g., Moore, 1972). This book refers to the symbol [S] as the concentration of S while recognizing that careful quantitative work requires consideration of activities instead.

According to the Nernst equation, ionic equilibrium potentials vary linearly with the absolute temperature and logarithmically with the ionic concentration ratio. As would be expected from our discussion of Figure 4, equilibrium potentials change sign if the charge of the ion is reversed or if the direction of the

gradient is reversed, and they fall to zero when there is no gradient. To correspond to the physiological convention, we now define side 1 as inside (intracellular), 2 as outside (extracellular), and all membrane potentials to be measured inside minus outside. Then we can write the equilibrium potentials for K^+ ions and for the other biologically relevant ions.

$$E_K = \frac{RT}{F} \ln \frac{[K]_o}{[K]_i} \tag{1-11a}$$

$$E_{Na} = \frac{RT}{F} \ln \frac{[Na]_o}{[Na]_i} \tag{1-11b}$$

$$E_{Ca} = \frac{RT}{2F} \ln \frac{[Ca]_o}{[Ca]_i} \tag{1-11c}$$

$$E_{Cl} = \frac{RT}{F} \ln \frac{[Cl]_i}{[Cl]_o} \tag{1-11d}$$

The subscripts o and i stand for outside and inside, respectively. The meaning of the numbers E_K, E_{Na}, and so on, can be stated in two ways. Using E_K as an example: (1) If the pores in a membrane are permeable only to K^+ ions, the membrane potential will change to E_K; (2) If the membrane potential is held somehow at E_K, there will be no net flux of K^+ ions through K^+-selective pores.

How large are the equilibrium potentials for living cells? Table 2 lists values of the factor RT/F in the Nernst equation; also given are values of $2.303(RT/F)$ for calculations with $\log_{10}$ instead of ln as follows:

$$E_K = \frac{RT}{F} \ln \frac{[K]_o}{[K]_i} = 2.303 \frac{RT}{F} \log_{10} \frac{[K]_o}{[K]_i} \tag{1-11e}$$

From Table 2 at 20°C an e-fold ($e \approx 2.72$) K^+ concentration ratio corresponds to

TABLE 2. VALUES OF *RT/F* (OR *kT/e*)

Temperature (°C)	*RT/F* (mV)	2.303 *RT/F* (mV)
0	23.54	54.20
5	23.97	55.19
10	24.40	56.18
15	24.83	57.17
20	25.26	58.17
25	25.69	59.16
30	26.12	60.15
35	26.55	61.14
37	26.73	61.54

TABLE 3. FREE IONIC CONCENTRATIONS AND EQUILIBRIUM POTENTIALS FOR MAMMALIAN SKELETAL MUSCLE

Ion	Extracellular concentration (m*M*)	Intracellular concentration (m*M*)	$\frac{[\text{Ion}]_o}{[\text{Ion}]_i}$	Equilibrium potential[a] (mV)
Na^+	145	12	12	+67
K^+	4	155	0.026	−98
Ca^{2+}	1.5	10^{-7} M	15,000	+129
Cl^-	123	4.2[b]	29[b]	−90[b]

[a] Calculated from Equation 1-11 at 37°C.
[b] Calculated assuming a −90-mV resting potential for the muscle membrane and that Cl^- ions are at equilibrium at rest.

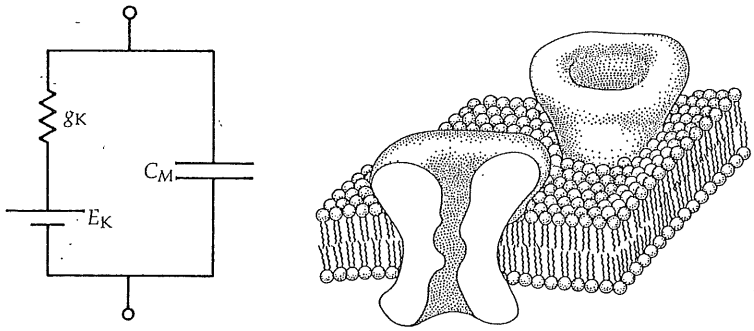
$E_K = -25.3$ mV, a 10-fold ratio corresponds to $E_K = -58.2$ mV, and a 100-fold ratio corresponds to $E_K = -58.2 \times 2 = -116.4$ mV. Table 3 lists the actual concentrations of some ions in mammalian skeletal muscle and their calculated equilibrium potentials ranging from −98 to +128 mV. E_K and E_{Cl} are negative, and E_{Na} and E_{Ca} are positive numbers. E_K sets the negative limit and E_{Ca} the positive limit of membrane potentials that can be achieved by opening ion-selective pores in the muscle membrane. All excitable cells have negative resting potentials because at rest they have far more open K-selective channels (and in muscle, Cl-selective channels, too) than Na-selective or Ca-selective ones.

Current–voltage relations of channels

Biophysicists like to represent the properties of membranes and channels by simple electrical circuit diagrams that have equivalent electrical properties to the membrane. We have discussed the membrane as a capacitor and the channel as a conductor. But if we try to test Ohm's law on the membrane of Figure 4, we would immediately recognize a deviation: Current in the pores goes to zero at E_K and not at 0 mV. The physical chemist would say, "Yes, you have a concentration gradient, so Ohm's law doesn't work." The biophysicist would then suggest that a gradient is like a battery with an electromotive force (emf) in series with the resistor (see Figure 5) and the modified current–voltage law becomes

$$I_K = g_K(E - E_K) \tag{1-12}$$

The electromotive force is E_K and the net driving force on K^+ ions is now $E - E_K$ and not E. This modification is, like Ohm's law itself, empirical and requires experimental test in each situation. To a first approximation this linear law is often excellent, but many pores are known to have nonlinear current–voltage relations when open. Some curvature is predicted, as we shall see later, by explicit calculations of the electrodiffusion of ions in pores, particularly when there is a higher concentration of permeant ion on one side of the membrane than on the other or when the structure of the channel is asymmetrical.



(A) EQUIVALENT CIRCUIT (B) INTERPRETATION

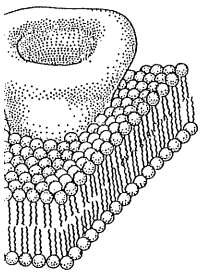
5 TWO VIEWS OF A K^+-SELECTIVE MEMBRANE

In electrical experiments the membrane acts like an equivalent circuit with two branches. The conductive branch with an EMF of E_K suggests a K^+-selective aqueous diffusion path, a pore. The capacitive branch suggests a thin insulator, the lipid bilayer.

Consider now how simple current–voltage measurements can be used to gain information on ionic channels. Figure 6 gives examples of hypothetical observations and their interpretation in terms of electrical equivalent circuits. Figure 6A shows three linear *I–E* curves. They pass through the origin, so no battery is required in the equivalent circuit, meaning either that the channels are nonselective or that there is no effective ionic gradient. The slopes of the successive *I–E* relations decrease twofold, so the equivalent conductance, and hence the number of open channels, differs correspondingly. Thus conductances give a useful measure of how many channels are open in an area of membrane.

6 CURRENT–VOLTAGE RELATIONS OF MEMBRANES

Measured *I–E* relations can be interpreted in terms of electrical equivalent circuits and the modified form of Ohm's law (Equation 1-12) that takes into account the electromotive force in the pores. Four hypothetical conditions are shown. (A) Membranes with 1, 2, and 3 pores open give *I–E* relations with relative slopes of 1, 2, and 3. (B) Pores with negative or positive electromotive forces give *I–E* relations with negative or positive zero-current potentials. (C) Pores that step from a low-conductance state to a high-conductance state (see inset graph of g versus E) give *I–E* relations consisting of two line segments. (D) Pores with smoothly voltage-dependent probability of being open (see inset graph of average g versus E) give curved *I–E* relations. The dashed lines, corresponding to a constant high conductance, are the same *I–E* relations as in part B. However, when the pores close at negative potentials, lowering g, the current decreases correspondingly from its maximal value.



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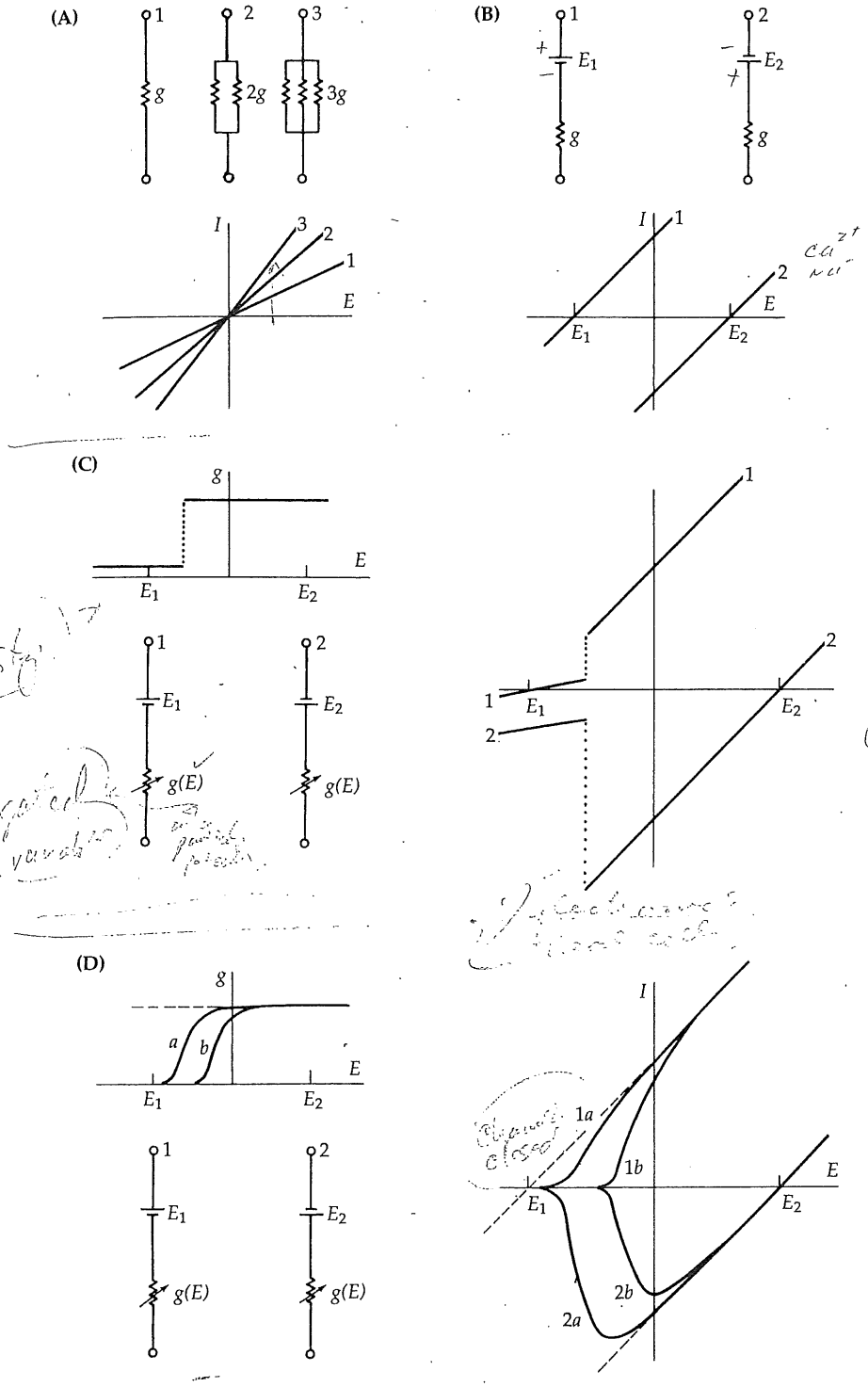

Figure 6B shows two *I–E* relations of equal slope but with different zero-current potentials. The corresponding equivalent circuits have equal conductances but different electromotive forces in their batteries. This could arise from different channels with different ionic selectivities or from the same channel bathed on the two sides by different concentrations of its permeant ions. Hence zero-current potentials are useful in studies of selectivity.

Figure 6C shows the effect of a CONDUCTANCE CHANGE. This is a little harder and needs to be analyzed in several steps. Since the *I–E* relations do not pass through the origin, we know again that there is an electromotive force in these channels. Both a negative emf, E_1, and a positive emf, E_2, are illustrated, as in Figure 6B. Unlike Figure 6B, however, here the *I–E* relations are not single straight lines. This tells us that the membrane conductance changes with voltage, a property called RECTIFICATION in electric circuit theory. In biological membranes, strong rectification usually means that the ionic channels carrying current are open at some membrane potentials and shut at others. We can imagine a voltage-gated switch that opens and closes the channels. In this example, the conductance is low at very negative membrane potentials and suddenly steps up to a higher level as the potential is made less negative. The low- and high-conductance segments of the *I–E* relation are each linear and extrapolate back to a zero-current point corresponding to the emf of the channels when open.

Figure 6C corresponds to measurements on a system with a sharp voltage threshold for opening of ionic channels. Real voltage-gated channels cannot measure the membrane potential this precisely and the voltage dependence of their opening is less abrupt, as in Figure 6D. The case illustrated in Figure 6D may seem difficult, but because it corresponds closely to practical observations, it is worth working through. First note that there is no ionic current at membrane potentials more negative than E_1. Hence the conductance is zero there, and the channels must be closed. Positive to 0 mV, the *I–E* relations are steep, straight lines like those in Figure 6B. Here the conductance is high, and the channels must be open. In the intermediate voltage range, between E_1 and 0 mV, the current is smaller than expected from the maximal conductance (dashed lines). Hence only some of the channels are open.

To determine how many channels are open at each voltage, we should calculate the ionic conductance at each potential. When this is done using the modified form of Ohm's law (Equation 1-12) and the appropriate channel electromotive force, E_1 or E_2, one derives the conductance–voltage (g–E) relations shown in the inset. The conductance changes from fully off to fully on over a narrow voltage range. As a first approximation, this continuous conductance–voltage relation reflects the steep voltage dependence of the open probability of the channel.[2] We can think of this channel as being electrically excitable, a voltage-gated pore.

The *I–E* relations in Figure 6 are representative of observations made daily in biophysical studies of ionic channels. Examples will appear in Chapter 2. Inter-

[2] Some nonlinearities may be due to other factors, including an intrinsic nonlinearity of the *I–E* curve for a single open channel, discussed above.

ested readers will want to work out for themselves how voltage-dependent channel opening accounts for the results by resketching each *I–E* relation and calculating the corresponding conductance–voltage relation point-by-point from Equation 1-12.

Ionic selectivity

It is essential for electrical excitability that different ionic channels be selective for different ions. However, no channel is perfectly selective. Thus the Na channel of axons is fairly permeable to NH_4^+ ions and even slightly permeable to K^+ ions. How can we determine ionic selectivity from electrical measurements? The simplest way is to measure the electromotive force or zero-current potential for the channel with, say, ion A^+ on the outside and B^+ on the inside. This is called a BIIONIC POTENTIAL. Suppose that A^+ and B^+ have the same valence. If no other permeant[3] ion is present, the permeability ratio. P_A/P_B is defined by the equation

$$E_{rev} = \frac{RT}{zF} \ln \frac{P_A\,[A]_o}{P_B\,[B]_i} \tag{1-13}$$

where the zero-current potential is often called the REVERSAL POTENTIAL (E_{rev}) since that is the potential around which the current reverses sign.

Equation 1-13 resembles the Nernst equation, but now with two ions. It expresses an important, simple idea: The permeability of a channel for A^+ is said to be half that for B^+ when you need two concentration units of A on one side and one concentration unit of B on the other to get zero electromotive force. Equation 1-13 is the simplest form of an expression derived from diffusion theory by Goldman (1943) and Hodgkin and Katz (1949). Unlike the Nernst equation, such expressions describe a steady-state interdiffusion of ions away from equilibrium. Therefore, the simplifying rules of equilibrium cannot be applied, and the derivation must make assumptions about the structure of the channel.

Signaling requires only small ionic fluxes

To close this chapter we can exercise our electrical knowledge by reconsidering the experiment in Figure 4 using biologically realistic numbers and the electrical equivalent circuit in Figure 5. Suppose that the membrane contains K-selective pores that contribute 20 pS (20×10^{-12} siemens) of electrical conductance apiece.[4] If an average of 0.5 pore is open per square micrometer, the specific membrane conductance is

$$g_M = \frac{0.5 \times 20 \times 10^{-12}\ \text{S}/\mu\text{m}^2}{10^{-8}\ \text{cm}^2/\mu\text{m}^2} = 1\ \text{mS/cm}^2$$

[3] The words "permeable" and "permeant" are sometimes confused. A channel is *permeable*: capable of being permeated. An ion is *permeant*: capable of permeating. In French a raincoat is *un imperméable*.

[4] Most biological ionic channels have an electrical conductance in the range of 1 to 150 pS.

Then the specific membrane resistance is $R_M = 1/g_M = 1000\ \Omega \cdot \text{cm}^2$, and the membrane time constant for $C_M = 1\ \mu\text{F/cm}^2$ is $\tau_M = R_M C_M = 1$ ms. Suppose that the concentration ratio of KA salt across the membrane is 52:1 so that E_K is $58.2 \log_{10} (\frac{1}{52}) = -100$ mV. Now what happens immediately after the salt solutions are introduced and K^+ ions start to diffuse? The voltmeter reports a membrane potential changing from 0 mV to −100 mV along an exponential time course with a time constant of 1 ms.

$$E = [1 - \exp(-t/1\ \text{ms})] \cdot 100\ \text{mV}$$

After a few milliseconds the system has reached equilibrium and an excess charge of $Q = EC_M = 10^{-7}\ \text{C/cm}^2$, all carried by K^+ ions, has been separated across the membrane. This amounts to a movement of $Q/F = 10^{-12}$ mol of K^+ ions per cm^2 of membrane, a tiny amount that would alter the original 52-fold gradient very little. Hence our calculation shows that full-sized electrical signals can be generated rapidly even with relatively few pores per unit area and with only minute ionic fluxes.

Notice that the size of the needed ionic flux depends on the *surface area* of the cell, whereas the effect of the flux on internal ionic concentrations depends on the *volume* of the cell. In a giant cell (a 1000-μm-diameter squid axon) the surface-to-volume ratio is the lowest, and electrical signaling with a 110-mV action potential changes the available ionic concentration gradient by only 1 part in 10^5. On the other hand, the smallest cells (a 0.1-μm axon or dendrite), the surface-to-volume ratio is 10^4 times higher and a single action potential might move as much as 10% of the stored-up ions.

Having reviewed some essential rules of physics, we may now turn to the experimental study of ionic channels.