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RUCH — PATTON

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Apartado 26370—Cedro 512
Mexico 4, D.F., Mexico

Rua Coronel Cabrita, 8
Sao Cristovao Caixa Postal 21176
Rio de Janeiro, Brazil

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CONTRIBUTORS

ALBERT M. GORDON, Ph.D., Professor, Department of Physiology and Biophysics, University of Washington School of Medicine.

BERTIL HILLE, Ph.D., Professor, Department of Physiology and Biophysics, University of Washington School of Medicine.

HARRY D. PATTON, M.D., Ph.D., Professor and Chairman, Department of Physiology and Biophysics, University of Washington School of Medicine.

J. WALTER WOODBURY, Ph.D., Professor, Department of Physiology, University of Utah College of Medicine.

PREFACE

This volume, chronologically the last of the 20th edition of *Physiology and Biophysics*, is really the first in logical order, since it contains material basic to that of the other volumes and in particular to that of Volume I. The literature in the broad area of excitable tissues and reflex physiology has grown so rapidly and so voluminously since the publication of the 19th edition that we have gone through several revisions and updates to arrive at the present one, which will itself no doubt be in part outdated by publication time. The result is a compendium of heavily amended or in several instances completely rewritten chapters and many new illustrations, tables and figures. Another new feature is the adoption of full bibliographic citation including titles and full pagination, which should be helpful to the reader seeking further information or documentation.

As in the previous edition, a major emphasis of the present volume is on cellular and molecular mechanisms and on the physical and quantitative basis of excitability; some parts, especially the first chapter, may be difficult for the student of biology and medicine. On the other hand, the authors have throughout emphasized those aspects of basic neurophysiology that bear directly on the understanding of disease states and which we believe should be part of the intellectual armamentarium of the practitioner of scientific medicine. We hope that the current tendency of medical curricula to depend heavily on syllabi and lecture-note handouts will not last forever; in any case, we have sought to produce a volume to which the seriously interested student who is dissatisfied with such cursory treatments can resort.

The authors are deeply indebted to Ms. Anita Olson and Ms. Cheryl Steiner for manuscript typing, to Dr. Remedios Moore for typing and editing some chapters, to Mr. Pat Roberts for his excellent photography and to Mrs. Helen Halsey for her splendid illustrations.

H. D. PATTON
Seattle

T. C. RUCH
Santa Barbara

CONTENTS

Chapter 1

THE CELL MEMBRANE: ION FLUXES AND THE GENESIS OF THE RESTING POTENTIAL

by J. WALTER WOODBURY

INTRODUCTION

This chapter has dual pedagogical goals: (1) to provide a clear, qualitative verbal description of the concepts essential for understanding membrane electrophysiology, and (2) to provide a vigorous mathematical and physical chemical basis for these concepts. For this reason, most of the sections consist of qualitative descriptions of the material which are preceded or followed by a more rigorous theoretical development based on mathematics and closely tied to the physical meanings of the equations. It is believed that a sound grasp of the essential concepts can be obtained without following the theoretical developments. In order to aid the beginning or casual reader, the theoretical sections are put in smaller print than the verbal descriptions. These fine print sections can in most instances be skipped with little loss in continuity. However, in *some* of the fine print (theoretical) sections, essential equations are developed (for example, Ohm's law, the Nernst equation). Understanding the implications of these equations (but not their derivations) is essential to understanding membrane electrochemical phenomena. The reader who skips the theoretical sections must be prepared to accept these equations and be ready to work at understanding their implications. The reader seeking a solid grasp of this field should be ready to work at understanding the derivations and their physical and chemical implications in detail.

Basic Postulate of Physiology. The study of physiology, like the study of most other observable phenomena, is based on a series of postulates. The basic postulate of physiology is that every specific function of the body is performed by a specific structure or structures. Progress in the understanding of bodily function comes from identifying the previously unknown structure that performs a known function (for example, the discovery of the cell surface membrane from measurements of the electrical properties of excitable cells) or from discovering the previously unknown function of a known structure (for example, the discovery that the function of the myelin sheath of nerve fibers is to reduce charge separation across the membrane and thus increase the conduction speed of the nerve impulse). Current research deals mostly with questions of these types at the molecular level. For example, what is the molecular structure of Na^+-ion channels in excitable membranes that gives rise to its ion selectivity and gating functions? What are the functions of the basement membrane on the cell surface in regulating ion movements across the membrane?

Preview of Excitability.[2, 6, 8, 9, 10] The first three chapters deal with the functions and underlying structures of the membranes of excitable cells in generating ion concentration and voltage differences between the intracellular and interstitial fluid and in generating and propagating action potentials. The first two chapters describe the conditions prerequisite for the generation of

an action potential. This chapter deals with the generation and maintenance of the transmembrane resting potential. The second chapter describes the properties of ion pumps driven by metabolic energy, primarily the Na^+–K^+ exchange pump. The third chapter describes the generation and propagation of the action potential in unmyelinated and myelinated nerve fibers.

Any animal tissue such as muscle or brain is composed of closely packed cells and the solution surrounding and bathing them, the *interstitial* fluid. The cell plasm, or *intracellular* fluid, and the interstitial fluid are similar; both consist largely of water, and both fluids have roughly equal numbers of particles per unit volume dissolved in them. The functional boundary between the intracellular and interstitial fluids is a thin (75 angstroms, 7.5 nanometers), highly organized, bimolecular lipoprotein layer that severely restricts the interchange of materials. It should be kept in mind that the cell interior is highly organized, containing the nucleus, nucleolus, mitochondria, endoplasmic reticulum, and so on. Nevertheless, it is convenient and meaningful to regard the intracellular fluid as a single aqueous phase when discussing ion exchange across cell surface membranes.

The differences between the intracellular and interstitial fluids are more striking than their similarities. This chapter deals with two of these differences: (i) The concentrations of ions are markedly different. The concentrations of sodium ions, $[Na^+]$, and chloride ions, $[Cl^-]$, are much higher in the interstitial fluid than in the intracellular fluid. The situation is reversed for the potassium ion; its concentration, $[K^+]$, is much higher in intracellular than in interstitial fluid (see Table 1–1). (ii) There is an electric potential difference (voltage) between the intracellular and interstitial fluids. In skeletal muscle cells, the cell plasm is about 90 mV (0.09 V) negative to the interstitial fluid; in nerve cells the voltage is about − 70 mV.

Substances tend to diffuse from regions of higher concentration to regions of lower concentration. Hence Na^+ and Cl^- ions tend to diffuse into, and K^+ ions out of, cells. The movements of ionized substances are influenced by an additional factor: The existence of a voltage across the membrane means that electrical forces exist inside the membrane. When the intracellular fluid is negative with respect to the interstitial fluid, the electrical forces within the membrane exert an inward force on cations (+) and an outward force on anions (−).

Since these large differences in concentration and potential appear across the thin functional membrane of the cell, it is reasonable to suppose that this membrane plays an important role in the maintenance of these differences. Two aspects of the cell membrane are largely responsible for the observed concentration and electric potential differences: (i) Ions diffuse through the membrane at a minute fraction of the rate at which they diffuse through water. This barrier to diffusion of ions is a result of the nonpolar, hydrophobic nature of the lipid portion of the membrane. In most cell membranes, the rate of diffusion of Na^+ ions is much slower than the diffusion rates of K^+ and Cl^- ions. (ii) Energy derived from metabolism is used by cells to transport Na^+ out of the cell and K^+ into the cell. These ionic movements just balance, on the average, the diffusion of Na^+ into and K^+ out of the cell. This *active transport* of Na^+ and K^+ maintains the intracellular Na^+ concentration at low values and the intracellular K^+ concentration at high values. The voltage arises primarily because potassium ions permeate the membrane much more readily than do sodium ions.

More generally, the role of the membrane in cellular function is to regulate the interchange of materials between a cell and its environment. The crucial functions of the cell surface membrane in regulating the interchange of ions and other substances between the cell and its environment make a description of membrane properties a useful starting point for a study of physiology. The passive transfer of nonionized substances across the membrane is only cursorily treated. The permeation and active transport of ions through the membrane and the consequences of these ion movements are the subject matter of this and the next chapter. Knowledge of these processes and the underlying concepts is necessary for an understanding of a wide range of physiological phenomena: (i) the electrical activity of nerve and muscle cells and the processes of synaptic transmission (Chaps. 3, 4, 5, and 6, Vol. IV); (ii) the distribution of ions and water between the various body fluid compartments (Chap. 26, Vol. II) and the regulation

of interstitial and intracellular *p*H (Chap. 27, Vol. II); (iii) the role of active ion transport in the secretive (Chap. 2, Vol. III) and absorptive (Chap. 3, Vol. III) processes of the gastrointestinal tract and in the formation of urine by the kidney (Chap. 25, Vol. II).

In this chapter the main ideas concerning the origins of transmembrane concentration and potential differences will first be sketched. Then the step-by-step development of present concepts of the origins of these potential and concentration differences will be described, and, where necessary, the underlying physical and chemical principles will be reviewed in the text.

THE IONIC HYPOTHESIS. The current view of the origin of ion concentration and electric potential differences across the membrane and the genesis of the action potential was formulated by A. L. Hodgkin[8] in a review, "The ionic bases of electrical activity in nerve and muscle." He discussed the evidence relating to

> . . . a general hypothesis, which may be regarded as the modern counterpart of the membrane theory of Bernstein (1912) and Lillie (1923). Briefly, the hypothesis is that the action potential depends on a rapid sequence of changes in the permeability to the sodium and potassium ions. It makes use of the observation that potassium is concentrated inside most excitable cells, whereas sodium and chloride are relatively dilute. The resting potential is explained by supposing that the cell membrane is moderately permeable to the potassium and chloride ions, but is relatively impermeable to sodium and the internal ions. Any sodium which leaks into the cell is assumed to be pumped out by a secretory process which must ultimately depend on metabolism. A large but transient increase in the permeability to sodium occurs when the fibre is depolarized by an electrical stimulus or by flow of current from an adjacent region of active nerve. Sodium ions therefore enter the fibre at a high rate, and reverse the potential difference across the cell membrane. They also provide the current for depolarizing adjacent regions of resting nerve. The increase in the permeability to sodium is followed by a similar rise in the permeabilty to potassium. This accelerates the rate at which these ions leave the fibre and helps to restore the membrane potential to its resting value. The interchange of sodium and potassium provides the immediate source of energy for the propagation of nervous impulses, but it must be reversed by a metabolic process during the period of recovery which follows a burst of electrical activity. Since nervous activity is usually intermittent, it must be supposed that nerve fibres spend a large part of their lives paying off the debt which they have incurred during the passage of electrical impulses.

Experiments performed since then have added much detail about the processes of ion permeation through membranes, the nature of the Na^+ pumping process and the kinetics of the changes in ionic permeability that occur during the action potential but have not appreciably altered the ionic hypothesis. A. L. Hodgkin and A. F. Huxley received the 1963 Nobel Prize in physiology for their pioneering efforts using voltage–clamp techniques to describe quantitatively the ionic currents flowing during the action potential.[14]

ELECTRIC POTENTIALS AND ION CONCENTRATIONS IN MUSCLE

As stated above, there are two striking characteristics of cells to be considered in this chapter: the large difference in ion concentrations and the large difference in electric potential between the inside of the cell and the interstitial fluid.

Although these facts have been known for several decades, refinements in old techniques and introduction of new techniques were required to establish them with sufficient accuracy to make possible crucial tests of the various possible interpretations. The advent of radioactive isotopes made it possible to show that, contrary to previous belief, sodium can penetrate the membrane; the development of intracellular potential recording techniques made possible accurate estimates of transmembrane voltages. In order to give a concrete picture of the existence and size of a transmembrane potential difference in muscle cells, one method for making such measurements is described here.

Intracellular Recording. Figure 1–1 is a schematic diagram showing how the difference in electric potential between the inside and the outside of a cell can be measured directly and accurately. The technique for this was perfected by Ling and Gerard.[20] A microelectrode is made by drawing a piece of glass tubing down to a small tip and then filling the tubing with a concentrated solution of KCl. If the electrode tip is not larger than about 0.5 μm, it can be inserted transversely through the cell membrane of a muscle fiber without detectable damage to

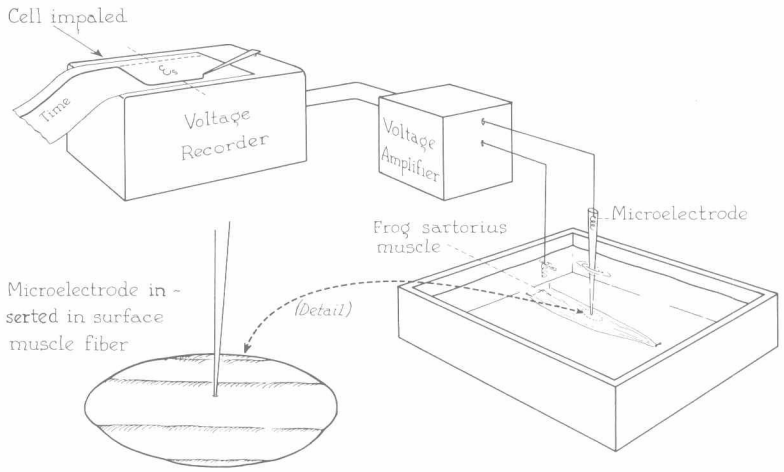


Figure 1–1 Intracellular recording. Schematic diagram of experimental arrangement for measuring transmembrane electric potential differences. A frog sartorius muscle is dissected free and pinned to a wax-bottomed chamber (right) filled with a physiologic (Ringer's) solution. A capillary ultramicroelectrode is held in position over the muscle with a micromanipulator (not shown). Electrical connection is made to microelectrode and chamber by means of spirals of chlorided silver wires. Potential difference between tip of microelectrode and bathing medium is amplified and displayed as a function of time by recorder. When electrode penetrates the cell membrane (arrow on record at left), pen is suddenly deflected, thus indicating the existence of a steady transmembrane potential ($\mathcal{E}_s$). Drawing at lower left is enlarged view of electrode inserted through membrane of a single cell to show that tip of electrode (0.5 μm) is much smaller than diameter of muscle fiber (100 μm).

the membrane. Any electrode larger than this at the tip appreciably damages the membrane and lowers the measured potential. The electric potential or voltage of the microelectrode tip when it is in the solution bathing the muscle is taken as zero. When the microelectrode is advanced toward the surface of the muscle, the voltage of the electrode does not change until the tip penetrates a cell membrane (*arrow* in Fig. 1–1). At this time the voltage drops abruptly to a value close to − 90 mV (inside negative) and remains at this value as long as the electrode is in the cell. This transmembrane electric potential is commonly called the *resting potential* but also will sometimes be referred to here as the *steady-state* voltage or potential, E_R, the E being derived from electromotive force.* Measured steady transmembrane voltages in different tissues vary from about − 10 mV to − 100 mV, but generation and maintenance are probably by the same mechanism in all tissues: active Na^+–K^+ transport.

Ion Concentrations in Muscle. The term *extracellular fluid* refers to all fluids that are not inside cells. Blood, lymph, cerebrospinal fluid, etc., are in this category. *Interstitial fluid* is the fluid in direct contact with the tissue cells, and therefore knowledge of the concentrations of ions in this fluid is necessary in the study of membrane phenomena. The concentrations of ions in the interstitial fluid are slightly different from those in blood plasma, because plasma contains an appreciable concentration of ionized protein; i.e., there is a Gibbs–Donnan equilibrium (Chap. 26, Vol. II) between plasma and interstitial fluid, maintained across the capillary wall. It is technically difficult to obtain samples of interstitial fluid, so ion concentrations are measured in blood plasma and interstitial concentrations are calculated from the Gibbs–Donnan ratio; the correction is about 5 per cent.

Table 1–1 gives the approximate concentrations of the more important ions in the interstitial fluid of mammals.* Intracellular concentrations are estimated from chemical analysis of a known weight of tissue and a measurement of the fraction of the tissue water that is in the interstitial space. The total amount of any ion in the interstitial fluid is then obtained by the product of the interstitial concentration and the fractional

*The term *steady* emphasizes that this unvarying voltage is one aspect of a steady state of cellular function maintained by metabolism. On the other hand, the term *resting* contrasts with *active* in describing impulse conduction in excitable tissues. The usual abbreviation is E_r for resting membrane voltage. Since E_r is used to mean reversal potential in Chap. 2, Vol. IV, E_R is used here for resting or steady voltage. The generic term, *membrane voltage*, is symbolized as E_M. In this chapter, voltage is used almost exclusively, because it has become synonymous with electric potential difference and is shorter. "Potential" has many meanings.

*Concentrations are given in the units of μmoles per cm^3 rather than the usual units of mmoles per liter (mM) because flux calculations are facilitated by taking the unit volume as 1 cm^3 rather than 1 liter (1 μmole per cm^3 = 1 mmole per liter). In some usages, e.g., calculation of current, equivalents are more convenient than moles. However, only monovalent ions are dealt with extensively, and since equivalents = valence × moles, the mole and the equivalent are the same size. In the pertinent literature, moles are commonly used.

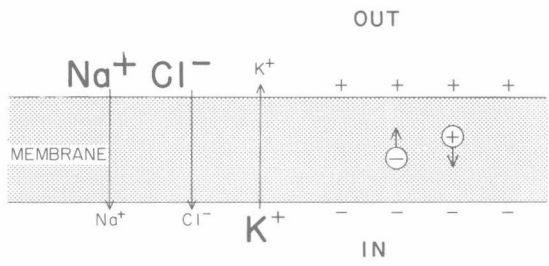
Table 1–1 *Approximate Steady-State Ion Concentrations in Mammalian Muscle Cells and Interstitial Fluid and Steady-State Transmembrane Voltage**

INTERSTITIAL FLUID		INTRACELLULAR FLUID			
	$[Ion]_o$ μmoles/cm^3		$[Ion]_i$ μmoles/cm^3	$\frac{[Ion]_o}{[Ion]_i}$	$E_{ion} = \frac{61}{z} \log \frac{[Ion]_o}{[Ion]_i}$ (mV)
Cations		*Cations*			
Na^+	145	Na^+	12	12.1	66
K^+	4.1	K^+	150	1/36.6	−96
H^+	3.8×10^{-5}	H^+	13×10^{-5}	1/3.4	−32
pH	7.4	pH	7.0		
others(1)	5				
Anions		*Anions*			
Cl^-	118	Cl^-	3.9(2)	30	−90
HCO_3^-	27	HCO_3^-	12	2.3	−21
others(1)	7	A^-(3)	146/z		
Steady-state voltage	0	−90 mV		1/30(4)	−90

*Double vertical line represents membrane.
(1) See Chap. 26, Vol. II, for more detailed listing.
(2) Calculated from membrane potential with the Nernst equation for a univalent anion, i.e., $z = -1$; $[Cl^-]_i = 118 \times 10^{-90/61}$ μmoles per cm^3. Direct measurements are inaccurate.
(3) A^- denotes the numerous organic anions that balance the inorganic cations. Their average valence, z, must be about −1.2 to give internal electroneutrality and osmotic balance with interstitial fluid.
(4) Calculated for a monovalent cation at equilibrium.

volume. This amount is subtracted from the total amount of ion in the tissue sample to give the amount of ion in the intracellular water. Intracellular concentration is the ratio of the amount of ion to the amount of water in the cells. The middle two columns in Table 1–1 show the concentrations of the more important ions in the intracellular water of mammalian skeletal muscle. Although intracellular concentrations vary from tissue to tissue, the electrolyte pattern of muscle is fairly representative. To summarize Table 1–1, interstitial fluid has high concentrations of Na^+ and Cl^-; the intracellular fluid has high concentrations of K^+ and the mixture of mostly organic anions (A^-) that are necessary for osmotic balance and electroneutrality. The right-hand columns are explained later.

Factors Affecting Ion Diffusion Through Membranes. PASSIVE FACTORS. Because of their random thermal motion, the individual molecules of a dissolved substance are continually intermixing (diffusing). If the concentration of the dissolved substance is higher in one region than in an adjacent one, more molecules will move from the region of higher to the region of lower concentration than in the reverse direction. Thus Na^+ and Cl^- ions tend to diffuse into cells, and K^+ and A^- ions tend to diffuse out of cells, as illustrated by the direction of the arrows in Figure 1–2*A*. Concentrations are indicated by the size of the symbol (e.g., Na^+) representing the ion. The rate of diffusion of these ions through the membrane depends not only on the external and internal concentrations but also on the ease with which they pass through the membrane. In



A. CONCENTRATION GRADIENTS B. VOLTAGE GRADIENT

Figure 1–2 Voltage and concentration gradients across the cell surface membrane exert driving forces on ions. *A*, Concentration inside (IN) and outside (OUT) the cell is indicated by the size of the symbol for each ion species. The direction of the diffusional force is indicated by arrows. *B*, Charges separated by membrane (+ outside and − inside) exert an inward force on cations and an outward force on anions in the membrane. The size of the transmembrane voltage is proportional to the number of charges separated by the membrane.

fact, the cell membrane so severely limits the rate at which substances diffuse through it that the rate of movement of ions is determined almost solely by the membrane. That is, diffusion of ions through water is so much faster than through the membrane that the ion concentrations near the membrane differ from those in the surrounding bulk medium by negligible amounts.

The rate of movement of ionized substances through the membrane is also affected by the transmembrane voltage. The existence of a transmembrane potential difference means that electric charges are separated by the cell membrane. This follows from the definition of electric potential difference between two points as the work done, against electric forces, in carrying a unit positive charge from one point to the other. No electrical work is done in carrying a charge through the membrane unless charges are separated by the membrane. These separated charges (inside negative, outside positive) exert a force on any ions in the membrane. This force tends to drive cations (+) into the cell and anions (−) out of the cell. As shown in Figure 1–2*B*, any cations that enter the membrane are attracted by the negative charges on the inside and are repelled by the plus charges on the outside of the membrane, and vice versa for anions.

K^+ ions tend to diffuse out of the cell because of their high internal concentration, but they tend to diffuse into the cell because of the electrical charges separated by the membrane. These two tendencies nearly, but not quite, cancel each other, so that there is a slight net tendency for K^+ ions to diffuse out of the cell. A similar argument holds for Cl^- ions, but in this instance the tendency for Cl^- ions to diffuse into the cell because of their high interstitial concentration is exactly balanced by the tendency of the electrical forces to keep the negatively charged Cl^- ions from entering the cell. Since there is no net tendency for Cl^- to diffuse through the membrane, the inside and outside concentrations of Cl^- are in electrochemical equilibrium.

ACTIVE TRANSPORT. The situation is quite different for Na^+ and A^-; both the concentration and the potential differences act to drive Na^+ ions into and A^- ions out of cells. The membrane is usually assumed to be impermeable to A^- ions and is much less permeable to Na^+ than to K^+ ions. Nevertheless, there is an appreciable steady leakage of Na^+ ions into cells. Despite this leakage, the internal concentration of Na^+ remains at low values inside living cells. Therefore, some mechanism present in the cell must carry Na^+ ions out of the cell as fast as they enter, on the average. Since work must be done to carry Na^+ from a region of lower to a region of higher concentration and from a lower to a higher electric potential, it must be concluded that energy derived from cellular metabolism is used to carry Na^+ out of the cell. This process is referred to as *active Na^+ transport*, as the *Na^+–K^+ pump*, or as the *Na^+ pump*. The word *pump* denotes that metabolic energy is required by the process. *Active transport* is a generic term referring to the process whereby metabolic energy is continuously expended to maintain transport of a substance in a direction opposite to that in which it tends to diffuse because of differences in concentration and voltage. Experimentally it is known that extrusion of three Na^+ ions is usually accompanied by an uptake of two K^+ ions. This linkage of K^+ uptake to Na^+ extrusion accounts for the slight unbalance in the distribution of K^+; the net outward diffusion of K^+ is balanced by the inward pumping of K^+. In muscle and nerve cells most of the transmembrane voltage arises because the membrane is much more permeable to K^+ than it is to Na^+ and because the Na^+–K^+ pump maintains the internal Na^+ ion concentration at a low value. Since the membrane is more permeable to K^+ ions than to Na^+ ions, K^+ ions would diffuse out of the cell faster than Na^+ ions would diffuse into it if there were no membrane potential; K^+ ions diffusing out must leave the nonpermeating A^- behind and thus the membrane is charged. The Na^+–K^+ pump is *electrogenic* because three Na^+ ions are ejected for every two K^+ ions taken up. This net outward current flow adds a small amount to the size of the transmembrane voltage. This electrogenic component, though small, is thought to play a significant role in the functioning of the nervous system. The molecular mechanisms of the Na^+ pump are described in Chapter 2, Volume IV.

SUMMARY. Four factors that together determine the rate of flow of ions through the membrane have been briefly described: (i) transmembrane concentration differences, (ii) transmembrane voltage difference, (iii)

active Na^+–K^+ transport, and (iv) the barrier to transmembrane ion movement imposed by the structure of the cell membrane, which can be thought of as a frictional retarding force. The remainder of this chapter describes in greater detail these forces, their interrelationships, and their role in the functioning of the cell.

DIFFUSIONAL FLUXES

Concentration Gradient. DIFFUSION.[17] All the molecules in a solution, both solute and solvent, move in random directions between collisions with other molecules. The average kinetic energy of the molecules attributable to random motion is directly proportional to the absolute temperature. The random motion of the molecules is such that the rate at which molecules leave a small volume is proportional to the concentration (moles per cm^3) of the substance in the small volume. Similarly, molecules from other regions diffuse into the region under consideration. If the concentration of a substance is everywhere constant, a molecule found in one region at one time may be found in any region at a later time, even though there is no net movement of the substance. This process of intermixing of solute (and solvent) particles is called *diffusion.*

GRADIENT AND FLUX. In a solution in which the concentration of a substance varies from one region to another, there will be a net movement of solute particles from regions of higher to regions of lower concentration, because more molecules per second leave than enter the region of higher concentration. This net diffusion is most conveniently expressed quantitively in terms of the *flux* (M), defined as the number of moles per second passing through an area of 1 cm^2 oriented perpendicularly to the direction of flow of the substance (moles per cm^2 second). The net diffusion of a substance from regions of higher to lower concentrations is analogous to the flow of water in a river. The rate of flow is proportional to the steepness of the stream bed; the steeper the grade, the faster the flow. Water flows directly downhill, i.e., in the direction of steepest slope; a substance diffuses "downhill" in the direction of "steepest slope." The magnitude of the steepest slope or rate of change of concentration and the direction of this steepest slope constitute a vector. This vector is called the *concentration gradient,* abbreviated "**grad**[S]" (Fig. 1–3*A*). Flux (**M**) is a vector

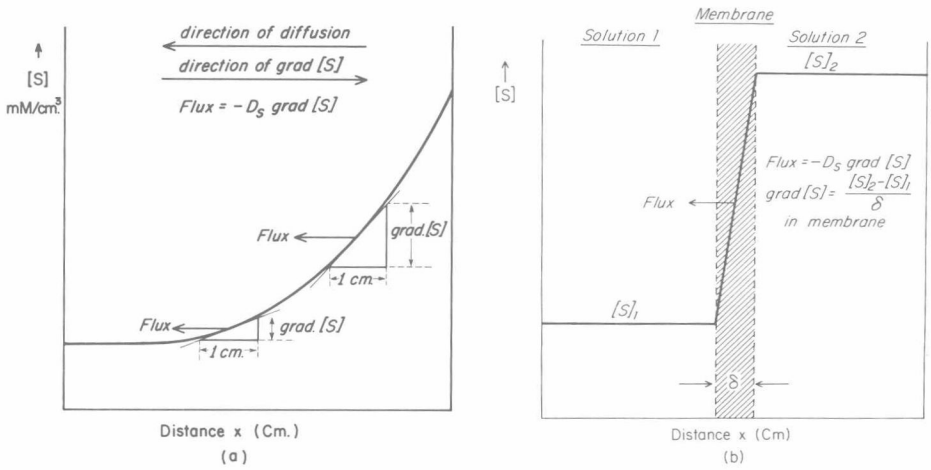


Figure 1–3 Concentration gradient and flux in one dimension. *a*, Graph of concentration of a substance ([S]) against distance in a one compartment aqueous system. [S] increases from left to right; consequently, net diffusion of S is from right to left. Rate of diffusion is proportional to concentration gradient, **grad** [S], *i.e.*, flux = $-D_S$ **grad** [S], where D_S is the diffusion constant. **Grad** [S] is defined, in one dimension, as the slope of the [S]-x curve as shown. *b*, A thin membrane divides the system into two compartments. S is assumed to diffuse much more slowly through the membrane than through the aqueous media on both sides. Therefore, **grad** [S] in the water is nearly zero, and **grad** [S] across the membrane is large. The average gradient of S in the membrane is the difference in [S] across the membrane ($[S]_2 - [S]_1$), divided by the membrane thickness, δ. **Grad** [S] is large if δ is thin, but flux is small because D_S is so small in the membrane.

whose direction is opposite to that of the concentration gradient. Vectors are indicated by **boldface** type.

The flux of a substance due to a concentration gradient is directly proportional to and in the opposite direction from the concentration gradient (Fick's first law), as illustrated in Figure 1–3*A*. The proportionality constant is called the *diffusion coefficient*, D. Thus, the diffusional flux, $\mathbf{M}_S$ (moles per cm^2 second), of a substance S is $\mathbf{M}_S = -D_S$ **grad** [S] where [S] is in moles per cm^3. D_S (cm^2 per second) is a measure of the ease with which molecules of S move through the solution; the greater the frictional resistance to their movement, the lower the diffusion coefficient.

Strictly speaking, activities rather than concentrations should be used here and throughout the remainder of the chapter. Concentrations will be used primarily for simplicity. The error is small in calculating transmembrane voltages for which concentrations appear only as outside/inside ratios. The error is larger in calculating fluxes, since these are proportional to activity. However, ion-selective microelectrodes slim enough to be inserted without damage into cells have recently been developed,[28] and there is a growing body of information on the activities of various ion species in different cells under various conditions. This information may well permit more accurate tests of flux equations.

Fick's first law is based on the assumption that the rate of diffusion of any solute particle is not influenced by the presence or absence of any other solute particle; i.e., the movement of one particle is *independent* of the movements of any other particle. This is called the *independence* principle[13] and is the basis for most theoretical calculations of expected ion movements. Although the movements of many ion species through membranes obey the independence principle, there are a number of known exceptions.[5]

The concentration gradient of a substance can be thought of as exerting a force on a substance. This force can be visualized by thinking of the solute molecules as a gas immersed in a viscous medium, the solvent water molecules. The ideal gas law, PV = NRT, can be rewritten to give P = RTN/V (where P is pressure, V is volume, R is the gas constant, T is absolute temperature, and N is the number of moles). For a substance, S, in solution, [S] = N/V; hence the partial pressure of S is $P_S = RT[S]$. In other words, a substance at concentration [S] exerts a partial pressure proportional to its concentration. The presence of a concentration gradient in a solution means there is a gradient of partial pressure that exerts a force on the substance in the direction from higher to lower pressure.

Fluxes through Cell Membranes. The presence of a cell membrane in a system greatly simplifies the description of the diffusion of a substance, because most dissolved substances diffuse through the membrane so much more slowly than they diffuse through water that the diffusion time in water usually can be neglected (Fig. 1–3*B*). More precisely, the rate of diffusion of a substance through the membrane is so slow that a negligibly small concentration gradient in the aqueous media suffices to bring the substance up to or away from the membrane as rapidly as it diffuses through the membrane. Thus, appreciable changes in concentration occur only in and near the membrane. The rate of penetration of a substance through a membrane depends on the properties of the membrane and on the concentration gradient of the substance in the membrane. Since the membrane is a thin, fixed structure and the concentration gradient in the solution is negligible, the average concentration gradient through the membrane is obtained by dividing the difference in concentrations between the interstitial and intracellular fluids by the thickness of the membrane (δ) as shown in Figure 1–3*B*. Thus, in the membrane, $\mathbf{grad}\ [S]_m = ([S]_o - [S]_i)/\delta$. (Distance increases in the direction from inside to outside.) The subscript "m" is used to denote the value of a quantity in the *m*embrane; the subscript "o" (for *o*utside the cell), the value in the interstitial fluid; and the subscript "i" (for *i*nside the cell), the value in the intracellular fluid.

PERMEABILITY. Fick's first law, $\mathbf{M}_s = -D_s$ **grad** [S] simplifies when applied to diffusion of a neutral substance through a membrane (Fig. 1–3*B*):

$$\mathbf{M}_s = -D_s\ \mathbf{grad}\ [S]_m = -(D_s/\delta)([S]_o - [S]_i) = P_s([S]_i - [S]_o)$$

The ratio D_S/δ is called the *permeability*, P_S, of the membrane to the substance S. D_S is the diffusion coefficient of S in the mem-