Physics of the nerve impulse

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After briefly presenting the history of the problem, we describe the fundamental properties of the nerve cell, its electrical characteristics, and the phenomenological pattern of the excitability phenomenon. We discuss in detail ion transport through biological membranes and models for them, in particular through bilayer lipid membranes. We present the fundamental views on transport mechanisms and discuss the molecular bases of this process. We present the experimental facts concerning the ion channels of biomembranes and describe the theory of single-file ion transport. We discuss in detail the possible physical mechanisms that govern the relationship of the conductivity of the channels to the electric field. We discuss the process of propagation of impulses along nerve fibers.

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CONTENTS

1. INTRODUCTION

Modern biology is developing no less swiftly than physics was at the beginning of the 20th Century. These advances have been made possible by using the most modern physical and physicochemical methods of research. Studies in the field of biological membranes are acquiring ever greater importance in connection with such branches of biology as molecular genetics, protein synthesis, and enzymatic catalysis. The functions of cell membranes are highly varied. One of the major ones is the generation and conduction of nerve impulses.

The problem of the nerve impulse has a rich, and at times dramatic, history. It would be naive to try in this review to throw light on it in more or less detail.[1)] Yet it is hard to resist the temptation of at least listing the fundamental stages of the path taken. This is also important because a brief historical digression into the problem of the nerve impulse makes it clear how closely physics and biology are intertwined here. The biophysics of excitable membranes is now experiencing a new boost that involves a shift to the molecular level of studies. This is expressed in attempts at reconstructing ion channels, at analyzing their composition and structure, and at developing new relaxation methods and measurements of electric fluctuation spectra. Theoretical views on the nature of bioelectrical phenomena are also developing in parallel with the experimentation.

An extensive literature has been devoted to the problem of the nerve impulse. The wealth of details, the fine distinctions in the characteristics of different systems, and the large number of empirical relationships that have been developed on the computer are characteristic of the physiological approach (and undoubtedly justified). Yet in order to understand the physics of the subject, we must abstract from the less important details, and try to see the general and principal points that are inherent in the problem as a whole. This is precisely the principle on which we have based the following presentation.

2. THE NERVE FIBER: FUNDAMENTAL PROPERTIES

a) On the history of the problem

It was thought as early as the 17th Century that nerve fibers serve as information-transfer channels from the brain to the muscels. Various hypotheses have been advanced on the nature of the information carrier that have reflected the level of scientific views of each epoch. Descartes spoke of "animal spirits." Depending on their quality, they cause contractions or softening of muscles. The physiologists, who were already armed with the microscope, were inclined to believe that the nerve fiber is a tube along which a "nervous fluid" flows. In his *Principia*, Newton wrote of an elastic wave that propagates along the fiber, as was quite natural in the century of triumph of mechanics. However, this hypothesis was soon refuted, and mechanical concepts came to be

[1)] One can find an excellent presentation of the history of the problem in the introductory article by A. V. Lebedinskiĭ in the Russian translation of *Selected studies on animal electricity of Galvani and Volta*. [1]

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replaced by electrical ones. The so-called electric fishes played a certain role in confirming the latter.

The ability of certain fishes to give a shock has been known for a long time. Even the Roman physician Scribonius Largus recommended using the discharges of the skate *Torpedo* as a remedy for gout, headache, and epilepsy. In 1776 Cavendish measured the electric field intensity distribution around the skate *Torpedo* held in a vessel containing water. These experiments served to prove that the shock given by the skate is an electric discharge, which they compared with the discharge of a Leyden jar.[2)] At about the same time, information had been collected on the effect of external electric fields on the organism. It was natural to assume from all these facts that there is a certain relation between the "nervous forces" and the external electric field. Yet the problem of the nature of the "nervous fluid" remained open. And in 1791 Galvani published the *Commentary on the Effect of Electricity on Muscular Motion*, for which a remarkable fate was in store. Upon bringing two different metals into contact with a nerve-muscle preparation of a frog, he observed contraction of the muscle.[3)] Since he already knew from his previous experiments about the effect of atmospheric electricity on muscular contraction, Galvani assumed that this twitching of the leg was caused by electricity. It remained unclear just what was the source of this electricity, the living object or the metals. Galvani assumed that the contraction of the muscle was caused by bioelectricity, whereby the nerve fiber played the role of a conductor. When combined with the metallic electrodes, it closed the circuit and facilitated a discharge of the muscle equivalent to that of a Leyden jar. As a recognized authority in the field of electricity, Volta interested himself in Galvani's experiments and duplicated them. However, his subsequent experiments established that the source of the electricity was the contact of unlike metals with an electrolyte solution. Thus a new class of current sources was discovered, which were later called galvanic elements. The scientific dispute of the physiologist and the physicist didn't stop at this.

In his treatise of 1797, Galvani described the phenomenon of contraction of a muscle upon contact with a nerve without inclusion of metals in the system. However, his experiment, which the founder of modern electrophysiology du Bois-Reymond later called "the fundamental experiment of nerve-muscle physiology" could not yet overcome the skepticism of the physicists—the victory in the polemic remained with Volta. The rehabilitation of bioelectricity, which dates back to the mid-19th Century, required considerably more sensitive measuring technique. The experiments of Matteucci (in the 1840's) played a large role in resurrecting galvanism. The latter tried to substantiate physically the appearance of electricity in living tissues "according to Volta" by contact phenomena at the surface of the muscle fiber.

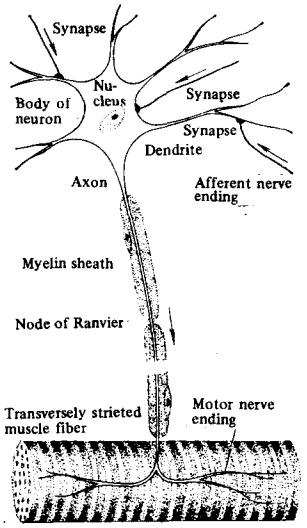


FIG. 1. Diagram of the structure of a nerve cell (spinal motor neuron of frog).[3]

Faraday showed great interest in the problem of bioelectricity. "However amazing the electrical phenomena are that inhere in inorganic matter," he wrote, "they do not in any way compare with those that involve the activity of the nervous system and vital processes." The full triumph of Galvani's ideas followed the studies of du Bois-Reymond and his school (1843), who measured the rest currents of muscle and nerve, and then the action currents that arose after stimulation. At the same time, Helmholtz and Bernstein measured the velocity of propagation of nerve impulses. The physical views that Bernstein developed on the source of bioelectricity were based on the studies of Gibbs, Helmholtz, Nernst, and Ostwald on the thermodynamics of the galvanic cell. We shall touch upon the fundamental views of this theory in the presentation below. Application of more refined experimental technique has led to a substantial development of Bernstein's views. The phenomenological theory of bioelectrical phenomena developed by Hodgkin and Huxley excellently describes a vast bulk of factual material. The most recent development in this field of science is characterized by deeper penetration into the molecular nature of membrane phenomena.

b) Structure and properties of the nerve cell

The fundamental structural element of the nervous system of higher organisms is the neuron, which consists of a cell body from which many processes emerge: the dendrites (Fig. 1). One of these processes in the peripheral neurons is much longer than the others—this is the axon, which can sometimes be as much as meters long, while its diameter is of the order of 1–100 μm. As a rule, the axons are surrounded by a thick fatty (myelin) envelope that is interrupted periodically (every 1–2 mm) by the nodes of Ranvier (1 μm). The meylin segments play the roles of insulating sleeves, and the nerve fiber in these regions is analogous to a passive cable. Only the part of the membrane in the nodes of Ranvier is electrically active. The nonmyelinated axons of squid offer great convenience for study, and they

[2)]The electric skate generates 50-A pulses at 60 V, while the electric eel creates pulsed fields of 500 V.

[3)]According to the testimony of one of his contemporaries, "the prepared frogs happened to be on his table on the occasion of his preparing soup for his ailing wife" (see Ref. 1).

TABLE I. Concentration of electrolytes ($mmol/l$) for squid axon.

Ion	Outside, c^0	Inside, c^i
Na^+	460	50
K^+	10	400
Cl^-	540	40–100

sometimes attain diameters of a millimeter. In these fibers, which are also called smooth fibers, the whole membrane possesses electric activity. Henceforth, unless specified otherwise, we shall be discussing smooth nerve fibers.

We can picture the axon as a hollow tube filled with an electrolyte solution. The wall of this tube (the axon membrane) consists of lipids and proteins. The thickness of the membrane amounts to ~70 Å. In a state of rest, the electric resistance of the membrane is very high (about 10^3 ohm·cm^2), while its capacitance amounts to about 1 μF/cm^2. The axon membrane separates the inner solution from the outer one, which has a different composition. Thus, the concentration of potassium ions is high inside in the axoplasm and the concentration of sodium and chloride ions is low as compared with the surrounding medium (Table I). The inside of the cell at rest is negatively charged with respect to the outer medium, and a potential difference is developed at the membrane of about 60 mV. In Bernstein's theory, the appearance of the rest potential is explained as follows. One assumes that the membrane is permeable only to potassium ions. Then, in order to equilibrate the diffusion current of potassium ions, the following potential difference must be established at the membrane:

$$\varphi_m = \frac{kT}{e} \ln \frac{c^0_{K+}}{c^i_K}. \qquad (1)$$

The experimentally obtained values of the rest potential do not fully agree with those calculated from (1), but this aroused no special concern until people were able directly to measure the currents of the different ions through the membrane, and thus to test Bernstein's fundamental postulate. It turned out that the membrane in a state of rest is permeable not only to potassium ions but also to sodium and chloride ions. Indeed the permeability to sodium and chloride is considerably smaller than for potassium ($P_K : P_{Na} : P_{Cl} = 1 : 0.04 : 0.45$), but this did not save the old membrane theory from crisis. Actually, the sodium ions, which occur in excess in the outer medium, enter the cell under the action of both diffusional and electrical forces. Therefore people had to assume that a specific mechanism acts in the membrane: a sodium pump that performs the so-called active transport. That is, it removes the sodium ions to the outside against the electrochemical potential gradient by expending metabolic energy.[4)] The pump is constructed in such a way that the sodium transport to the outside is coupled with potassium transport into the cell. The stoichiometry of the pump with respect to sodium and potassium differs from 1 : 1. That is, the process of active transport is electrogenic. However, its contribution to the rest potential of the giant squid axon amounts to only 2.5 mV. Therefore we can treat the problem of calculating the rest potential while digressing from the existence of active transport. Since the membrane is permeable to an entire set of ions, the rest state is not one of thermodynamic equilibrium. It is stationary because of the action of the ion pumps, and here the membrane potential under open-circuit conditions is found from the condition that the total electric current should be zero. Hence the calculation of the rest potential must be based on the definite pattern of ion transport through the biomembrane.

[4)]We shall henceforth not deal with the problems of active transport, since the process of generation of the nerve impulse is an independent phenomenon. The fundamental role of active transport consists in maintaining a concentration drop at the membrane. When the pumps are turned off, the concentration is gradually equalized, both in the excited and in the resting cell.

c) The excitability phenomenon

If one passes through an axon a small current impulse that gives rise to a subthreshold depolarization of the membrane, i.e., it shifts the potential of the cell to the positive side, then the potential monotonically returns to the original level after the external stimulus has been removed. That is, the axon behaves like a passive electric circuit consisting of a capacitor and an approximately constant resistance.

However, everything looks different if the current impulse is large. Then the potential continues to vary even after the perturbation has been removed; it passes through zero, becomes positive, and only later does it return to the rest level (Fig. 2). The response of the membrane no longer depends on the perturbation. Such a response is called a nerve impulse or action potential. Hodgkin and Huxley[5] have given a correct phenomenological interpretation of the mechanism of generation of the nerve impulse that is based on voltage-clamping experiments. The essence of this method is that one imposes a potential on the membrane in the form of a step function of varying amplitude and records the current as a function of time. Special experiments showed that the total ion current (curve 1 in Fig. 3) is made of three components, a potassium current, a sodium current,

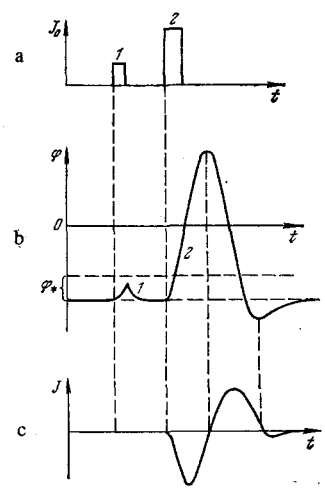


FIG. 2 Response of a nerve fiber (membrane potential (b) and ion current (c)) to an external current impulse (a). 1—subthreshold effect, 2—superthreshold effect.

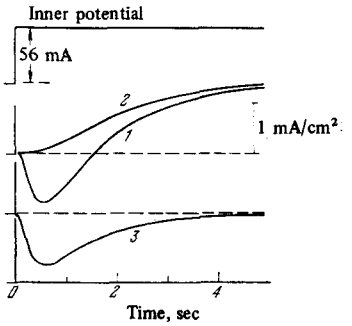


FIG. 3. Separation of the membrane current (1) into the potassium (2) and sodium (3) components. The variations in the currents are elicited by a rapid shift in the potential inside the fiber by + 56 mV (upper garph). [2]

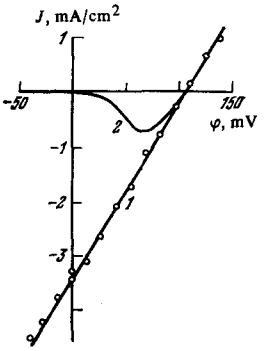


FIG. 5. Direct measurement of the instantaneous sodium conductivity (1). Curve (2) corresponds to the peak volt-ampere characteristic. [6]

and a leakage current. The potassium current sets in after a lag, and it reaches its steady-state value within a time of the order of several milliseconds (curve 2 in Fig. 3). The sodium current, which is directed inward, increases rapidly in size, reaches a maximum, and then slowly declines (curve 3). The latter phase is called the inactivation of the sodium current. Upon measuring a series of J–t curves for different amplitudes of the potential fixed at the membrane, one can construct the volt-ampere characteristics of the system (Fig. 4). Evidently, the steady-state volt-ampere characteristic will coincide with the potassium characteristic. As regards the sodium current, one customarily describes it in terms of the relationship of the peak value of J_{Na} to the potential (Fig. 4b). The current J_{Na} vanishes at a potential that coincides with the equilibrium sodium potential, $\varphi_{Na} = (kT/e)\ln(c^0_{Na}/c^i_{Na})$. The fact draws attention that the J_{Na}–φ curve is nonmonotonic. In the vicinity of φ_{Na}, the sodium volt-ampere characteristic is close to linear, and the sodium current is proportional to $(\varphi - \varphi_{Na})$. The volt-ampere characteristic shows no hysteresis in the linear region. We can convince ourselves of this by imposing short voltage impulses of any amplitude and sign on a membrane that has preliminarily been depolarized to potentials lying to the right of the minimum point in Fig. 4b. Figure 5 shows the corresponding instantaneous volt-ampere characteristic. To the left of the minimum point, the sodium current shows hysteresis and it is characterized by a more complicated dependence on φ. Yet even in this region, the system behaves ohmically in response to fast voltage pulses. The falling region on the curve of the J_{Na}–φ relationship can be naturally explained by an increase in sodium conductivity with increasing shift of the potential to the positive side. Here this conductivity, just like that for potassium, shows hysteresis. The sodium conductivity reaches a constant value to the right of the minimum point in Fig. 4b.

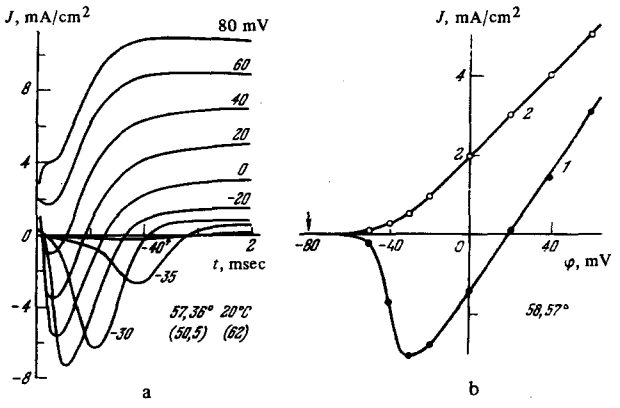


FIG. 4. Typical data on voltage clamping. a) Ion current density at the different voltage values indicated in the diagram, b) the peak (1) and steady-state (2) volt-ampere characteristics. [6]

Pharmacological agents (especially tetraethylammonium—TEA) and toxins (especially tetrodotoxin—TTX) have been a powerful tool for studying the nature of the ion currents. When TEA is introduced into an axon, the potassium current is completely suppressed, while the sodium current is not altered. Conversely, introduction of TTX suppresses the sodium current. These experiments practically prove the existence of separate (sodium and potassium) conduction mechanisms. We should also recall some experiments on intracellular introduction of the enzyme pronase, which abolishes the sodium inactivation without changing either the kinetics of the potassium current nor the kinetics of sodium activation. En masse, all these facts convincingly indicate two independent systems of ion transport in the membrane, the sodium system being regulated by two separate mechanisms: activational and inactivational. The presented results allow one to give a qualitative explanation of the excitability phenomenon. In the course of development of a nerve impulse, the permeability of the membrane to sodium sharply increases, and a current of these ions rushes into the fiber. It is highly essential that the process proves to be self-accelerating—the increase in sodium permeability facilitates an increase in potential on the inner side of the membrane, which in turn increases the sodium permeability. Consequently the system approaches the equilibrium sodium potential. Simultaneously the potassium-transport system is turned on, and it removes the positive charge from the cell, and restores the membrane potential to its original value.

d) The Hodgkin-Huxley equations

The electrical phenomena in excitable membranes are quantitatively described by the equations proposed by Hodgkin and Huxley. Although these equations are empirical in nature, they play an extremely large role in biophysics, and actually comprise the language in which

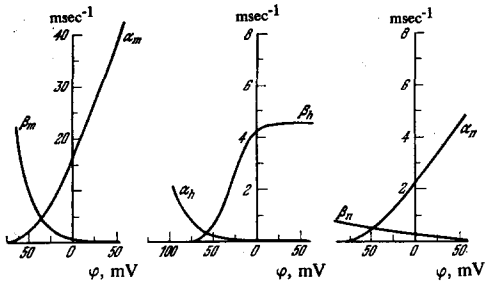


FIG. 6. Curves of the relationship of the rate constants α and β to the membrane potential. [6]

all the experimental material is discussed. Apparently the success of the Hodgkin-Huxley formalism indicates that it is based on a correct physical picture of the processes that occur in excitable biological systems. Hence it is reasonable to take up this problem briefly.

The time behavior of the membrane potential under spatially uniform excitation of the fiber is described by the equation

$$C\frac{d\varphi}{dt} = -J, \tag{2}$$

Here C is the capacitance of the membrane, while the ion current J is composed of two partial currents, a potassium and a sodium current:

$$J = J_K + J_{Na}.$$

As experiment implies, each of these currents can be treated as a current produced by a battery of constant emf and variable hysteretic conductivity:

$$\begin{aligned} J_{Na} &= g_{Na}(\varphi - \varphi_{Na}), \\ J_K &= g_K(\varphi - \varphi_K). \end{aligned} \tag{3}$$

The separation of the linear hysteresis-free coefficients $(\varphi - \varphi_i)$ is experimentally justified (see the right-hand line in Fig. 5). The central problem is how to describe the dynamics and the potential-dependence of the hysteretic conductivities g_{Na} and g_K. Hodgkin and Huxley[5] introduced the "unobservable" variables m, h, and n, which obey the following linear dynamic equations:

$$\begin{aligned} \frac{dm}{dt} &= \alpha_m(1-m) - \beta_m m, \\ \frac{dh}{dt} &= \alpha_h(1-h) - \beta_h h, \\ \frac{dn}{dt} &= \alpha_n(1-n) - \beta_n n. \end{aligned} \tag{4}$$

The conductivities g are defined as nonlinear functions of these variables:

$$\begin{aligned} g_{Na} &= \bar{g}_{Na} m^3 h, & \bar{g}_{Na} &= 0.12\ \Omega^{-1}\ \text{cm}^{-2} \\ g_K &= \bar{g}_K n^4, & \bar{g}_K &= 3.6\cdot 10^{-2}\ \Omega^{-1}\ \text{cm}^{-2} \end{aligned} \tag{5}$$

The relationship of the kinetic coefficients α and β to the membrane potential (Fig. 6) was chosen by the condition of best match of the J–t curves as calculated and as measured by the voltage-clamp method. These same arguments dictated the choice of the exponents in the relationships (5). For example, the relationship $g_K \sim n^4$

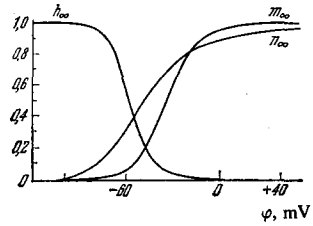


FIG. 7. Relationship of the steady-state values of m, n, and h to the membrane potential. [6]

describes well the hysteresis of the potassium current. The introduction of two variables m and h to describe the dynamics of sodium conduction involves the existence of two independent processes, activation and inactivation, while the concrete exponents also arise from the hysteresis of the sodium current. Figure 7 shows the relationship of the steady-state values of m, n, and h to the membrane potential. It is evident here that a steady-state potassium current can flow over a broad potential range, whereas the steady-state sodium current is restricted to the narrow region in which m^3 and h simultaneously differ from zero.

We shall discuss below the physical interpretation of the Hodgkin-Huxley equations. Here we shall restrict ourselves solely to a general conclusion that amounts to the idea that the membrane of a nerve fiber constitutes a nonlinear ionic conductor whose properties depend substantially on the electric field. Hence it will be useful to examine the features of ion transport through biological membranes and models for them.

3. ION TRANSPORT THROUGH MEMBRANES

a) Bilayer lipid membranes

People have represented the structure of cell membranes over the last several decades by using the Danielli-Davson model.[7] A double layer of lipid molecules lies in the middle of the membrane. This lipid sandwich is covered on the outside by a layer of proteins, which can penetrate into the interior of the membrane to form various functional structures, e.g., polar pores (Fig. 8a). The thickness of this structure is of the order of 100 Å. The Danielli-Davson model, which has recently been considerably refined, has played a substantial role in the development of membrane studies. As early as the thirties of this century, the first attempts were undertaken to prepare thin, stable lipid or proteolipid

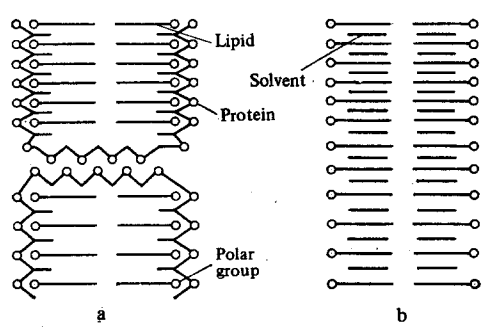


FIG. 8. The Danielli-Davson model. a) cell membrane, b) lipid bilayer.

model structures. These attempts were crowned with success in 1961, when Mueller *et al.*[8] found that a suspension of oxidized beef-brain phospholipids in aqueous solution spontaneously forms a bimolecular (black) film.

The idea of the experiment proved to be amazingly simple. A certain amount of phospholipid is dissolved in a liquid hydrocarbon, e.g., *n*-decane. A teflon diaphragm having a small aperture is placed in a vessel containing water. A drop of the lipid solution is introduced at this aperture. Upon gradually spreading, it is first converted into a thick film showing rainbow patterns, and then it thins down and becomes black.

First of all, it was interesting to determine the thickness of the black films. The simplest and most widespread method consists in measuring the electric capacitance of the film. If we know the dielectric constant of the lipids (it lies in the range from 2 to 3), we can calculate the thickness directly from the formula for a plane condenser. While slightly varying as a function of the composition of the lipids, the thickness proved to be approximately 50 Å. This exactly matches twice the length of the chains of the lipid molecules. Optical measurements give the value 70 Å, which apparently includes also the polar heads of the lipids.

Thus we can represent a bilayer lipid membrane (BLM) in the way shown in Fig. 8b. The question arises of the state and arrangement of the solvent molecules in the membrane. In principle, they can lie either between the two lipid layers, or within each layer between the molecules of the lipids. The obtained estimates rule out the first possibility. It has been shown by studying a BLM made of phosphatidylcholine[9] that one lipid molecule in the film takes up an area of about 75 $Å^2$, whereas the minimum possible area is 58 $Å^2$. This made it possible to rule out the idea that the solvent molecules exist in each layer between the lipid molecules in about a 1 : 1 ratio. This is just the situation depicted in Fig. 8b. Apparently the role of the simple solvent molecules is played in natural membranes by the more polar fillers.

Thus a BLM is a thin film of hydrocarbons stabilized in the aqueous phase by the lipid molecules, which in themselves contribute substantially to the volume of this film. Thermodynamic analysis of these films implies[10] that molecules that can form such structures must possess a high energy of adsorption, both on oil and on water. These conditions are naturally satisfied by lipids that contain long hydrocarbon chains and short, strongly polar groups. Another requirement that the lipids also satisfy is the condition that their heads should not strongly differ in cross section from the hydrocarbon chains.

A quite unique feature of the BLMs is that they have molecular dimensions in one direction, but macroscopic dimensions in another. Usually these distances differ by a factor of 10^6. Rather many problems arise thereby that have no exhaustive answers as yet. First of all, this pertains to the structure, stability, and phase transitions in these films. In spite of individual studies, much yet remains to be done in this field.

Bilayer lipid membranes are a model for the skeleton of the cellular membrane, which amounts to a barrier between two volumes of liquid. If one deposits on this skeleton or matrix suitable functional groups, then one can confer definite functions on it that are inherent in cell membranes. Thus, by using alamethicin together with the surface-active protein protamine, Mueller and Rudin have been able to reconstruct the phenomenon of electric excitability in an artificial membrane.[11] Subsequently various authors have been able to reconstruct a whole series of membrane phenomena by using different substances and cell fractions.

b) Charge transport through membranes

Even the first measurements of electrical properties of BLMs showed that they differ considerably from cell membranes. While the electric conductivity of cell membranes amounts to about 10^{-3} ohm^{-1} cm^{-2}, the electric conductivity of BLMs varies over the range from 10^{-6} to 10^{-10} ohm^{-1} cm^{-2}, depending on the experimental conditions. We can take 10^{-8} ohm^{-1} cm^{-2} as the most typical value. This is a very low conductivity. For the sake of illustration, we shall compare it with the conductivity of the surrounding electrolyte solution. If this solution contains KCl in 0.01 *M* concentration, the conductivity of an aqueous layer of the same thickness as the BLM amounts to 10^4 ohm^{-1} cm^{-2}. The difference is as great as a factor of 10^{12}.

A very important discovery has been a class of substances that can radically change the electrical properties of membranes (for a review, see Refs. 12 and 13). They have been called ionophores. Their presence increases the conductivity of membranes by many orders of magnitude. The ionophor themselves are needed in small amount; they only effect transport of other ions existing in the solution through the membrane. Here the conductivity is selective in nature. The ionophors include fat-soluble acids (2, 4-dinitrophenol, dicumarol, tetrachlorotrifluoromethylbenzimidazole (TTFB), etc.), polypeptides (valinomycin, the group of nactins, gramicidins A, B, and C, and alamethicin).

The overwhelming majority of studies on electrical properties have treated lipid membranes as a homogeneous phase. The limitations of this approach are quite obvious, but still it permits one to obtain rather important results. The conductivity of the membrane is determined by the concentration of current carriers existing in it and by their mobility. The extremely low dielectric constant of lipids (it lies in the range from 2 to 3) is very unfavorable for incorporation of charged particles into the membrane. The distribution coefficient of the particles between the lipid and the aqueous phases is

$$\gamma = \frac{c_m}{c_W} = e^{-W/kT}, \qquad (6)$$

Here W is the energy of a particle in the lipid, as referred to its energy in water. It is composed of the electrostatic energy and the energy of hydrophobic interaction:

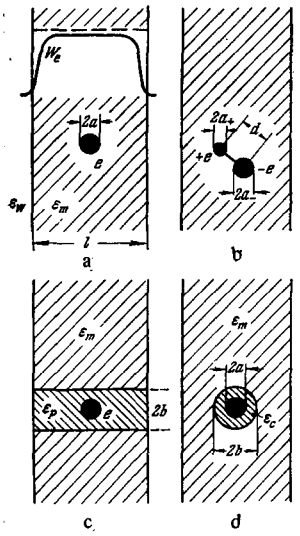


FIG. 9. Illustrating the calculation of the energy of an ion in a membrane. a) Effect of the image forces; b) formation of ion pairs; c) hydrophilic pore in a membrane; d) effect of complex formation.

$$W = W_e + W_n.$$

The major term is the first one. For spherical particles of radius a, it takes on the form

$$W_e = \frac{q_0}{a}\left(\frac{1}{\varepsilon_m} - \frac{1}{\varepsilon_W}\right), \qquad q_0 = \frac{z^2e^2}{2kT}. \tag{7}$$

At the temperature 25 °C for a valency of one, $q_0 = 282$ Å. If we assume the radius of the ion to be 2 Å, and the dielectric constant of the membrane $\varepsilon_m = 3$, then the distribution coefficient proves to be 10^{-20}. Then the conductivity of bilayer membranes would be substantially lower than the values indicated above. The situation can be somewhat improved by the hydrophobic interactions, which elevate the distribution coefficient to favor the lipid phase. Moreover, there is an entire set of circumstances that can diminish the energy of a particle inside a membrane. We shall now proceed to discuss them.

There are at least four factors that diminish the energy of an ion in a membrane[14]:

1) the membrane has a finite thickness;

2) the ions can form ion pairs inside the membrane;

3) the membrane can have pores of high dielectric constant through which the particles pass;

4) the ion can be wrapped in a neutral carrier molecule of high polarizability that solvates it (increases its effective radius a), and thus facilitates its solution in the membrane phase.

Calculation of each of these effects yields the following results.

a) Image forces arise at the boundary between the membrane and the aqueous phase. The electrostatic energy W_e of the ion in the membrane is diminished, and it acquires the form of the curve shown in Fig. 9.[15] At the center of the membrane, the image forces decrease energy by the following amount:

$$\frac{e^2}{\varepsilon_m l}\ln\frac{2\varepsilon_W}{\varepsilon_W+\varepsilon_m}. \tag{8}$$

When $\varepsilon_W = 80$ and $\varepsilon_m = 2$, the relative difference from the energy of the ion in an infinite medium amounts to 1.4 a/l. That is, it does not exceed several percent. This example makes it pictorially evident that the hydrocarbon part of the membrane constitutes a substantial barrier to the passage of ions. The height of the barrier amounts to several tens of kcal/mole.

b) Formation of ion pairs from two close-lying spheres upon ion interaction also does not yield an appreciable gain. The electrostatic energy of two particles of radii a_+ and a_- separated by the distance d (see Fig. 9b) is

$$W = \frac{e^2}{2\varepsilon_m a_+} + \frac{e^2}{2\varepsilon_m a_-} - \frac{e^2}{\varepsilon_m d}. \tag{9}$$

Hence we see that the maximum reduction in energy will be no more than twofold. Only a covalent bond between the charged particles will substantially diminish the electric field around them, but this would now imply the discharging of the two associated particles.

c) Pores having high polarizability can substantially diminish the energy of a charge in a membrane (see Fig. 9c). When $b \ll l$, the energy of a particle on the axis of the pore is

$$W_p = \frac{e^2}{2\varepsilon_p a} + \frac{e^2}{\varepsilon_m b} P\left(\frac{\varepsilon_m}{\varepsilon_p}\right). \tag{10}$$

The second term in this formula arises from the image forces in the walls of the pore. It is inversely proportional to the radius b of the pore. The function $P(x)$ has been calculated numerically.[14] Its maximum value does not exceed 0.25. If, for example, the value of ε_p is comparable with ε_W, then the height of the barrier for an ion passing through the membrane is given simply by the second term. When $\varepsilon_m = 2$, it is

$$\frac{e^2}{2b} P\left(\frac{1}{40}\right) \approx \frac{28}{b}\text{ kcal/mole}, \tag{11}$$

Here b is expressed in Ångström units.

d) Finally let us examine the possibility of complex formation. Let a neutral molecule of high polarizability form a spherical complex with the ion. If the outer radius of the complex is b, then its energy in the medium has the form

$$W_c = \frac{e^2}{2\varepsilon_m b} + \frac{e^2}{2\varepsilon_c}\left(\frac{1}{a} - \frac{1}{b}\right). \tag{12}$$

In the case in which the molecules of the complex-former have high polarizability and we neglect the second term, the energy of the complex is still considerable in comparison with the thermal energy, though considerably smaller than the energy of a "bare" ion. For example, if $b \sim 5$–10 Å, then at 25 °C we have

$$W_c = 16.5\text{–}8.2 \text{ kcal/mole or } 9.8\text{–}4.9\ kT/\text{ion}.$$

We should note another circumstance of no small importance. If the increase in electrostatic energy of the ion in the hydrocarbon phase is large, then a powerful

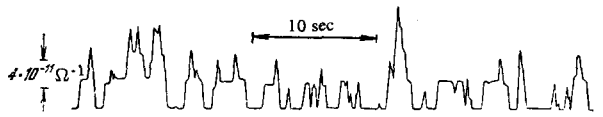


FIG. 10. Conductivity fluctuations of a bilayer membrane containing gramicidin A.

electrostriction can arise in the membrane. Estimates show that pressures arise of the order of an atmosphere. It is not ruled out that this can locally thin the membrane, and thus substantially affect ion transport.

Of course, the estimates made are very crude in nature. Yet they clearly indicate that the barrier for passage of an ion through a membrane can be considerably reduced by complex formation or by special pores. Now returning to the ionophores, which substantially facilitate ion transport through membranes, we can assume that they act by one of these two mechanisms.

An extensive literature has been concerned with the theory of induced ion transport.[13] Methods have been developed in detail there for "electrical diagnostics" of mechanisms of ion transport. However, it is of interest to study the mechanisms by directly determining the activation energy of conduction. In Ref. 16, they measured the temperature-dependence of the conductivity of membranes in the presence of a number of ionophores. In the studied temperature range from 17 to 45 °C, the conductivity is described by the formula

$$g = Ae^{-H/RT},$$

Here H is the activation enthalpy of the overall transport process. Here for monactin, H proved to be 32.5 kcal/mole, for valinomycin 55 kcal/mole, and for gramicidin A 9.3 kcal/mole. Studies of a different type had shown even earlier that valinomycin and monactin are mobile carriers, while gramicidin A must form a polar pore inside the membrane. The numbers given above confirm this conclusion. Moreover, Hladky and Haydon[17] studied the conductivity of membranes at low concentrations of gramicidin, and observed discrete fluctuations of conductivity (Fig. 10). Each step in this diagram corresponds to creation or destruction of a single channel.

The difference between the two mechanisms has been demonstrated also in Ref. 18. Specially selected lipid membranes could be "frozen" or "melted" by changing the temperature. In the presence of valinomycin and nonactin, the conductivity sharply declined upon freezing the membrane, while the properties of gramicidin A were not altered. A very simple explanation of this phenomenon again consisted in the idea that valinomycin and nonactin, in contrast to gramicidin A, act as mobile carriers whose mobility is sharply diminished upon freezing the membrane. At the same time, the status of the surrounding lipid molecules need not exert an appreciable influence on the properties of a pore.

Thus the induced transport that arises in the presence of ionophors can be effected by the mechanism of mobile carriers[19] and with the aid of special pores. The latter mechanism is also called the relay mechanism, since the pore can be formed of several molecules lying in sequence, between which the ion is transferred. A variety of the mobile-carrier mechanism is possible in which the ion is transported not by one molecule, but by several together—this variant is called collective transport. If we add to this the mechanism of direct passage of large fat-soluble ions such as tetraphenylborate and dipicrylamine, then we get all the fundamental types of mechanisms that have been treated in the literature (Fig. 11).[13] The simplest mechanism is that of direct passage of ions through the membrane. We shall begin there the presentation of the laws of ion transport. However, at first we shall spend a little time on a more detailed molecular description of membranes.

c) The molecular approach

Thus far we have been treating the lipid membrane as a perfectly homogeneous, continuous phase. Evidently this is only an approximation to reality, and it does not fit many important properties of membranes. Therefore attempts have been made at more detailed description of the membrane, and in particular, of the process of transport of particles through it.

First of all, we note that a bilayer lipid membrane exists in the liquid-crystalline state. On the one hand, it is very fluid—its individual components are mobile, while on the other hand, the molecules of the lipids are ordered to a very high degree—they lie in two layers, and their hydrocarbon tails are extended. A greater or lesser degree of order can exist within each layer. In Ref. 20, they studied electron diffraction from bilayers

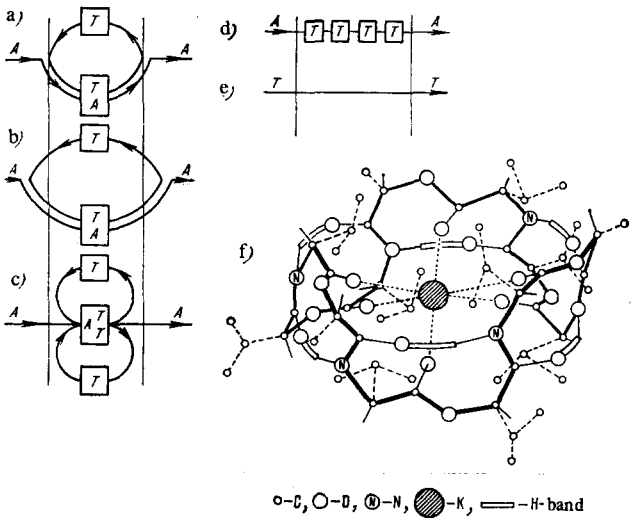


FIG. 11. Mechanism of transport of ions through membranes. a) Mobile carriers with a "small turnstile" (the carrier T is confined within the membrane, while complex formation occurs at the membrane-solution phase boundaries; b) mobile carriers with a "large turnstile" (the carrier T occurs both in the membrane and in solution, and complex formation occurs in solution); c) collective transport (the ion A is transported by several particles of the carrier T); d) relay transport; e) direct passage; f) structure of the molecular of valinomycin.[12]

made of phosphatidylcholine. The diffraction patterns showed that the bilayer consists of a large number of small but well-packed regions whose orientation differs somewhat from the orientation of their neighbors. The size of the crystallites is estimated to be several hundred Ångström units. Such structures should be dynamic in nature, and should change in form as time passes.

The mobility of the lipid molecules that constitute the membrane is important from the standpoint of transport of particles through the membrane. The movement can be parallel to the surface of the membrane or transverse. Even Langmuir[21] had studied the transverse movement of molecules with the example of polylayers of barium stearate. In recent years the development of sensitive methods, and in particular the use of spin labels, has made it possible to study this process in greater detail. It has been shown[22] that barium stearate molecules exchange with one another between the individual layers with a characteristic "half-decay" time of 25 minutes at 25 °C. They also advanced the hypothesis that exchange processes involving lipids in the "fluid regions" of biological membranes must occur at a high rate.

The transfer of lipid molecules from one layer to another has one essential feature: the molecule must not only move to a new site, but must also turn around as well, since its polar head must be directed to the opposite side. Apparently this situation can play a substantial role in transport processes. We shall illustrate it with the example of transport of chlorine through lipid membranes.

It has been noted in a number of studies that the flux of chloride ions as calculated from the electric conductivity of the membrane and as measured with labeled atoms has different values. Here the isotope flux proves to be three orders of magnitude larger.[23] An electric potential applied to the membrane exerts no effect on it. Thus some process acts in the membrane that is not manifested electrically, and which leads to intensive ion transport. Similar phenomena are rather well known in biology, and they are attributed to exchange processes in which ions are exchanged from one reservoir for an equivalent number of the same type of ions in another. Exchange diffusion is most easily effected with carriers that can migrate through the membrane only in the loaded state. However, the studied membranes contained nothing but the lipids themselves. Hence the hypothesis naturally arose that the lipids themselves carry out the exchange function. Chloride ions combine with the polar heads of the lipids, and the complexes that are formed are transported through the membrane. Such a mechanism explains not only the difference between the electrical and isotope fluxes, but also the saturation of the fluxes with increasing concentration. Such a turnover or flip-flop of the lipid molecules runs at varying rates, depending on the composition of the membrane and the experimental conditions. Thus the characteristic flip-flop time for phosphatidylcholine amounts to several hours.

Flip-flop is geometrically a rather complicated process. In order to turn over a large molecule, a large space must be freed in advance. Hence the most probable course is the flip-flop of not one molecule, but simultaneously of two from different layers.[24] Such a simultaneous flip-flop of two molecules frees a site in each of the layers for incorporation of new molecules. The entrainment of two, and possible even more, molecules apparently can explain the rather large activation energy of the process, which is as much as 19.4 kcal/mole.

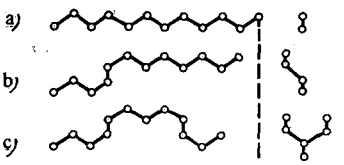


FIG. 12. Conformational rearrangement of a hydrocarbon chain. a) Extended chain, b) chain with a simple kink, c) combination of two $2g_1$ kinks. One should note the effective shortening of the chain. The lateral view of the chains is shown at the right.

Of course, not every type of membrane transport involves flip-flop of lipid molecules. Yet some displacement of membrane components must occur in every case. This problem is closely connected with the diffusion of molecules in polymeric materials. Experimental and theoretical studies of mechanical relaxation in polyethylene, paraffins, and other polymeric materials show that polymeric materials, both in the crystalline and in the liquid-crystalline states, contain definite types of mobile structural defects. These are the so-called kinks,[25,26] which stem from conformational rearrangements of the hydrocarbon chains. When existing in the hydrocarbon part of a membrane, the kinks form small mobile pockets or bubbles of free space of varying dimensions, depending on their type and position. The molecule to be transported from the aqueous phase near the membrane can enter the free space of a kink at the surface of the membrane and then diffuse through the membrane along with the mobile kink.[27]

The origin of the kinks involves the specific nature of the hydrocarbon chain. In an extended chain (Fig. 12), all the bonds adopt the *trans* conformation. However, each C–C bond can rotate by an angle of ±120°. This state is called the *gauche* conformation. If one rotates an extended hydrocarbon chain about any C–C bond by a 120° angle, and then about another C–C bond lying one unit away from the original one by the angle −120°, then one gets the conformation (Fig. 12) that has been called the elementary kink $2g1$.[25] The linear molecule becomes as though doubly bent; it consists of two regions whose axes are mutually displaced. Figure 13 shows a photograph of molecular models of such chains.[27]

In kink formation the chain is effectively shortened by the length of one CH_2 group. Therefore it is improbable that kinks would be formed or disappear in the middle of a chain, since then the rather long ends of the molecule would have to be drawn in. Kinks can be formed (or disappear) far more easily at the surface, whence they can migrate into the interior of the membrane.

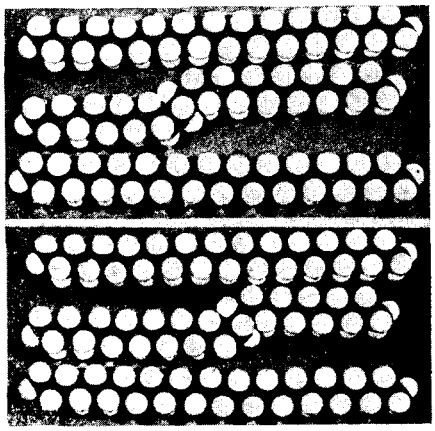


FIG. 13. Molecular model of a hydrocarbon chain having a kink.

The energy difference between the *trans* and *gauche* conformations can be calculated with the example of the butane molecule,[28] and it proved to be 0.8 kcal/mole. Hence formation of a single kink increases the intrinsic energy of an isolated chain by $2\times0.8=1.6$ kcal/mole. The activation energy of the transition from the *trans* to the *gauche* conformation amounts to 2.4 kcal/mole. This gives 4.8 kcal/mole per two bonds. However, kink formation considerably increases the entropy of the system. Hence kinks are rather favored thermodynamically The free energy of formation and migration of kinks is comparable with the thermal energy at room temperature ($kT=0.6$ kcal/mole). Hence we can expect that the hydrocarbon part of a membrane in equilibrium contains rather many kinks, which constantly move along the nolecular chains.

A statistical calculation has been performed[26] of the concentration of kinks. In the general case, the fraction of the CH_2 groups occurring in the *gauche* conformation increases with the temperature. In paraffins and linear polymers, just as in lipids, the concentration of kinks undergoes two sharp jumps with increasing temperature. One rotational phase transition occurs at the temperature T_t, which lies below the melting point of the material, and a second one at the melting point T_m. When $T\leqslant T_t$, the concentration ξ of kinks is no greater than 0.05, while when $T>T_t$, ξ lies in the range from 0.1 to 0.5. For the most membrane lipids, room temperature lies above the transition temperature T_t. Hence the concentration of kinks in a lipid membrane lies in the range 0.1–0.5. If we know this quantity, we can calculate the absolute concentration of kinks, which in the general case is $c_k=8.5\times10^{-2}$ mole/cm^3. A suitable combination of two $2g1$ kinks can generate rather large cavities that contain water and other particles. Most of these cavities have a length of several links of the hydrocarbon chain. Therefore they do not form penetrating pores.

An increase in the concentration of kinks must elevate the specific volume of the lipids. Actually x-ray study has shown that the rotational phase transition in a paraffin is accompanied by a volume increase of 2–4%.[29]

And finally, there is the mobility of the kinks. Estimates show that the diffusion coefficient of the kinks must be about 10^{-5} cm^2/sec.[27] This value is of the same order of magnitude as the diffusion coefficient of particles in water.

The kink model treats the hydrocarbon phase of the membrane as an ordered structure containing defects. The kink mechanism will be inapplicable if the degree of disorder is so large that the hydrocarbon phase is more reminiscent of a liquid than of an ordered structure. Most likely, the proposed mechanism pertains to the passage through a membrane of small neutral molecules having an effective volume equal to, or somewhat exceeding the volume of a CH_2 monomer. It can participate also in passage of relatively large ions through the membrane. However, the permeation of large molecules like the cyclic antibiotics cannot be described by a simple mechanism. The presence of large molecules must strongly distort the hydrocarbon chains. However, such a distortion can be described in terms of formation of combination of kinks in adjacent chains.

d) Direct passage

Now we shall proceed to a phenomenological description of the laws of ion transport through thin membranes. The simplest form of ion transport that has been studied in greatest detail is direct passage of charged particles. There are two approaches to describing it: discrete and continuous. In the discrete approach, which is based on the Eyring theory of absolute reaction rates,[30] one assumes that the particle gets through the membrane by making several discrete jumps through activation barriers. The continuous approach is based on the concept of free diffusion and migration of particles in the membrane, which is treated as a continuous, homogeneous phase. Then the flux of ions of type k is given by the expression

$$J_k=z_k u_k RT\left(\beta z_k c_k E-\frac{dc_k}{dx}\right); \tag{13}$$

here z_k is the charge of the ion of type k (in units of the charge of a proton), u_k is the mobility, which is related to the diffusion coefficient D_k by the Einstein relationship $D_k=u_k/\beta$ ($\beta=e/kT=F/RT$), and E is the electric field intensity, which satisfies the Poisson equation

$$\frac{dE}{dx}=\frac{F}{\varepsilon}\sum_k z_k c_k; \tag{14}$$

Here ε is the dielectric constant of the membrane and c_k is the concentration of the ions of type k. In the steady-state case we have $J_k=$const., i.e., Eq. (13) is a nonlinear first-order differential equation that contains the unknown functions c_k and E and the unknown constant J_k. In the non-steady-state case, we have the continuity equation

$$\frac{\partial J_k}{\partial x}+Fz_k\frac{\partial c_k}{\partial t}=0, \tag{15}$$

while the total current J_0 is now composed of a displacement current and an ion current:

$$J_0 = \varepsilon \frac{\partial E}{\partial t} + \sum_k J_k. \tag{16}$$

In the steady-state case, we can derive from Eq. (13) the important integral relationship

$$J_k = -g_k(\varphi - \varphi_k), \tag{17}$$

Here φ_k is the equilibrium membrane potential for the ions of type k, while the conductivity g_k is given by

$$g_k = Fz_k^2 u_k \Big/ \int_0^\delta \frac{dx'}{c_k(x')}. \tag{18}$$

The conductivity g_k depends on the potential via the concentration profile. That is, the volt-ampere characteristic is nonlinear. Neverthelss, it makes a certain sense to write the equation in the form of (17), where the factor linear with respect to the potential shift has been isolated. First, one can use the equilibrium concentration profile in calculating the conductivity in the limit of a small external field, whereupon the solution is reduced to a single quadrature. Second, when the external field is changing rapidly, the concentration distribution in the membrane cannot readjust. Therefore, for short times, Eq. (17) predicts a linear current-potential relationship.

The electrodiffusion problem is considerably simplified when the space charge in the membrane is small. In order to construct and substantiate the corresponding approximation, we must find the equilibrium potential distribution in the membrane and in the surrounding solutions for the case of a single permeating ion. In the self-consistent-field approximation, the ion concentrations are related to the potential by the Boltzmann relationships. That is, the Poisson equation (14) proves to be closed. Figure 14a, curve 1 shows the result of the solution. The potential jump in the membrane, which is defined as the potential difference between its center and either of the boundaries is

$$\psi(0) - \psi\left(\frac{\delta}{2}\right) = -\frac{\varkappa_m^2 \delta^2}{16}, \tag{19}$$

Here we have $\psi = e\varphi/kT$, δ is the thickness of the membrane, $\varkappa_m^2 = \varkappa^2 \gamma \varepsilon/\varepsilon_m$, $\varkappa = \sqrt{8\pi e c \beta/\varepsilon}$ is the Debye parameter, and c is the bulk concentration of the permeating ion in the solution. Thus the potential jump in the membrane is determined by the relationship between its thickness and the shielding distance, which depends primarily on the number of carriers. With typical values of the parameters, the shielding distance exceeds the membrane thickness by several orders of magnitude. That is, the potential is constant (the dotted straight line (2) in Fig. 14a). This means that when an external field is imposed, the resulting field in the membrane can be considered to be constant (see case (b) in Fig. 14).

The Nernst-Planck equation is considerably simplified when $E = \text{const}$. In this approximation, the partial volt-ampere characteristic takes on the form

$$J_k = \frac{z_k^2 RT u_k \psi}{\delta} \frac{c_k(0) - c_k(\delta) e^{-z_k \psi}}{1 - e^{-z_k \psi}}, \tag{20}$$

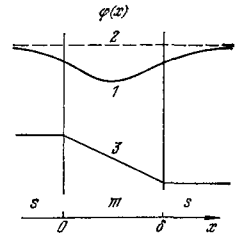


FIG. 14. Potential distribution in the solvent (s)—membrane (m) system. 1—general case, 2—equipotential distribution, 3—constant-field approximation.

Here $c_k(0)$ and $c_k(\delta)$ are the concentrations in the membrane phase, which are related to the corresponding concentrations c_k^0 and c_k^i in the solutions by the distribution coefficients γ_k, e.g.,

$$c_k(0) = \gamma_k c_k^i.$$

This is precisely the formalism, which is based on the concept of diffusion and migration of ions in a continuous, homogeneous phase, that has been used to describe the state of rest of biological membranes. For example, one can easily calculate from Eq. (20) the membrane potential, which is one of the fundamental characteristics of the nerve cell. As we have mentioned, the membrane of smooth fibers is permeable to sodium, potassium, and chloride ions. Hence the state of rest is a steady state, rather than a thermodynamic equilibrium. For an open circuit, the steady-state condition is reduced to compensating the partial ion currents: $\sum_k J_k = 0$. Actually this condition is the equation for determining the membrane potential, whose solution has the form

$$\psi = \ln \frac{P_{K^+} c_{K^+}^0 + P_{Na^+} c_{Na^+}^0 + P_{Cl^-} c_{Cl^-}^i}{P_{K^+} c_{K^+}^i + P_{Na^+} c_{Na^+}^i + P_{Cl^-} c_{Cl^-}^0}, \tag{21}$$

where we have introduced the so-called permeabilities $P_i = u_i \gamma_i / \beta\delta$.

The relationship (21) describes satisfactorily the experimental data, provided only that the interval of concentrations is not too broad. This can hardly be considered as a serious argument in favor of the applicability of the electrodiffusion theory for describing the electrical characteristics of nerve cells in a state of rest, since the unknown parameters P_i are determined from the same set of experiments. Moreover, the entire set of existing facts indicates that the treatment of the membrane as a continuous homogeneous phase that is adopted in the electrodiffusion theory is not adequate. Therefore, actually the grounds for using the relationships (20) and (21) is their simplicity and our lack of reliable information on the true mechanism of ion transport in a state of rest. Considerably more is known on the phenomenon of ion transport through membranes in the process of excitation, when the conductivity of the system is sharply elevated. It has been firmly established that the ion transport is effected in this case through specialized lipoprotein structures that are called the ion channels. We shall proceed to describe the transport through the channels, which requires a special formalism.

TABLE II. Relative permeabilities of the sodium channels of the nodes of Ranvier for univalent cations.

Ion	P_{ion}/P_{Na}	Ion	P_{ion}/P_{Na}
Sodium	1.00	Formamidine	0.14
Hydroxylamine	0.94	Guanidine	0.13
Lithium	0.93	Hydroxyguanidine	0.12
Hydrazine	0.59	Potassium	0.09
Thallium	0.33	Aminoguanidine	0.06
Ammonium	0.16		

4. ION CHANNELS

a) Facts and hypotheses

A multitude of arguments have now been amassed in favor of the idea that the conducting structures of biomembranes act as channels, rather than mobile carriers.[31] We recall in this regard the experiment of Chandler and Meves,[32] who studied the high-frequency conductivity of membranes under conditions in which the permeating ions sodium and potassium were absent in the solution. If the membrane contained charged mobile groups, then in an ac field one would record an electric current that was equal in order of magnitude to the ion current under ordinary conditions. However, the high-frequency conductivity proved to be zero within the limits of accuracy of experiment. Hence they concluded that mobile carriers were absent, and they gave an upper estimate of the number of channels. Subsequent experiments on TTX and TEA binding, together with measurements of electrical fluctuations (see Sec. c of Chap. 5) confirmed these data: a number of the order of hundreds of sodium channels occurs per 1 μm^2. The conductivity of a single sodium channel is estimated to be 4×10^{-12} ohm^{-1}, and that of a potassium channel is 12×10^{-12} ohm^{-1}.[33] As yet the ion channel remains to a considerable degree an undefined concept in the structural sense. It has not yet been possible to distinguish and characterize the membrane proteins of excitable biological membranes that are responsible for controllable ion transport. Hence highly varied concepts are widespread in the literature on the nature of the ion channels. Some authors rely on the analogy with the artificial channels in BLMs, where the electric field affects the statistical process of assembly of a channel from subunits. They consider that biological membranes contain no preexisting ion channels, while the observed process of elevated conductivity upon depolarization involves their assembly. Another viewpoint is more widespread, which treats a channel as a rather rigid macromolecular system that is capable of small conformational rearrangements. An entire series of arguments that we shall not undertake to analyze favors the latter viewpoint.[5)] The concept of an ion channel as being a lipoprotein complex that is characterized by a set of conformational states allows us to treat it as a "vector" enzyme that catalyzes an ion-transport reaction. A feature of this transport enzyme is that its activity is controlled by the electric field. The analogy of membrane transport systems with enzymes extends also into the field of specificity. As studies in the field of membrane-active complexons have shown,[12] the mechanism of interaction of an ion with a complexon initiates a conformational rearrangement of the entire complex, and it is a good model for the induced fit between a substrate and the active center of an enzyme.

An ion channel performs two fundamental functions: it makes the membrane selective and field-controllable. The natural question arises of whether a correspondence can be established between these functions and any specific molecular groups. In a more modest (and more realistic) formulation of the problem, this comes out as: can one affirm that the different functions are performed by different groups of the channel? It does not yet seem possible to give a categorical answer, though it is deemed most likely that different regions of the channel perform the transport-selective and regulator functions. We shall begin to analyze the different functions of the channel with studying the transport system. In other words, we shall treat ion transport through open channels.

The ion channels are highly selective. Nevertheless, if one replaces the sodium ions with other cations in the outer solution and blocks the potassium channel with tetraethylammonium, one can realize an aritifical solution in which other cations are transported through the sodium channel. As a result one can find the set of permeabilities for the series of cations. Table II,[4] which is based on the data of Hille,[34] gives an example of such a series.

Analogous experiments have recently been performed also for the potassium channel (Table III). By using a set of cations with different crystallographic radii, estimates could be made of the geometric characteristics of the sodium and potassium channels.

Figure 15 shows the model of the sodium channel proposed by Hille.[34] It consists of entrance regions, where the ion is gradually dehydrated and the water molecules are replaced by the polar groups that line the interior of the channel. The main barrier lies in the selective center of the channel, which is estimated to have a cross section of 3×5 Å. The dimensions of the cross-section are chosen from considerations of geometric exclusion of the nonpermeating cations. The longitudinal dimension (of the order of several Å) is very crudely estimated by starting with the maximum admissible ohmic resistance of the channel as a conductor having the specific characteristics of the surrounding solutions. The sodium channel has a very high conductivity. In the open state, the current through it amounts to $\sim10^7$ ions/sec. This compels us to narrow the main barrier region as much as possible in order to get high permeability along with high selectivity.

The high permeability of the sodium channel poses the problem of whether the limiting process is the diffusion of the ions in the space adjoining the membrane. Estimates show that the limiting diffusional flux is about

[5)] Perhaps the most convincing argument in favor of preexisting channels is the fact of binding of TTX to a closed sodium channel.

TABLE III. Relative permeabilities of the potassium channels of the nodes of Ranvier for univalent cations[35]

Ion	P_{ion}/P_K	Crystallographic radius of the ion, Å	Ion	P_{ion}/P_K	Crystallographic radius of the ion, Å
Potassium	1.000	2.66	Hydroxylamine	0.025	3.30
Thallium	0.300	2.80	Methylamine	0.021	3.60
Rubidium	0.910	2.96	Formamidine	0.020	3.60
Ammonium	0.130	3.00	Lithium	0.018	1.20
Cesium	0.077	3.38	Sodium	0.010	1.90
Hydrazine	0.029	3.33			

an order of magnitude higher than the flux through the channel. That is, the intramembrane transport is limiting. This is all the more true for the potassium channels, whose transport capacity is $\sim 10^6$ ions/sec.

Upon turning to the selectivity problem, we note that the existing theory is a thermodynamic one, and it is based on calculating the equilibrium distribution coefficient of the ions between the aqueous solution and a certain state in the membrane. If we calculate the energy difference of solvation simply by Born's formula (7), this yields a selectivity series that increases monotonically with the radius of the ion, whereas real channels are characterized by a more complicated variation. Hence Eisenman[36] has introduced into the treatment also the interaction of the ion with a fixed charge localized in the membrane. It is physically obvious that ions of small radius now take an advantageous position, since their coulombic interaction with the fixed charge of opposite sign will increase their affinity for the channel. The selectivity series that one observes in the sodium channel compels us to assume that an anionic group lies in the vicinity of the selective center.

Figure 16 shows a hypothetical model of the potassium channel. The discrimination against ions of larger size is explained by steric factors. However, one asks why the permeability of the potassium channel to sodium is so low when the latter has a smaller crystallographic radius. If the cavity of the channel is rigid, the state of the sodium ion shown in Fig. 16a is energetically unfavorable with respect to its situation in aqueous solution (Fig. 16b), where it is fully hydrated. The case is more favorable for potassium, since the polar groups of the channel form a compact solvation shell around it. The purely thermodynamic approach to the selectivity problem does not seem satisfactory. The process of ion transport is a kinetic phenomenon. That is, as we shall show in the treatment below, selectivity also depends on kinetic parameters.

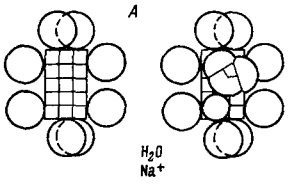


FIG. 15. Model of the sodium channel. The oxygen atoms that line the channel are shown, together with a partially hydrated sodium ion.[34]

b) Single-file transport

The ion channels allow only single-file movement of ions. Consequently, the ordinary electrodiffusion description of (13), which is based on the notion of diffusion of noninteracting point particles, loses force. This is also indicated by analysis of the experimental data on one-way ion currents. We can easily derive from (20) an expression for the one-way ion currents by assuming that $c_k(\delta)$ and $c_k(0)$ are alternately equal to zero:

$$\vec{j}_k = \frac{z_k u_k \psi c_k(0)}{\beta\delta(1-e_{\cdot}^{-z_k\psi})}, \quad \overleftarrow{j}_k = \frac{z_k u_k \psi e^{-z_k\psi} c_k(\delta)}{\beta\delta(1-e^{-z_k\psi})}. \tag{22}$$

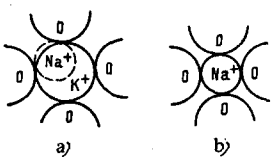
Equation (22) implies that the one-way currents are independent in the constant-field approximation, and their ratio is determined by the Ussing formula:

$$\frac{\vec{j}_k}{\overleftarrow{j}_k} = \frac{c_k(0)}{c_k(\delta)} e^{z_k\psi} = e^{z_k(\psi-\psi_k)}. \tag{23}$$

The experimental results indicate that the entering and exiting currents are independent in the sodium channel when sodium ions are moving through the channel.[37] Yet if the sodium in the outer solution is replaced by other cations, the sodium channel ceases to obey the independence principle. The transport of potassium ions through the potassium channels does not obey the independence principle.[38] Thus, for example, when the external potassium concentration is elevated by a factor of 10, the entering current is increased by a factor of 30, while the exiting current diminishes by a factor of 3–4. The ratio of the entering and the exiting potassium currents does not obey the Ussing formula (23), and it is described by the empirical relationship

$$\frac{\vec{j}}{\overleftarrow{j}} = e^{n(\psi-\psi_k)}, \tag{24}$$

where $n \approx 2.5$.



a) b)

FIG. 16. Model of the potassium channel. a) Arrangement of potassium and sodium ions in the selective center of the potassium channel. b) The sodium ion in aqueous solution lies in closer contact with oxygen atoms.[4]

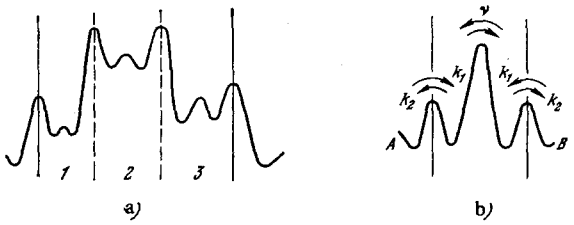


FIG. 17. Schematic drawing of the energy profile of an ion in a channel. a) General case, b) three-barrier model.

According to the model of the ion channel shown in Fig. 15, the profile of the potential energy of an ion in the membrane can be pictured in the form of the curve shown in Fig. 17a. Regions 1 and 3 correspond to the entrance regions of the channel, and region 2 to the selective center. Until the number of potential wells, their depth, and the height of the barriers are concretized, this picture remains rather general. If the complete set of barriers includes several major, highest ones, then we can conveniently treat the transport in a discrete formalism. In the converse case in which the number of barriers is large, while they are similar in height, one can use a continuous description. The variant in which the potential profile contains only three high barriers proves to be sufficiently interesting (Fig. 17b). Let a solution containing the ions A lie to the left of the membrane, and a solution containing the ions B to the right. Figure 17 shows the rate constants of all the transitions through the barriers. The states of the channel are characterized by the binary functions $F(X_1, X_2)$, $(X=A, B, O)$ in terms of which all the current are expressed. The functions $F(X_1, X_2)$ satisfy a system of differential equations that can be conveniently solved by the method of directional partial diagrams.[39] Figure 18 shows the base diagram for the three-barrier problem. The points denote the different states of the channel, and the lines denote the transitions. Arrows are not drawn on links along which a transition can occur in both directions. Each line is matched with a certain analytical expression. For example, the line $OO \to AO$ corresponds to $k_1^A A\, e^{\beta\varphi_1/2}$. Here A is the concentration of ions in solution, and φ_1 is the potential jump at the first barrier. The probability of any state i of the system is

$$F_{(i)} = \frac{\sum \text{partial diagrams directed toward state } i}{\sum_j \sum \text{partial diagrams directed toward state } j}. \tag{25}$$

The partial diagrams are obtained from the base diagram by breaking the minimum number of links in such a way that the obtained diagram contains no cycles. The directional partial diagrams are obtained from the partial diagrams by orienting all the lines toward the sought node. The one-way ion currents can be calculated especially simply in the case of a high middle barrier:

$$\begin{aligned} &j_A = nG\gamma_A A v_A e^{\psi_2/2}, \qquad j_B = nG\gamma_B B v_B e^{-\psi_2/2}; \\ &G^{-1} = (1+\gamma_A A)(1+\gamma_B B), \quad \gamma_A = \frac{k_1^A}{k_2^A} e^{\psi_1}, \quad \gamma_B = \frac{k_1^B}{k_2^B} e^{-\psi_2}. \end{aligned} \tag{26}$$

The combination of coefficients $\gamma_A A$ determines the probability of filling the entrance well when it is vacant; the coefficient G characterizes the probability that the channel is empty, while the product $\nu e^{\psi_2/2}$ is the rate constant of the transition through the central barrier.

TABLE IV. The coefficients ν_i and γ_i^0 for a for a series of cations.

Ion	γ_i^0, M^{-1}	ν_i/ν_{Na}
Sodium	2.6	1
Thallium	21.7	0.042
Hydroxyguanidine	18.0	0.037
Aminoguanidine	9.3	0.038
Methylguanidine	16.3	0.039

The ratio of the one-way currents of (26) is described by the Ussing formula

$$\frac{j_A}{j_B} = e^{\psi - \psi_0}, \quad \psi_0 = \ln \frac{\nu_B \gamma_B^0 B}{\nu_A \gamma_A^0 A}, \tag{27}$$

where $\gamma_i^0 = k_1^i / k_2^i$. As Eq. (26) implies, the one-way currents depend nonlinearly on the concentrations on the two sides of the membrane. That is, the independence principle does not hold. We have $G \approx 1$ only in the case in which $\gamma_A A \ll 1$ and $\gamma_B B \ll 1$, i.e., the concentration in the solution or the affinity of the given ions for the entrance regions of the channels is small. Then the one-way currents prove to be independent, and as in the electrodiffusion theory, they are determined only by the product of parameters $\nu_i \gamma_i$. Autoblockage effects are manifested as the concentration or the affinity is increased. Equation (26) implies that the permeability in the general case becomes a function of the concentration. This also implies that the selectivity as expressed as the ratio of one-way currents is determined not only by the thermodynamic quantity, the affinity, but also by the kinetic characteristic ν_i.

Actually the presented formulas also describe a more general variant of the energy profile in which the entrance regions correspond to a series of shallow potential wells that are in equilibrium with the surrounding solutions. Analysis of the experimental data on transport of different cations through the sodium channel allows us to conclude that this type of energy profile having a single high barrier reflects certain qualitative features that are characteristic of the sodium channel. Without taking up the details of the processing of the experimental material, we present in Table IV the found coefficients ν_i and γ_i for a set of cations. It implies that the substituted ions bind better to the entrance region of the channel than sodium does, but their rate constants ν for passage through the selective center are much smaller than the corresponding constant for the sodium ion. When the sodium ions are replaced by other cations in equivalent concentration, the terms $\gamma_B B$ in the blockage

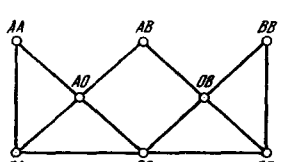


FIG. 18. Base diagram of the three-barrier model.

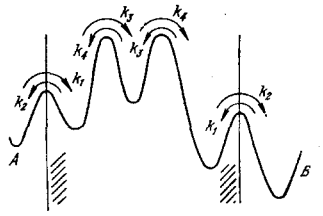


FIG. 19. Energy profile of an ion in a four-barrier channel.

coefficient G increases, since the distribution coefficient γ is minimal for sodium. Hence, as we go to other cations, we can expect deviations from the independence principle, as is observed experimentally. We should add to this the fact that calculation of the volt-ampere characteristic of one-way transport allows one to get a linear current-potential relationship over a rather broad region, in line with the experimental data.

Let us take up briefly the allowance for Coulomb effects in one-way transport. The potential energy of an ion in the channel is composed of its interaction with the transport system itself and with the other permeating particles that lie in the channel. Consequently the overall energy profile depends on how the channel has been filled. In calculating the currents, we must now renormalize certain of the rate constants with account for the Coulomb ion-ion interaction, whereas the scheme of calculation remains as before. As a result, we get the following expression for the one-way currents:

$$j_A = n\tilde{G}\gamma_A A v_A e^{\psi_2/2}, \quad j_B = n\tilde{G}\gamma_B B v_B e^{-\psi_2/2}, \quad \tilde{G}^{-1} = 1 + \gamma_A A + \gamma_B \qquad (28)$$

The Ussing formula remains in force. The difference between Eqs. (26) and (28) amounts to the term $\gamma_A A \gamma_B B$ in (26), which is lacking in (28). This involves the disappearance of the state (AB) owing to Coulomb repulsion. Correspondingly, the one-way currents are elevated, and the blockage effects are diminished.

c) The sodium channel with a modified energy profile

An energy profile having a high central barrier does not contradict the measured electrical characteristics of the sodium channel. However, it conflicts with data that indicate a fixed anionic group in the region of the selective center. The channel becomes sharply less permeable for cations when it becomes protonated. Hence we can naturally suppose that the selective region in the protonated state is equivalent to a high barrier that becomes lowered and split for cations when it becomes deprotonated. Thus transport through the channel is now a four-barrier process (Fig. 19), and the state of the channel should be described by the three-dimensional vector (X_1, X_2, X_3), with $X_i = A$, B, or O. Now the base diagram has the form of Fig. 20a. Here the solid lines denote transitions between the solutions and the entrance regions, while the dotted lines denote transitions between the entrance regions and the selective center. The calculation of the probability of any state is considerably simplified if one uses perturbation theory, while assuming the transitions k_3 and k_4 to be slow (dotted lines) in comparison with the transitions k_1 and k_2 (solid lines). However, the physical pattern of the transport process proves to be more perspicuous if we use the method of current diagrams. Upon considering all possible current cycles, we can get information on the different "mechanisms" of transport of an ion through the membrane. We should view a transport "mechanism" as a sequence of states through which the channel passes in this process. For illustration, Figs. 20b, c show two examples of current cycles. The first one corresponds to one-particle transport (b), and the second one to two-particle transport (c). Coulomb effects cause two-particle transport to become the fundamental transport process in a certain range of the parameters of the original energy profile. We can explain this physically as follows. The fixed anionic center facilitates entrance of cations into the channel by attracting them. However, it simultaneously hinders their exit from the channel. Evidently, exit from the central well would be facilitated if a second cation entered the left-hand well (Fig. 21), since repulsion by the ion 2 now weakens the interaction of the ion 1 with the fixed charge. In other words, the well has lost depth, and the possibility has arisen that the ion 1 will transfer to the right-hand well. Correspondingly, the ion 2 can fill the central well, as though "dislodging" ion 1. Quantitative analysis of this problem as performed by the method of current diagrams pre-

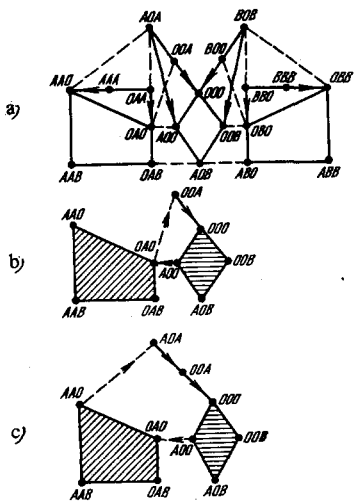


FIG. 20. Base diagram of the four-barrier model (a) and the current cycles that correspond to one-particle (b) and two-particle (c) transport.

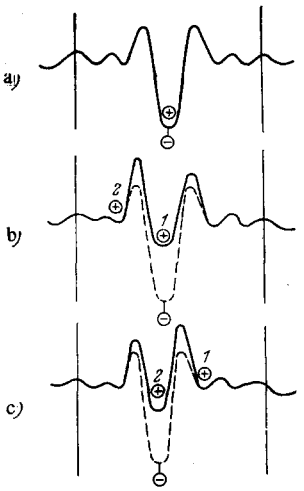


FIG. 21. Two-particle transport of ions through a channel. The deep potential well (a) corresponds to the one-particle state in the channel. Coulombic repulsion in the two-particle state makes the well not so deep (d) and facilitates transport of ion 1 into the next well (c).

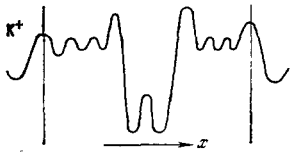


FIG. 22. Energy profile of an ion in the potassium channel.

sented above shows that such a "billiard" mechanism does not violate the validity of the Ussing formula, and it leads to qualitatively the same results as single-file transport in a channel having a single high barrier.

d) The potassium channel

In all the cases mentioned above, in spite of deviations from the independence principle, the Ussing formula has continued to hold. However, this formula is no longer valid for the potassium channel. Therefore we must expand the class of potential-energy profiles of an ion in the channel in order to find conditions under which deviations will arise.

Properly speaking, the mechanical model of Huxley, and also the studies of Heckmann[41] have shown the way to seek the solution of this problem. The required modification of the energy profile must consist in introducing into the selective center not one, but at least two strongly correlated deep potential wells (Fig. 22). The expression for the ratio of one-way currents in this case has the form

$$\frac{j_A}{j_B} = e^{2(\psi - \psi_0)}. \tag{29}$$

The second power in the exponential seems to indicate a doubling of the order of the ion transport reaction through the channel. This happens because the channel exists in a filled two-particle state. Therefore exit of a particle into the solution is accompanied by a successive shift of ions throughout the channel. As we see from Fig. 23, Eq. (29) satisfactorily agrees with experiment. One can also explain quantitatively the observed deviations of the one-way currents from the independence principle. This indicates that the energy profile in Fig. 22 correctly reflects the fundamental qualitative features of the energy profile in the potassium channel.

e) A continuous description

If we neglect correlations, then we can write the transport between the wells m and $m+1$ in an inhomogeneous chain in the form

$$j_{m,\,m+1} = n\nu_{m,\,m+1}\theta_m(1-\theta_{m+1})\,e^{\psi_m\delta_{m+1}/2} - n\nu_{m+1,\,m}(1-\theta_m)\,\theta_{m+1}e^{-\psi_m\delta_{m+1}/2}, \tag{30}$$

Here θ_m is the filling number of the well m. Since $\psi_{m,m+1}$ is small when there are a large number of wells, then if we assume that θ_m varies smoothly, we can transform to a continuous description:

$$j(x) = -n\nu(x)\,\delta\frac{d\theta}{dx} - n\,\delta\nu(x)\,\theta(1-\theta)\frac{d(\psi+\mu_0)}{dx}, \tag{31}$$

Here δ is the distance between adjacent barriers, and $\mu_0(x)$ is the dimensionless standard chemical potential of the ion. Equation (31) is a simple generalization of the Nernst-Planck equation that takes account of the limited number of sites that are vacant for diffusion, as well as the inhomogeneity of the membrane.

5. DEPENDENCE OF THE CONDUCTIVITY OF THE CHANNEL ON THE ELECTRIC FIELD

a) Interpretation of the Hodgkin-Huxley equations

The Hodgkin-Huxley equations describe the dynamics of certain macroscopic quantities, the conductivities of regions of the membrane that contain a large number of individual ion channels. Therefore a given relationship can be derived as a result of averaging the conductivity of channels that are undergoing saltatory changes, or else simply as the continuously varying conductivity of a single channel multiplied by the density of channels. Let us examine the first possibility with the example of the potassium conductivity. As Eq. (5) implies, this quantity is proportional to n^4, where n varies from 0 to 1. We can naturally treat n as the probability that some particle belonging to a channel should lie in a certain position that facilitates the onset of the conductive state. The exponent 4 then will mean that there are four of such particles and that they are all independent, whereby the channel exists in the conductive state only whenever all four particles simultaneously lie in the "active" position. As the form of the kinetic equation for n implies, only one state exists for the n-particle besides the active state, and its probability is $1-n$. Thus a very simple interpretation of the Hodgkin-Huxley model gives the following picture for the potassium channel. For the sake of definiteness, let all of the n-particles bear a certain positive charge. Then in a state of rest, all four n-particles lie on the inner side of the membrane, and the channel is closed. If we increase the membrane potential, then the energy of the n-particle on the inside is increased, and that on the outside is reduced. Therefore the probability of finding a particle on the outside of the membrane increases, and the conductivity of the membrane increases accordingly. Finally, at large potentials the n-particles spend almost all the time on the outside of the membrane, and the conductivity reaches its highest possible value. One can interpret analogously also the conductivity for the sodium channel, with the distinction that here one must introduce two types of particles, m and h, whereby one channel contains three m-particles and one h-particle that do not interact with one another. The kinetic equation for the m-particles is linear. This corresponds to the notion of a single-barrier transition, or to the existence of only two states

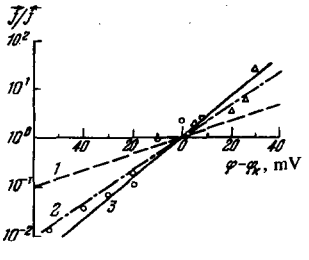


FIG. 23. Relationship of the ratio of one-way currents to the potential jump at the membrane. 1—Ussing formula, 2—Eq. (29), 3—experiment.[38]

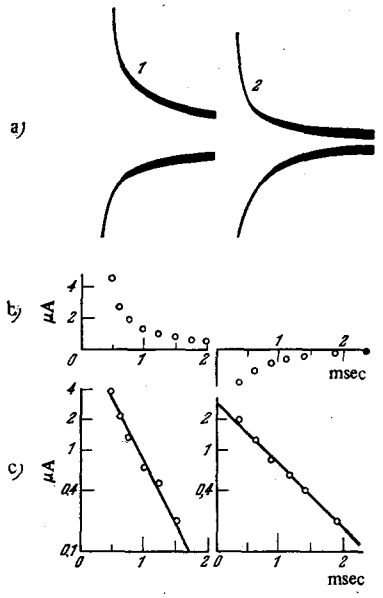


FIG. 24. Isolation of the asymmetric component of the displacement currents (gating currents) when a stepwise voltage of ±120 mV is clamped at the membrane. The axon is perfused with a solution of 55 mM CsF and placed in a K- and Na-free saline solution that contains 110 mM Ca and 300 pM tetrodotoxin. A voltage of -70 mV was preliminarily clamped across the axon. a) Record of the displacement currents; b) their difference, which determines the asymmetric component of the currents; c) logarithm of this component after correction for the leakage currents. The curves 1 correspond to turning on the step potential, and the curves 2 to turning it off.[43]

of the m-particle. The equilibrium constant between these states depends on the potential as follows:

$$\frac{\beta_m}{\alpha_m} \approx e^{-35\psi/9}.$$

One usually assumes that the potential difference between the initial and the final states of the m-particle coincides with the membrane potential φ. Then the charge of the m-particle as determined near the rest potential proves to be ≈4. However, it is hard to become reconciled with the idea of several particles jumping in the activation process over a distance of the order of 100 Å, if one starts with the idea that the channel amounts to a rather rigid macromolecular system. One can assume that the m-particles undergo small displacements, but then one has to assume their charge to be reasonably large. One can obviate this difficulty only by rejecting the independence of the subunits of the channel. Undoubtedly, this idea is related to the earlier attempts at accounting for the cooperative nature of the interaction between the channels.[42]

b) Gating currents

The structural rearrangement of a channel that occurs when the external electric field varies must be manifested in an additional component of the displacement current that has been given the graphic name of the "gating current." It has been possible to measure it only in recent years.[31,43] The problem consisted in isolating the extremely small useful signal. Hence people have usually made the measurements on nerve fibers in which the inner medium was replaced with a potassium-free solution, and sodium ions were removed from the outer solution. Moreover, they have added specific poisons, TEA and TTX, which suppress the conductivity of the potassium and the sodium channels. Yet even under these conditions, the fluctuations were rather large in comparison with the useful signal, so that often one had to resort to a storage device that automatically averaged the records. A typical experiment consisted in the following. One applied to a fiber that had been preliminarily hyperpolarized to −90 to −100 mV a positive square-wave voltage impulse. Here one recorded the transition currents at the beginning and at the end of the impulse that were directed toward the outside and the inside of the fiber, as is shown in Fig. 24a. In order to allow for the capacity-charging current, measurements were performed for a negative voltage impulse (Fig. 24b), whereupon the results of the first and second experiments were algebraically added. As a result they isolated the asymmetric component of the displacement current, which had the same sign as the current in response to a positive voltage impulse, although the amplitude was considerably smaller (Fig. 24c).

A characteristic feature of the obtained asymmetric currents is their exponential time-dependence. Analysis of the characteristic relaxation time of the asymmetric current as a function of the potential during a positive voltage impulse showed that this time very closely matches the relaxation time of the variable m in the Hodgkin-Huxley model. This coincidence allowed them to assume that the observed asymmetric current involves particles that open and close the sodium channel. Subsequently additional facts have been found that favor the direct relationship of the asymmetric displacement currents and the gate particles.

By integrating the gating current over the time, one can get the size of the equivalent charge that is transported from one side of the membrane to the other. Figure 25 shows the relationship of the displaced charge to the membrane potential. The solid curve was obtained semiempirically[43] under the assumption that the gate particles have one stable state near each side of the membrane, and that they have a charge of $1.3e$. This curve is described by the formula

$$\frac{Q(\psi)}{Q_{\max}} = \frac{1}{1+e^{-z\psi}} \qquad (z=1.3), \tag{32}$$

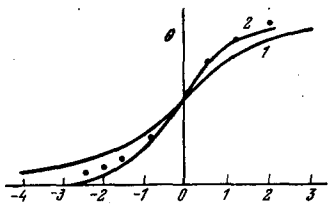


FIG. 25 Fraction of transported charge as a function of the potential. Curve 1 is constructed by Eq. (33) with a linear approximation of $w(\theta)$. Curve 2 is by Eq. (32), and the dots are from experiment.[43]

where Q_{max} is the maximum displaced charge, which is approximately 2400 $e/\mu m^2$.

One can consider the gating currents to result from jumping of the m-particles during the activation of the membrane. From the physical standpoint, as we have noted, the notion of jumping of several charged-particles through the whole thickness of the membrane seems ill convincing. Hence we must examine other possibilities of interpretation of the gating currents. We shall take up one of them that ascribes the gating current to a change in the total dipole moment of the system.[44] If the lipoprotein complex is rigid enough, then the reorientation of the elementary dipoles corresponding to individual bonds will be cooperative. This is very essential in explaining the steep dependence of the displaced charge on the membrane potential that is observed experimentally (see Fig. 25). If we assume that every elementary dipole has only two possible orientations, then we can describe the fraction θ of dipoles that have changed orientation by the relationship

$$\frac{\theta}{1-\theta} e^{-w\theta} = ke^{\varepsilon\psi}, \tag{33}$$

Here w is a cooperativity parameter, $\varepsilon = d/e\delta$, d is the change in the projection of the dipole moment, δ is the thickness of the membrane, e is the unit charge, and k is a constant. Figure 25 shows that $\theta(\psi)$ relationship calculated for $w = 3.5$ and $\varepsilon = 0.2$. The deviation of the calculated curve from experiment at small and large θ can be removed if we assume w to be a function of θ that is defined by the condition that (32) and (33) should coincide. This means that the ion channel in terms of the dipole moment is a system with variable cooperativity in which the latter increases in the regions of small and large θ. For example, when $\theta = 0.1$ (or 0.9), $w = 4.6$. The gating current, which is proportional to $\dot{\theta}$, and the voltage-dependence of its relaxation time agree qualitatively with experiment.

The change in the ion permeability during the reorientation of the dipoles can arise from various factors. If the reorientation is associated with appreciable conformational changes in the system, then the "opening" or "closing" of the channel can have its literal meaning. That is, it amounts to a rearrangement of the molecular geometry of the channel. Another case can also occur in which the dipole reorientation doesn't change the geometry of the channel, but affects the electrostatic component of the energy of the ions in the channel. Thus it affects both their concentration in the membrane and their effective mobility. Simple estimates show that the electrostatic mechanism of regulation can in principle give rise to the observed potential-dependence of the conductivity.

We can make the final choice between these two possibilities after we have found out whether the conductivity of an individual channel varies discretely or continuously. It is physically evident that the "all-or-none" rule will favor the conformational hypothesis, while a continuous variation of the conductivity should indicate rather the functioning of an electrostatic regulation principle. The Hodgkin-Huxley model postulated that an ion channel, e.g., the potassium channel, can exist in one of five conformational states, only one of them being conductive. Of course, this picture of the channel is not the only possible one, if we start with the condition of matching the data of the voltage-clamping experiments. For example, one can assume that all the conformational states are conductive, while one seeks the conductivity distribution function from the condition of best match with experiment. Another possibility consists in introducing a single conformational state whose conductivity is described by the following nonlinear equation:

$$\frac{1}{4}\dot{g} = \alpha(\varphi)(g_0^{1/4} g^{3/4} - g) - \beta g. \tag{34}$$

One can distinguish these schemes by studying the spectrum of the electrical fluctuations.

c) Electrical fluctuations

The spectral characteristics of membranes can be arranged as follows in order of increasing intensity: thermal noise < Lorentzian or shot noise, which involves "opening" or "closing" of gates—$(1/f)$-type noise (flicker noise). A $(1/f)$-type spectrum has been observed in the most varied systems[45]: at microelectrodes, at small-diameter apertures that separate two electrolyte solutions, at porous membranes, and also at artificial phospholipid and biological membranes. In all cases, a necessary condition for a $(1/f)$ relationship was either the transmission of an electric current through the system or the existence of a concentration difference of a permeating ion on two sides of a membrane. There is as yet no exact theory that explains the spectral density of $(1/f)$-type fluctuations. We should note the two most widespread ones among the various attempts at theoretical explanation of this type of relationship. One of these attempts amounts to adducing a diffusion equation to describe the autocorrelation function. This equation gives an asymptotic relationship that can be described by the inverse square root of the time, and a corresponding Fourier transformation gives a $(1/f)$-type spectrum. An essential point is that one gets a $(1/f)$ relationship only in a certain frequency range. The second way of explaining the $(1/f)$ relationship uses the idea of inhomogeneity. In other words, one assumes the existence of many independent noise sources, each of which has a Lorentzian spectrum. By selecting a certain density distribution of these sources with respect to frequency, one can bring the overall spectrum close to a $(1/f)$ relationship, naturally, over a certain frequency range. Thus, although the nature of $(1/f)$-type noise has apparently not been reliably established, we can assume that this relationship itself is approximate and valid only over a restricted interval.

For a long time, the shot noise directly involved in the action of the gates in biological membranes could not be measured against the background of the $(1/f)$-type noise. Only in 1973 did Fishman[46] get difference spectra of the potential fluctuations of a native membrane and of a membrane having potassium channels blocked with TEA. Evidently, the difference spectrum

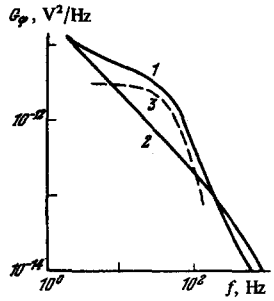


FIG. 26. Spectra of fluctuations of the membrane potential in a state of rest.[46] 1—normal curve, 2—spectrum after adding TEA, 3—difference between curves 1 and 2, which corresponds to the potassium channels.

corresponds to fluctuations of the electrical characteristics of the potassium channels, and it is exactly of Lorentzian type (see Fig. 26):

$$G_1(f) = \frac{f(\varphi)}{f^2 + f_0^2(\varphi)}. \tag{35}$$

We know from the thermodynamic theory of fluctuations that one gets a Lorentzian spectrum with one characteristic frequency for two-level systems, or in other words, for systems that can be described by one thermodynamic variable. Yet if the ion channel possesses a set of conformational states as in the Hodgkin-Huxley model, where they are assumed to be five in number, then several Lorentzian terms arise in the spectrum at multiple frequencies.[47] In fact, if the channel consists of four subunits, each of which exists in one of two states, then we can draw a kinetic diagram of this channel as follows:

$$\boxed{0} \underset{\beta}{\overset{4\alpha}{\rightleftarrows}} \boxed{1} \underset{2\beta}{\overset{3\alpha}{\rightleftarrows}} \boxed{2} \underset{3\beta}{\overset{2\alpha}{\rightleftarrows}} \boxed{3} \underset{4\beta}{\overset{\alpha}{\rightleftarrows}} \boxed{4}. \tag{36}$$

The numbers in the squares correspond to the number of active subunits, and the arrows and the expression attached to them show the possible transitions and the corresponding rate constants; the last square corresponds to the conductive state. We can easily calculate the spectrum of fluctuations in this model:

$$G_g(\omega) = 2\,\mathrm{Re}\int_0^\infty e^{i\omega t} G_g(t)\,dt, \tag{37}$$

Here $G_g(t)$ is the correlation function of the conductivity:

$$G_g(t) = \langle g(0)\, g(t)\rangle - \langle g(0)\rangle^2. \tag{38}$$

Let the potassium channel have the conductivity g_0 in the conductive state, and let $P(1,0)$ be the probability of the conductive state at the initial instant of time, while $P(1,0;1,t)$ is the probability that the channel will be open at the instants of time 0 and t. Then

$$\langle g(0)\, g(t)\rangle = g_0^2 P(1,0;1,t) = g_0^2 P(1,0)\, P(1,t/1,0), \tag{39}$$

where $P(1,t/1,0)$ is the conditional probability of the conductive state of the channel at the time t under the condition that the channel had been open at the initial instant of time.

We can easily find expressions for the probabilities P by solving Eq. (4) while using (5). Consequently, we get:

$$G_g(\omega) = 24 g_0^2 n_\infty^4 (\alpha_n + \beta_n) \sum_{k=1}^{4} \frac{n_\infty^{4-k}(1-n_\infty)^k}{(k-1)!\,(4-k)!} \frac{1}{\omega^2 + k^2(\alpha_n+\beta_n)^2}. \tag{40}$$

These formulas are easily generalized to the case having a given distribution of the states of the channel with respect to the conductivity.[48] Thus the elucidation of the type of frequency-dependence of the spectrum together with the potential-dependence of the characteristic frequency allows one in principle to solve the problem of the number of variables that are needed to describe the channel. In spite of rapid progress along this line, the level of accuracy of experiment does not allow us yet to draw final conclusions. It has already become possible to estimate the mean distance between channels from the amplitude of the fluctuations and the mean value of the fluctuating variable. This distance proved to be of the order of several hundred Å for the potassium channels in the squid axon.

6. PROPAGATION OF IMPULSES ALONG NERVE FIBERS

a) Velocity and shape of the impulse

A very important property of the nerve impulse is its ability to propagate along the fiber without attenuation at a constant velocity. In the one-dimensional case the membrane potential distribution $\varphi(x,t)$ is determined by the cable equation, which is a differential form of Ohm's law:

$$C\frac{\partial \varphi}{\partial t} = \frac{1}{R}\frac{\partial^2 \varphi}{\partial x^2} - J, \tag{41}$$

Here C is the capacitance of the membrane per unit length of fiber, R is the sum of the longitudinal intracellular resistances, and J is the ion current flowing through the membrane. The electric current J is a functional of the potential, which in turn depends on the time and on the coordinate. This relationship is defined by Eqs. (3)–(5). This form of the functional J is specific for a biological excitable medium. However, apart from the form of J, Eq. (4) is more general in nature, and it describes many physical phenomena, e.g., combustion. Therefore the transmission of the nerve impulse is often likened to the burning of a gunpowder fuse. While the ignition process in the running flame arises from the heat conductivity, in the nerve impulse the excitation involves the so-called local currents (Fig. 27).

These concepts date back to the end of the past century. Then many attempts followed to calculate the transmission of the nerve impulse mathematically. However, people had to know the excitation law in order to do this. While lacking factual data on the nature of nervous excitation, many investigators restricted themselves to constructing theoretical models whose properties recalled the excitation process. One can find the most interesting of them in the review[49].

It became clear as experimental data accumulated that the propagation effect itself is but little sensitive to the details of the excitation process, and that one can get the answer to problems involving propagation by using very simple models that reflect only the general proper-

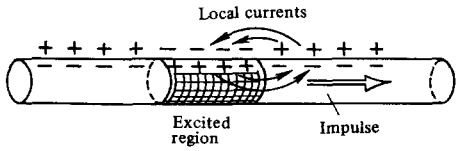


FIG. 27. Local currents that effect the propagation of the nerve impulse.

ties of excitation. The problem has interested investigators of calculating the velocity of the nerve impulse in a homogeneous fiber, as well as complicated regimes of propagation of excitation in active media such as cardiac muscle. As we know, the phenomenon of fluttering or fibrillation can arise in the myocardium, and it involves the spontaneous electrical activity of the medium. Wiener and Rosenblueth[50] have dealt with problems of fibrillation. They introduced the concept of a formal excitable medium, in which the nature of the excitation was inessential, and they obtained ground-breaking results. Their ideas have been subsequently developed considerably and refined in the studies of Gel'fand, Fomin, Tsetlin, *et al.*[51,52]

Another group of studies was somehow involved with calculating the velocity of the impulse. We can include here the well-known study of Kolmogorov *et al.*[53] on the diffusion equation with a nonlinear source. In this set of studies, it was no longer allowed to dispense with any notions, however simple, on the physics of the process. They usually amounted to the idea that either discharging of the membrane capacitance began when certain critical conditions were attained, or else a membrane generator that had certain given properties was turned on.[54–57] Such a physical modeling allowed people to get analytical results and to elucidate the physical laws. Another group of studies involved solving a detailed system of Hodgkin-Huxley equations on computers.[58] However we shall not analyze in detail these numerical methods of solution nor the formalized excitable media, and shall directly proceed to discussing the physical models and approaches that admit an analytical solution.

As we know, the ion current that flows through the membrane while a nerve impulse passes changes sign. Therefore we shall approximate the ion current J_B that flows through the membrane upon excitation with a piecewise constant function or two "square waves" (Fig. 28). In other words, we shall assume that a current is turned on at a certain instant that corresponds to the onset of excitation. It is directed inside the fiber and is equal in modulus to j'. After the time τ, the current reverses and is equal to j''. This phase continues during the time τ''. Moreover, we shall allow for the passive conductivity r_m of the membrane. Then the total current will be $J = J_{exc} + (\varphi/r_m)$. Now it is no longer hard to find an automodeling solution of Eq. (41). Since the velocity of the running wave is initially unknown, we get an eigenvalue problem. Its solution shows that there are two admissible values of the velocity, each of which corresponds to a running impulse of a definite shape. One of them proves stable while the other is unstable. The velocity of the stable impulse is

$$v_1 = \left(\frac{j'}{\varphi^* RC^2} - \frac{2}{r_m RC^2}\right)\left(\frac{j'}{\varphi^* RC^2} - \frac{1}{r_m RC^2}\right)^{-1/2}. \quad (42)$$

If we neglect the conductivity of the membrane in a state of rest, we get the simple formula

$$v_1 = \sqrt{\frac{j'}{\varphi^* RC^2}}, \quad (43)$$

which was first derived by Kompaneets and Gurovich.[55] The result included only the parameters of the first phase of the excitation current. That is, the velocity of propagation is determined by the leading front of the running impulse. The relationship of the velocity to the diameter of the nerve fiber is of interest. Experiments on smooth fibers show that the rate is approximately proportional to the square root of the diameter. The given formula gives the same result.

We can use the obtained solution to calculate the form of the action potential for concrete objects. A small variation of the excitation threshold and of the amplitude of the first phase of the current is allowable in the comparison with experiment. Figure 28 shows a nerve impulse in a squid axon. We see that the simple analytical model gives a very good approximation of the action potential that is not inferior to that obtained from the exact Hodgkin-Huxley equations. A choice of reasonable values of the parameters that figure in Eq. (43) gives velocities very close to the experimental values.[59] For example, one gets a velocity of about 21 m/sec for the giant axon of squid.

The obtained solution somewhat recalls the solitons, which have been intensively studied in various systems. Just as in the case of solitons, the nerve impulse has a set of allowed velocities, a stable form that does not depend on the conditions of formation, etc. However, there are also substantial differences. Solitons are excitations that arise in dynamic systems, whereas the nerve impulse arises and propagates in an active medium and is essentially a dissipative process. Another feature of the nerve impulse is its "refractory tail," which rules out repeated excitation immediately after passage of an impulse. It is therefore impossible, for example, for impulses to overtake one another. Nevertheless, it would be interesting to try to apply the methods of the theory of solitons to this field as well.

b) Propagation of impulses along inhomogeneous fibers

The nerve fibers along which impulses propagate are not homogeneous. Wherever the fibers branch or

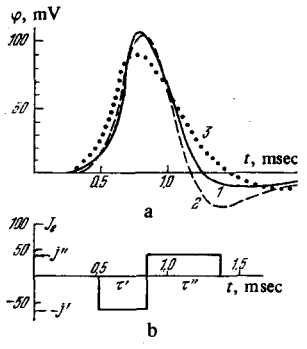


FIG. 28. Running impulse. a) Form of a spike (1—squid axon at 18.5 °C, 2—the studied model, 3—theoretical model of Hodgkin and Huxley); b) approximation of the ion current during excitation.

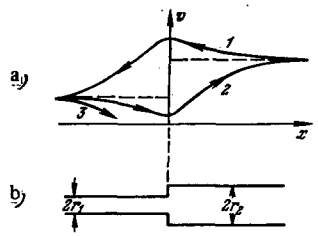


FIG. 29. Passage of impulses along an expanding fiber. a) Variation in the velocity of the impulse as a function of its direction (1,2—the impulses pass, 3—the impulse is blocked); b) schematic drawing of the expanding fiber.

change in cross-section, the passage of impulses can be hindered and sometimes even simply impossible. This problem has been studied by different authors by various methods. They have applied both analytical methods[60] and computer calculations.[58,61]

Let us examine the passage of an impulse along an expanding fiber (Fig. 29). Analysis shows that the velocity of the impulse declines as we approach the expansion, while it begins to increase after the expansion until it reaches a new steady state that exceeds the original one. Thus, the transition to a higher velocity does not occur monotonically, but after a delay. This delay increases with increasing difference in the cross-sections. With a great enough expansion, the impulse can fail to be established at all. It is of interest to calculate the critical expansion of a fiber that will not transmit an impulse. A calculation performed on the simple analytical model shows that the condition for blockage has the form

$$\left(\frac{r_2}{r_1}\right)^{3/2} > \varkappa + 1.11\sqrt{\varkappa} - 1.69 = K(\varkappa), \qquad (44)$$

here r_1 and r_2 are the radii of the two parts of the fiber, and $\varkappa$ is a safety factor, i.e., the ratio of the amplitude of the action potential to the threshold. Estimates with the parameters adopted for the giant axon of squid show that the critical expansion is somewhat larger than fourfold. Calculations in the Hodgkin-Huxley model give a value of the order of five.

Blockage does not occur when the impulse moves in the opposite direction. An impulse can always be transmitted from a broad to a narrow fiber. Yet the change in velocity upon passing the inhomogeneity is opposite in character. Upon approaching a constriction, the velocity of the impulse increases and then begins to decline to a new steady-state value (see Fig. 29). Thus we get a distinctive hysteresis loop on the velocity graph. The source of the "hysteresis" is physically quite understandable. A forward-lying broad fiber having a large capacitance is a powerful sink for charge. Therefore the potential rises toward the threshold more slowly. Yet if a constriction lies ahead, then it will not be in a condition to absorb a large charge, and will amount to a reflecting screen near which the potential rises more rapidly. Hence, the velocity of the impulse increases.

Another inhomogeneity is a branch in the fibers. Here different variants of passage and blockage of an impulse can occur. If the impulse approaches the branching node along one fiber (Fig. 30), then the condition for blockage has the form

$$\frac{\sqrt{r_2^3}+\sqrt{r_3^3}}{\sqrt{r_1^3}} > K(\varkappa).$$

Of greater interest is the approach to a branching node of impulses together along two fibers. If the impulses move synchronously, then the blockage condition has the form

$$\frac{\sqrt{r_3^3}}{\sqrt{r_1^3}+\sqrt{r_2^3}} > K(\varkappa).$$

Yet the problem is far more complicated when impulses apporach at different times toward a branching node. The blockage condition depends on the time offset. Since it has a rather cumbersome form, we shall restrict ourselves to a graphic illustration. If the impulses approach the node along fibers 1 and 2, which have the same radius r, then the critical radius of the third fiber that effects blockage depends on the time offset in the way shown in Fig. 30.

A synchronization effect arises in the approach of impulses at different times to a branching node. It is expressed in a decrease in the time offset between the impulses as they approach the node. If the time offset between the impulses is small, the impulse help one another in penetrating into the broad third fiber. Yet if the offset is large enough, the impulses begin to hinder one another. This involves the fact that the impulse that arrives first, but is not able to excite the third fiber, partially converts the node to the refractory state.[52]

c) Interaction of impulses

Thus far we have been treating transmission of a nerve impulse along a single fiber of more or less complicated shape. However, the nerve fibers in the organisms are usually combined into bundles or nerve trunks. This is quite visible in cross-sections of them, which look just like the cross sections of many-stranded cables. Each fiber in such a bundle amounts to an independent communication line, but they all have "one conductor in common," the intercellular fluid. Consequent-

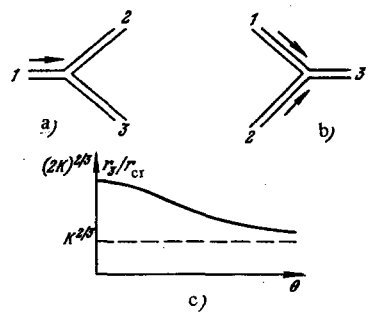


FIG. 30. Passage of impulses through a branching node. a) An impulse approaches along one fiber; b) impulses approach together along two fibers; c) relationship of the critical radius of the third fiber to the time offset between the impulses.

ly, when a nerve impulse runs along one of the fibers, it creates an electric field in the intercellular fluid, which can affect the membrane potential of the adjacent fibers. Of course, under normal conditions the different lines must operate without mutual interference, but the possibility of interaction exists in principle and it can be manifested under special, pathological conditions. Actually, if one treats nerve trunks with special chemical substances, one can observe not only mutual interference but also transmission of excitation to adjacent fibers. Perhaps the clearest experiment of this type was that performed by Katz and Schmidt.[82] Under laboratory conditions they placed two separate nerve fibers in a limited volume of external solution. The ends of the fibers emerged in different directions, and one could excite and control them separately. They found that an impulse running along one of the fibers simultaneously altered the excitability of the second fiber. The change occurs in three clearly marked stages. At first the excitability of the second fiber declines (the excitation threshold is raised). This decrease in excitability outruns the action potential that is running along the first fiber, and it lasts approximately until the potential in the first fiber reaches a maximum. Then the excitability increases. This stage coincides in time with the process of declining potential in the first fiber. And finally, the excitability again declines when a small final hyperpolarization of the membrane occurs in the first fiber.

They also studied experimentally the simultaneous passage of impulses along both fibers. Interaction of the impulses synchronized them under certain conditions In spite of the fact that the intrinsic velocities of impulses differed in the different fibers, a collective impulse could arise when they were simultaneously excited. If the intrinsic velocities of the impulses were the same, then the collective impulse had a lower velocity. When the intrinsic velocities differed appreciably, the collective velocity had an intermediate value. Only those impulses can be synchronized whose velocities do not differ too greatly—by no more than 10% in Katz and Schmidt's experiment. Here the interaction depends very strongly on the resistance of the external medium, and it increases with increasing resistance.

Let us proceed to describing this phenomenon mathematically.

The membrane potentials of two parallel fibers satisfy the following system of equations:

$$C_1\frac{\partial\varphi_1}{\partial t}=\frac{R_2+R_3}{\gamma}\frac{\partial^2\varphi_1}{\partial x^2}-\frac{R_3}{\gamma}\frac{\partial^2\varphi_2}{\partial x^2}-J_1,$$
$$C_2\frac{\partial\varphi_2}{\partial t}=-\frac{R_3}{\gamma}\frac{\partial^2\varphi_1}{\partial x^2}+\frac{R_1+R_3}{\gamma}\frac{\partial^2\varphi_2}{\partial x^2}-J_2; \quad (45)$$

here R_1 and R_2 denote the longitudinal resistances of the axoplasm of the first and the second fiber, while R_3 is the longitudinal resistance of the external medium, and $\gamma=R_1R_2+R_1R_3+R_2R_3$. One can fix the values of the ion currents in some way, depending on the adopted model of nerve excitation. If one uses the simple analytical model described above, then one can rather quickly solve the system written above. The results amount to the following. When one fiber is excited, a membrane

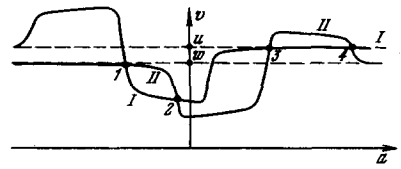


FIG. 31. On seeking the velocity of a collective impulse in interacting fibers. a—distance between impulses, v—velocity of the collective impulse, u and w—velocities of the individual noninteracting impulses. Curves I and II—theoretical velocities of impulses in each of the fibers when interacting with its neighbor; their intersection points 1, 2, 3, and 4 are the collective states.

potential that changes in sign is induced in its neighbor: at first the fiber is hyperpolarized, then depolarized, and finally hyperpolarized again. Evidently these three phases correspond to the depressed, elevated, and again depressed excitability of the fiber. They made a special study of the possible transmission of excitation between adjacent fibers—the so-called ephaptic transmission. Substitution of typical values of the parameters that enter into the final formulas shows that under normal conditions ephaptic transmission is absent in nerve trunks. As the radius of a fiber increases, the probability of transmission to it increases. The case of inhomogeneous trunks was studied especially. Transmission of excitation from fiber to fiber proved most probable in inhomogeneous regions, e.g., wherever fibers branch or leave the trunk.

The problem is more interesting of the simultaneous excitation of two adjacent fibers, and we shall treat it in somewhat greater detail. We shall seek an automodeling solution of the problem in which two impulses move at the same velocity at a constant distance from one another. In this case the problem contains two unknown parameters, the velocity v and the distance a between the impulses. Since the impulses affect one another, the velocity of each of them is formally a function of the distance between them (Fig. 31). The intersection points of the curves constitute a collective state, since under these conditions both impulses move at the same velocity at a constant distance from one another. The presented diagram contains four collective states, which are indicated by the numbers 1, 2, 3, and 4; only the states 1 and 3 are stable.

State 1 corresponds to the case in which the impulse lies ahead that has the lower intrinsic velocity. It retards the second, fast impulse, and doesn't let it pass, and they both move at a relatively low velocity. In state 3 the "fast" impulse lies ahead, and it drags the "slow" one after it. The collective velocity proves to be close to the intrinsic velocity of the fast impulse.

d) Accounting for the dynamics of development of excitation

The fundamental results of this chapter have been derived by using a simple analytical model. Physically this approach is quite justified, but here the problems remain of how exact this approximation is, and how the true dynamics of development of excitation affects the

propagation of an impulse.[63–65] We can answer these questions by using the Hodgkin-Huxley equations (3)–(5) and (41). The velocity of propagation of an impulse is governed only by its leading front. Hence we need not treat the potassium conductivity at all. Moreover, we need not treat the sodium inactivation, since the time for this process is substantially longer than the time of activation. Then, in line with the Hodgkin-Huxley equations, we get

$$\frac{d\psi}{d\xi}=\frac{1}{u^2}\frac{d^2\psi}{d\xi^2}+m^3, \quad -\infty<\xi<\infty,$$
$$\frac{dm}{d\xi}=-\frac{1}{\tau(\psi)}[m-m_0(\psi)], \tag{46}$$

Here we have introduced the automodeling coordinate $\xi = t + (x/v)$ and the following notation:

$$u=v\sqrt{\frac{RC^2\varphi_*}{j'}}, \quad \psi=\frac{\varphi}{\varphi^*};$$

Here $\tau(\psi)$ and $m_0(\psi)$ are known functions. We can derive from the system (46) simple expressions for the velocity of propagation of excitation in two limiting cases: fast and slow relaxation of the sodium variable m. In the former case we get upon assuming $\tau \to 0$:

$$u=1 \quad \text{or} \quad v=\sqrt{\frac{j'}{RC^2\varphi_*}}.$$

This result coincides with the expressions (43) for the velocity of propagation of an impulse in the simple model that we have treated above. We can assume that it corresponds quantitatively to the fast relaxation of the sodium current. In the limiting case of slow relaxation we get

$$v\approx 1.25\left(\frac{j'}{\tau^3C^5\varphi_*R^4}\right)^{1/8}. \tag{47}$$

Substitution of the known values of the parameters into (47) gives a value of the dimensionless velocity $n\approx 0.39$. The experimental value of u amounts to 0.27. It hardly makes sense to stop to discuss the ways of more exactly solving the system (46), which lead to a better agreement of the theory ($u\sim 0.3$) with experiment. It suffices to stress that the dynamics of the sodium current exerts a substantial influence on the velocity of propagation of the nerve impulse.

7. CONCLUSION

Study of mechanisms of functioning of biological systems is usually based on structural studies. The situation is complicated in the case of excitable membranes by the fact that no such structural-chemical foundation yet exists. Hence we must view the most pressing problem of the near future as being the isolation, study, and reconstruction of the membrane components that are responsible for selective ion transport regulated by the electric field.

The progress attained in studying ion transport involves partly the model experiments on bilayer lipid membrane. Yet there remain many unclear points in the very important field pertaining to problems of structure, stability, and phase transitions in membranes. Apparently the structural defects are precisely what is responsible for the background conductivity of the BLM; they can govern the laws of electrical breakdown and flicker noise that are characteristic of these systems. There is as yet no theory of such phenomena.

Although the quantitative description of ion transport through membranes that contain various complexons that act as mobile carriers or fixed channels has been developed with almost exhaustive thoroughness, it is phenomenological in nature. A shift to the molecular level involves great difficulties that stem from the very nature of the studied object. It seems evident that the binding and subsequent movement of an ion involves conformational rearrangement of the system. Yet the complete calculation of the structure of even an isolated ion complex (e.g., potassium with valinomycin) presents a yet unsurmounted problem. Apparently a possible pathway consists in broader application of computers. Such calculations are also needed for a correct solution of the problem of selectivity.

A most intriguing problem in the physics of the nerve impulse remains that of the mechanism of action of the ion channels of excitable membranes that are controlled by the electric field. The change in the conductivity of the channel involves either an appreciable rearrangement of the molecular geometry of the system or a change in the electrostatic component of the energy of the ion. It seems natural that in the former case the channel will operate discretely by an "all-or-none" principle, while in the latter case its conductivity will vary continuously. In principle a measurement of the spectra of current fluctuations will permit one to choose between these two possibilities. Therefore the fluctuational analysis of ion currents of biological membranes acquires a special meaning. It is hard to overestimate the importance also of another experimental method of studying excitable membranes: measuring the gating currents. If we describe an ion channel as being a certain macromolecular system that is characterized by a set of possible conformational states, each of which corresponds to a definite value of an electric variable, then the gating currents reflect the variation of this variable, and thus reflect the conformational rearrangement. However, the nature of the electric parameter that characterizes the state of a channel still remains unclear. A method cannot yet be seen that would permit one to distinguish whether transport or rotation of certain charged groups to large distances occurs, or a cooperative reorientation of a set of dipoles of atomic scale. Elucidation of this problem remains one of the pressing problems of the biophysics of the nerve impulse.

Measurement of the gating currents is acquiring ever more importance. If we treat ion transport through the channels as being a vector enzymatic reaction, then measurement of the asymmetric displacement currents is actually a new and very convenient method of studying conformational transitions. In modern biophysics the problem is being vigorously discussed of whether these transitions play a decisive role in the process of enzymatic catalysis,[66] or simply accompany it. The regulative role of conformational transitions in membrane transport systems is quite evident. However, the mech-

anism has not been studied of ion-conformational interaction involved with the elementary event of displacement of particles along the channel. Along this line, the fundamental problem of the relationship of the dynamic and statistical factors deserves serious study. It is topical not only in connection with transport problems, but is of general interest for biophysics.[67] The manifestation of the dynamic laws that determine the certain degree of "machine-like quality" in the behavior of the system[68] can prove important in studying the active-transport channels.

The theory of propagation of impulses along individual fibers of varying geometry has been developed in rather great detail. The next step is to study dense networks of electrically connected nerve fibers as excitable media. Similar systems have been studied in physics in connection with the problem of flame-front propagation. However, a biological excitable medium, which "burns but is not consumed," is characterized by a great variety of possible regimes of activity, among which fibrillation or the so-called electrical turbulence is of especial interest. Development of the theory of excitable media involves considerable difficulties since one must consider the nonlinear nature and memory of the function of the source, as well as the random distribution of the parameters. Therefore it is not remarkable that no one has yet been able to develop a suitable formalism. Such a problem as the applicability of the ergodic hypothesis to excitable media also demands special analysis. The complexity of this problem is compensated not only by its practical importance, but also by the inner beauty that appears in the model approaches that have already been developed in this field.

The epoch of unified science has long since passed, yet the influence of physics on the progress of biology is as great as before. And this involves not only the new methods of study, but also the very style of thought. Therefore it seemed natural to us to share with an audience of physicists the successes and difficulties of one of the fascinating fields of modern biology.

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