A MOLECULAR STRUCTURAL BASIS FOR THE EXCITATION PROPERTIES OF AXONS

DAVID E. GOLDMAN
From the United States Naval Medical Research Institute, Bethesda

ABSTRACT A structural model is suggested for axon membranes consisting of a double layer of lipid and phospholipid molecules in which the polar ends of certain phospholipids change their orientation and combining properties under the influence of an electric field. The phosphate groups act as ion exchange "gates" for the control of ion flow through the membrane. Expressions are developed for the calculation of membrane current components as functions of time, potential, and ionic environment. Approximate solutions show fairly good agreement with existing experimental data in a number of different respects such as steady-state current-voltage relations, the effect of calcium on steady-state current, potassium tracer flux ratios, initial current and rate of change of current, and the dependence of the time constants of current change on membrane potential.

INTRODUCTION

With a complete knowledge of the molecular structure of an axon and its membrane one should be able to calculate its behavior in any desired degree of detail. This cannot now be done not only because the structure is not precisely known, but also because, even if it were, many of the procedures for such calculation are complicated and in some cases not well understood. There is, however, much information available on the electrical and chemical behavior of axons and this, together with what can be surmised about membrane structure, provides strong encouragement for investigating approaches to the desired goal. The wealth of experimental material places rather severe restrictions on freedom of speculation and demands that any serious proposal account for a variety of phenomena. Foremost among these is the way in which ion transfer and its time characteristics depend on membrane potential, temperature, and on the ionic composition of the environment. These dependences have been formulated for squid axon in a very compact and useful way by Hodgkin and Huxley (1952). The formulation is based on simple physical concepts although the equations governing permeability changes are empirical. Other relevant studies include observations on the effects of environmental calcium concentration (Frankenhaeuser and Hodgkin, 1957; Frankenhaeuser,

1957), on tracer fluxes of sodium and potassium (Hodgkin and Keynes, 1955*a*, *b*), and on the effects of axon prehyperpolarization on the time characteristics of membrane current flow after subsequent depolarization (Cole and Moore, 1960).

A variety of suggestions has been made as to possible mechanisms of ion transfer control (Karreman, 1951; Hodgkin and Huxley, 1952; Frankenhaeuser and Hodgkin, 1957; Mullins, 1959; Tobias *et al.*, 1962; Shanes, 1962). However, there is a real need for a model specific enough to provide quantitative relations which can be checked experimentally and which show clearly their physical basis. This study develops such a model based on an examination of what is known of the structure of the axon membrane, on a consideration of ways in which ion transfer can take place, and on the application of simplified procedures of physical chemistry and classical statistical mechanics. The model is not complete but the results encourage further developments along the lines indicated. The membrane will be treated as an inert continuous medium on whose outer surface there is a layer of ion exchange sites attached to phospholipid molecules. These sites act as gates through which ions enter, to be driven through the membrane by electrical and diffusion forces. This approach incorporates features of the simple diffusion model, the single barrier model, and the ion-exchange model (see Teorell, 1953), none of which seem capable by themselves of explaining the complex phenomena which are observed and on which the essential behavior of axons depends. The functions and detailed behavior of the phospholipids of the membrane are not well understood. However, certain general properties are obvious consequences of their structure and it is these properties which will be used in constructing the model. The interaction of calcium and other ions with some of the phospholipid elements provides a basis for the analysis although little is yet known about specific binding characteristics.

Parts of this material have been reported in preliminary form (Goldman, 1961, 1962).

MEMBRANE STRUCTURE

Much work has been done on the structure of axon membranes with electron microscopy, optical microscopy, and x-ray diffraction (see Robertson, 1960). Chemical analyses of nerve tissue (see Folch-Pi and LeBaron, 1957) have also produced useful information. It now seems likely that there is a limiting membrane, roughly 100 A thick, which consists of a protein-coated bimolecular layer of lipid and phospholipid. Studies on the effects of lytic enzymes (Tobias, 1955) indicate that the presence of the bimolecular layer is essential since function is destroyed by phospholipases. Proteolytic enzymes appear to have little effect. The fact that the phospholipases can attack the layer also suggests that at least some large molecules can penetrate the protein layer and that the flow of small particles in and out of the axon is controlled by the bimolecular layer itself. The protein may be expected

to interact with large molecules and to participate in antigenic and enzymatic activity and may thus be important in maintenance activities. Its role in ion transfer is uncertain. The Schwann cell layer, important for the over-all functioning of the axon, does not now seem likely to exert a direct influence on ion transfer as related to impulse generation.

The axoplasmic surface of the membrane may well contain polar end groups and have a protein coating. However, the orientation of the polar groups on the axoplasmic side is geometrically opposite to that on the exterior and they react to electric field changes in a different way. We shall refer excitatory phenomena to the external surface layer, although this is at present a convenience rather than a demonstrated necessity. Recent experiments (Baker, Hodgkin, and Shaw, 1961) also appear to exclude the axoplasm itself from any direct role in excitation.

The bimolecular layer appears to contain cholesterol, galactosides, and phospholipids. Among the latter are sphingomyelin, phosphatidylethanolamine, phosphatidylcholine, and phosphatidylserine; diphosphoglyceroinositide may also be present. These phospholipids may occur individually at intervals along the membrane surface or they may occur in small clusters. The hydrocarbon chains are directed toward the interior of the membrane and the polar groups, where present, are exposed on both sides and in contact with the protein. Such groups as are not bound are dipoles and can be oriented by an electric field of sufficient strength. They may take on a variety of shapes and so expose, conceal, or distort the phosphate groups whose binding properties for various ions can thereby be modified significantly. On the other hand, the hydrocarbon chains are saturated or nearly so and the membrane interior then appears as a relatively inert lipid medium, some 50 to 60 A thick. The polar groups provide a layer 5 to 10 A thick on either side and the rest of the membrane thickness is provided by the protein.

ION TRANSFER PROCESSES

The phosphate groups act as exchange sites through which ions pass as they traverse the membrane. Interstitial leakage is probably small. The ions transferred are, of course, primarily sodium and potassium and the details of the transfer process must be different for the two ions. For practical purposes, at least until definite evidence to the contrary is produced, it is convenient to assume that the same sets of sites are involved in the transfer of both ions but in different configurations. The difference in ion size and hydration must be critical in this connection although detailed understanding must await an appropriate study of the structural chemistry of phospholipids.

The evidence (Hodgkin and Keynes, 1955*b*) that potassium ions, at least, passing in one direction seem to interfere with those passing in the opposite direction has immediate relevance. It suggests that the surface sites can absorb or combine with some of the penetrating ions as well as with calcium and thus form a "bottle-

neck." The sites are embedded in a relatively dense molecular complex which forms a barrier on each side through which penetrating ions must pass to reach or leave the combining sites. The potential profile then appears as a double barrier with a well in between (Fig. 1). Ions entering the site must also displace any ion already present. The process differs from an ordinary chemical exchange reaction in that the exchange can occur from two sides of the partially constrained site provided that it has the proper configuration. Changes in the external field as well as thermal agitation can reorient and distort the polar chain and change its relationship with neighboring molecules. These changes can be expected to modify the binding properties of the phosphate groups.

The membrane interior may conveniently be treated as a continuous medium which, although very thin, is nevertheless a number of ion diameters in thickness. A completely rigid structure would tend to establish individual channels for the ions. This is unlikely since the interaction between influx and efflux, while definite,

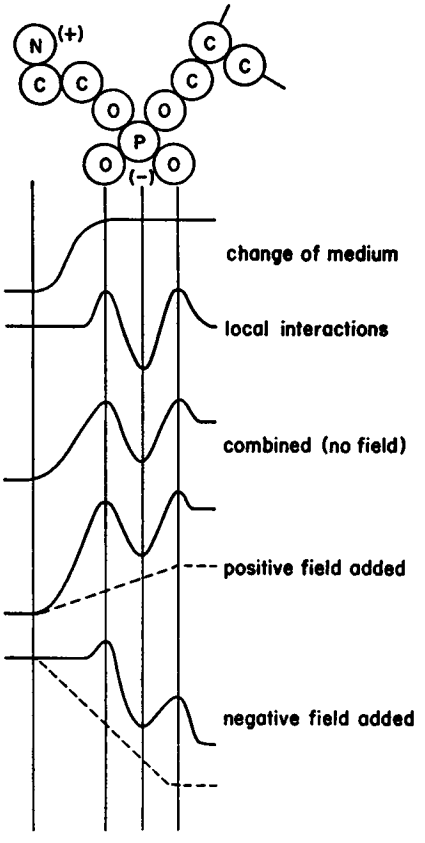


FIGURE 1 Schematic diagram of local energy barrier in the neighborhood of the ion exchange group in a direction perpendicular to the membrane surface.

is not extreme. The form in which the ions pass is likely to be a partly hydrated one although their transfer in combination with some other molecular element cannot be ruled out at present. The most that can be said is that as far as excitation is concerned, no carrier particles have yet been identified and no compelling evidence for their existence has been produced.

The major driving forces appear to be electrical and concentration gradients. Solvent drag and electroosmosis are also possible factors (Teorell, 1953). On the other hand, water permeability seems too small, at least in squid (Villegas and Villegas, 1960), to be important for rapid excitation processes although it may be significant for maintenance phenomena especially in relation to the resting potential.

KINETICS

On the basis of the general picture outlined we can formulate the characteristics of the ion transfer processes precisely enough to permit detailed study or at least to establish definite functional relations. Clearly the system is very complex and it is necessary to work out the separate aspects one at a time and then combine them. In order to keep in touch with elementary realities we use highly simplified model elements and procedures, keeping in mind both the advantages and disadvantages obtained thereby.

The Surface Layer. As a basis for kinetic analysis consider a liquid medium adjacent to a wall. The rate at which molecular particles in the medium strike the wall is $M\bar{v}$, where M is the concentration of the particles and $\bar{v}$ is the appropriate mean molecular velocity. If there are in the wall n available binding sites per cm^2, each with an effective area, s,[1] then the number of particles striking these sites is $snM\bar{v}$/cm^2/sec. If there is also a minimum energy, W, needed for the particle to enter a site, the rate is $snM\bar{v}\exp(-W/kT)$, k being the Boltzmann constant and T, the absolute temperature. If the site is already occupied by another particle, the incident particle must have sufficient energy to displace it. In addition, if a molecular complex can have more than one configuration, a corresponding relation exists with respect to the exchange. Since the sites are parts of flexible polar complexes, s and W may vary with the configuration.

The distribution of such configurations may be treated by considering them as systems of charges partially constrained by interatomic bonds. The number of complexes of a particular kind in a given configuration range is

$$A \exp\left(-\frac{U}{kT}\right) dx_1\, dx_2 \cdots$$

[1] The quantity s may contain steric and quantum factors as well as other elements of the relevant partition function (see Glasstone, Laidler, and Eyring, 1941). Detailed treatment is inappropriate here.

where U is the potential energy of the configuration expressed in terms of the distribution parameters x_1, x_2, etc., and A is a normalizing constant. In this case we have a flexible dipole in the presence of an external electric field, E, which generates an orienting field, F (Fig. 2). If the dipole has a separation r and makes an angle ψ

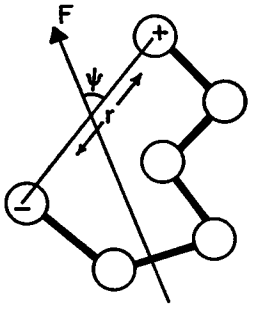


FIGURE 2 Flexible dipole in an electric field. F is the orienting electric force due to the applied field; r is the dipole length.

with the direction of the force, we can write

$$U = -eFr \cos \psi + \frac{e^2}{Dr} + U_0 \tag{1}$$

where e is the electronic charge, D is the dielectric constant, and U_0 represents energy terms not directly concerned with the dipole configuration. The relation between E, F, and D is a complicated function of the molecular structure and environment. In general, F is proportional to E, larger in polar media, but of the same order of magnitude. A simple treatment for a rigid dipole is given by Debye (1929). A molecule like phosphatidylserine requires a more extended treatment.

An idea of the relative values of the two energy terms may be gained by a simple calculation from a molecular model. The dipole of phosphatidyl choline, for example, has a minimum length of about 1.9 A and a maximum length of about 4.6 A. This corresponds to dipole moments of 9 and 27 Debye units, coulomb-stretching energies of 170 and 60 k cal/mol, and to field strengths of 4.0×10^8 and 4.6×10^7 volts/cm, respectively. The presence of a high dielectric constant medium with polar components may reduce the coulomb attraction by a factor of 10 to 100. For comparison, a potential of 50 mv across a 5 A layer produces a field of 10^6 volts/cm. Thus, the electric field present in a nerve membrane might be sufficient to hold the dipole in its extended state were it not for thermal agitation. When the chain is shortened by thermal effects, the attractive force becomes greater and the dipole is thus drawn into its shortest form. Except in very intense fields, therefore, the coulomb-stretching energy probably plays little part in variation of the dipole configuration and we are concerned mostly with rotation.

The orienting field, F, can be estimated, though not precisely calculated, from the relation

$$F = \left(\frac{1}{1 - f\alpha}\right) \frac{3D}{2D + 1} E,$$

α being the polarizability and f the so called factor of the reaction field (Böttcher, 1952). For large values of D, in a number of polar liquids at least, $1/(1-f\alpha)$ appears to have values in the range 1.2 to 1.5 so that we can write $F = \gamma E$ with $\gamma = 2$, approximately.

The above discussion applies to the polar complexes when they are in the dipole state. Certain cations when present combine with or are adsorbed by the phosphate groups, and the chain is then no longer a dipole. If the cation is univalent, there will be an ionized nitrogen group at the end of a flexible chain. If the cation is divalent, the chain contains a pair of like charges which then tend to separate. The binding energies of the various cations of importance are different and also depend on the configuration of the complex.

With any given ionic environment and electric field strength there is a definite distribution of configurations of the phospholipid polar complexes in both free and bound form. However, it will be more convenient to consider discrete configurational states between which exchange occurs (Fig. 3). For example, the chain connecting

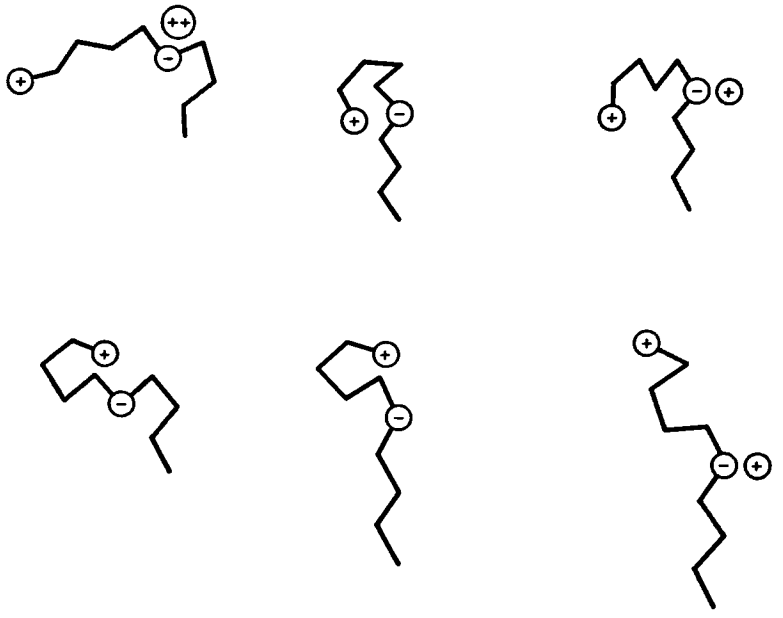
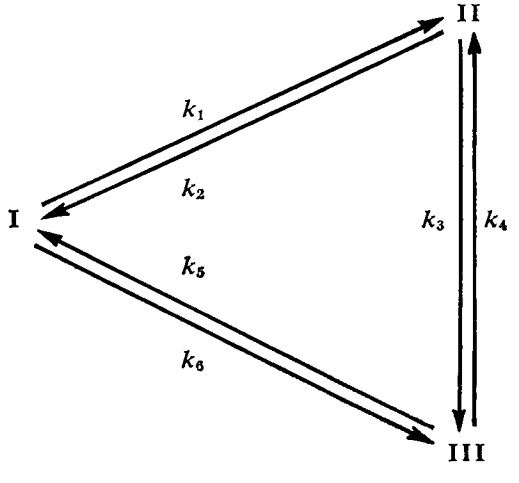


FIGURE 3 Skeleton diagram of some possible configurations of a flexible dipole. If the supporting chains are fixed in position, the upper row represents some structures in the presence of a downward electric field and the lower one, some structures with the field directed upward. The chains are three-dimensional and the diagram is schematic only.

the two ends of a polar group can be folded or extended. If folded, the positive gegenion of the phosphate group will be close to it. If extended, some other ion such as calcium, sodium, or potassium will be present. If the occupant is calcium, there will be positive charges at both ends of the chain—unless, indeed, the calcium ion were to link two separate phosphate groups. Either way, the electric field of a resting axon membrane will tend to keep the polar chains oriented with the positive ends tucked into the membrane. If the field is removed, many of these will tend to swing free. This produces a new configuration which we have assumed to have different binding properties; specifically, these are unfavorable to calcium but suitable for the passage of sodium. The site could be occupied nearly enough by the positive gegenion for it to act as a temporary, weak shield against other ions; alternatively it could bind sodium. We also suppose that the complex, when free of the surface, can exist in another configuration as well, one which favors the binding of potassium; this latter exchange being independent of the electric field. Thus, many of the sodium-favoring elements will be reversibly converted to a potassium-favoring state and the transfer of sodium, having risen to a maximum, will decline and be replaced by potassium transfer. If the field is now reimposed, the dipoles are pushed down into the surface again and can adsorb calcium as before. We thus indicate a particular interest in three configurations (although there may be more), each of which can bind various positive ions but to markedly different degrees. We tentatively regard the adsorption and desorption of ions on the sites as being very rapid so that the configurational changes represent the rate-limiting steps.

To formalize this, let there be three major interchangeable configurations: I, which binds calcium, II, with which the gegenion is closely associated (or which, perhaps, binds sodium), and III, which preferentially adsorbs potassium. Let there be n_T sites per cm^2 of membrane surface of which n_I, n_{II}, and n_{III} are in configurations I, II, and III, respectively. We represent this as



Four of the six rate constants, k_1, k_2, k_5, and k_6, depend on the field strength. To estimate their field dependence we consider the process of rotation of the chain about the phosphate end. The energy required for the process is raised or lowered by the rotational term of equation (1). Thus the rate constants must include the expression

$$\exp\left(\frac{eF}{kT}\overline{r\cos\psi}\right)$$

where the bar indicates an average value, r_0 which is different in the different configurations. Since $F = \gamma E$ and $r_0 E$ is the potential drop, ϕ_1, across the dipole, we can express the rate constants in the form

$$m_1 e^{\gamma\theta_1},\ m_2 e^{-\gamma\theta_1},\ k_3,\ k_4,\ m_5 e^{-\gamma\theta_1},\ m_6 e^{\gamma\theta_1} \quad \text{where} \quad \theta = \frac{e\phi}{kT},$$

a convenience which we use from here on, and the m's represent the remaining factors in the rate constants. It should be noted that, with reference to the behavior of an axon, the preferred direction of transfer in the above diagram is clockwise around the loop. Thus, the relative values of the coefficients must be properly chosen and the choice must, of course, be consistent with the results of experiment.

Next consider the interaction of the various configurations with the different ions present. Configuration I binds calcium in preference to other ions and the bound form does not participate in the rotation process brought about by field changes. If we denote the number of sites of type I which are calcium-bound as n_M and the rest by n'_{I}, we find $n_M = pM\, n'_{\mathrm{I}}$, where M is the calcium concentration in the adjacent external medium and p is a constant. There seems to be very little calcium on the axoplasmic side of the layer (Hodgkin and Keynes, 1957). For potassium, there is also a rapid attainment of equilibrium once the site achieves the proper configuration. However, since potassium is available to both sides of the site, the equilibrium constant has two parts: one corresponding to exchange with the external medium and the other corresponding to that with the potassium in the membrane. If K_e is the concentration of the medium and K′ is that in the membrane adjacent to the site layer, $n_K = (qK_e + q'K')n'_{\mathrm{III}}$. For configuration II we tentatively assume $n_N = n_{\mathrm{II}}$. If sodium is significantly adsorbed, a suitable correction must be made.

This picture implies that calcium is strongly bound, potassium less so, and sodium weakly, if at all, each to its proper configuration. There is, however, a strong possibility that other combinations also exist and that they may become important when the relative ion concentrations are extreme. For example, if the potassium-calcium ratio in the external medium becomes very large, configuration I may adsorb significant amounts of potassium and thus contribute to an increased potassium conductance even when the membrane has an appreciable negative potential across it. Magnesium, ammonium, lithium, etc., may participate under certain conditions. Detailed examination of this must await further study. The recent development of

techniques for perfusing squid axons (Baker, Hodgkin, and Shaw, 1961) should permit further analysis of some of these factors.

With the choices made above, the kinetic equations for the exchange processes become

$$\frac{dn'_{\mathrm{I}}}{dt} = -(k_1 + k_6)n'_{\mathrm{I}} + k_2 n_{\mathrm{II}} + k_5 n'_{\mathrm{III}} \tag{2}$$

$$\frac{dn'_{\mathrm{III}}}{dt} = k_6 n'_{\mathrm{I}} + k_3 n_{\mathrm{II}} - (k_4 + k_5)n'_{\mathrm{III}} \tag{3}$$

$$n_{\mathrm{II}} = n_T - n'_{\mathrm{I}} - n_M - n'_{\mathrm{III}} - n_{\mathrm{K}} \tag{4}$$

$$n_M = pMn'_{\mathrm{I}} \tag{5}$$

$$n_{\mathrm{K}} = (q\mathrm{K}_e + q'\mathrm{K}')n'_{\mathrm{III}} \tag{6}$$

$$n_N = n_{\mathrm{II}} \tag{7}$$

Initial conditions can be based on the preexistence of a steady-state at some original membrane potential which is suddenly changed at $t = 0$.

Ion Transfer across the Surface Layer. Potassium transfer occurs when a site holding a potassium ion is struck by another ion with sufficient energy to drive out the occupant in the direction toward which the incident ion is travelling. Because of the assumed structural and binding characteristics of the different configurations the displacing ions will be nearly all potassium. Thus the inward flux is

$$\vec{r}_{\mathrm{K}} = \sigma_{\mathrm{K}} n_{\mathrm{K}} \mathrm{K}_e \exp\left(-\frac{W_{\mathrm{K}}}{kT}\right) \tag{8}$$

and the outward flux is

$$\overleftarrow{r}_{\mathrm{K}} = \sigma_{\mathrm{K}}' n_{\mathrm{K}} \mathrm{K}' \exp\left(-\frac{W_{\mathrm{K}}'}{kT}\right) \tag{9}$$

where σ and σ' are constants involving molecular velocity and area factors. In the case of sodium which is weakly bound, if at all, the expressions are similar although the interpretation of the energy terms may be somewhat different.

Since the current density is simply the net flux multiplied by the charge,[2]

$$I_{\mathrm{K}} = en_{\mathrm{K}}\left[\sigma_{\mathrm{K}}'\mathrm{K}' \exp\left(-\frac{W_{\mathrm{K}}'}{kT}\right) - \sigma_{\mathrm{K}}\mathrm{K}_e \exp\left(-\frac{W_{\mathrm{K}}}{kT}\right)\right] \tag{10}$$

$$I_N = en_N\left[\sigma_N' N' \exp\left(-\frac{W_N'}{kT}\right) - \sigma_N N_e \exp\left(-\frac{W_N}{kT}\right)\right] \tag{11}$$

To evaluate the energies in these expressions we recall that the barrier is double

[2] Electric currents are positive when flowing from the axoplasm toward the external medium. Potentials are referred to an external ground.

with a well in between (Fig. 1). The energy of the incident ion must be sufficient to drive out the occupant or to get completely through. Thus, the higher of the two barriers is the relevant one. The barriers include energies of change of medium, change of hydration, local electron interactions, etc. We can condense these by noting that the ratio of the appropriate factors on the two sides constitutes the distribution coefficient of the ion between the aqueous medium and the membrane material. A rough estimate of the distribution coefficients can be made by calculating, both for the aqueous solution and for the lipid membrane material, the energy required to bring an ion from a distant vacuum into a medium of specified dielectric constant, hydrate it, and arrange its ion atmosphere. We use an effective ion radius of 5 A in water (2 water shells), 2 A in the membrane (1 water shell), and a dielectric constant of 10 for the membrane interior. The resulting values are of the order of 10^{-4}. Further, if an electric field is superimposed, the barriers are raised or lowered by some fraction of the potential drop across the site region. This potential drop is not necessarily the same as that across the dipole but should be nearly so and for present purposes the two will be considered the same. Hence,

$$I_K = en_K\lambda_K e^{-\beta\theta_1}(K'e^{\theta_1} - \zeta_K K_o) \tag{12}$$

$$I_N = en_N\lambda_N e^{-\beta\theta_1}(N'e^{\theta_1} - \zeta_N N_o) \tag{13}$$

with ζ the distribution coefficient, β the applicable fraction of the potential drop (assumed to be the same for both ions though this is not essential), and λ containing the appropriate area and energy term for outward passage of the ion.[3]

Ion Transfer through the Membrane Interior. The membrane interior has been approximated by an inert, continuous medium. Thus, the classical flow equations apply:

$$I_N = e\alpha_N\left(\frac{\partial N}{\partial x} + N\frac{\partial\theta}{\partial x}\right) \tag{14}$$

$$I_K = e\alpha_K\left(\frac{\partial K}{\partial x} + K\frac{\partial\theta}{\partial x}\right) \tag{15}$$

$$I_C = e\alpha_C\left(-\frac{\partial C}{\partial x} + C\frac{\partial\theta}{\partial x}\right) \tag{16}$$

The α's are the diffusion coefficients of the ions in the membrane and the usual assumptions have been made with respect to activity coefficients. Equation (16) has been included to represent any negative ion flow, partly chloride (at concentration C). The phosphate groups presumably form so high a barrier for anions that very few use the gates. Anionic current is more likely to result from interstitial leakage; there may, of course, be some interstitial leakage of cations as well. On the

[3] The dual use of e for electronic charge and for exponential base should present no difficulty.

other hand, the membrane interior should contain at least some anions to provide over-all charge balance. The thickness of the diffuse layer of Gouy, equivalent to Debye's $1/\kappa$, is several tens of Angstrom units in the membrane interior as compared with 4 to 5 A in the aqueous medium. Thus, the internal charge is distributed through the membrane whereas the external charge is essentially in a monolayer.

The equations of continuity

$$\frac{\partial I_N}{\partial x} = e\frac{\partial N}{\partial t} \tag{17}$$

$$\frac{\partial I_K}{\partial x} = e\frac{\partial K}{\partial t} \tag{18}$$

$$\frac{\partial I_C}{\partial x} = -e\frac{\partial C}{\partial t} \tag{19}$$

and Poisson's equation,

$$\frac{\partial^2\theta}{\partial x^2} = -\frac{4\pi e^2}{DkT}(N + K - C) \tag{20}$$

complete the system. At the external boundary, the barrier flow relations apply, and at the internal axoplasmic boundary it seems reasonable, with the simplifications made, to consider fixed concentrations and potential. The total current at the external boundary must also equal that at the internal boundary.

ANALYSIS OF RESULTS

Given a knowledge of the parameters of the system and the aid of modern computing devices, we should now be able to derive the various ion currents as explicit functions of membrane potential, time, and environmental ion concentrations. Before engaging in so ambitious a program we look into some of the more simply derived consequences for situations where experimental evidence is available or may reasonably be expected to become available. In this way we may evaluate the model in part and estimate some of the parameters.

The Steady-State. From equations (2) through (7) we get

$$\frac{n_T}{n_K} = \frac{1 + qK_o + q'K'}{qK_o + q'K'} + \frac{k_4(m_1 + m_6)e^{\gamma\theta_1} + m_1m_5 + (1 + pM)[(m_2k_4 + k_3m_5)e^{-\gamma\theta_1} + m_2m_5e^{-2\gamma\theta_1}]}{(qK_o + q'K')[k_3(m_1 + m_6)e^{\gamma\theta_1} + m_2m_6]} \tag{21}$$

$$\frac{n_T}{n_N} = 1 + \frac{(1 + qK_o + q'K')[k_3(m_1 + m_6)e^{\gamma\theta_1} + m_2m_6]}{k_4(m_1 + m_6)e^{\gamma\theta_1} + m_1m_5} + \frac{(1 + pM)[(m_2k_4 + k_3m_5)e^{-\gamma\theta_1} + m_2m_5e^{-2\gamma\theta_1}]}{k_4(m_1 + m_6)e^{\gamma\theta_1} + m_1m_5} \tag{22}$$

These relations are to be used with the boundary conditions for the steady-state solution of the flow equations (14) and (15). If the potential profile, $\theta(x)$, in the membrane is known, the solution may be obtained directly. Calculation of the profile requires, however, complete solution of the entire set of equations. The solution of equation (15), for example is

$$I_K = \frac{e\alpha_K}{aS}(\zeta_K K_i e^{\theta_m} - K' e^{\theta_1}) \tag{23}$$

where

$$S = \frac{1}{a}\int_0^a e^{\theta} dx,$$

and K_i is the potassium concentration in the axoplasm. θ_m is the total membrane potential and a is the membrane thickness measured from the binding site to the axoplasmic side.

By combining (12) with (23), we get

$$I_K = \frac{e\zeta_K(K_i e^{\theta_m} - K_e)}{\frac{a}{\alpha_K} S + \frac{e^{\beta\theta_1}}{\lambda_K n_K}} \tag{24}$$

Evidently, the two terms in the denominator correspond to the resistance of the membrane interior and surface layer, respectively, acting in series. To simplify this expression further, we use some rough approximations at the extremes of the potential range.

Suppose that θ_m is large and negative so that the axon is in a resting state or is hyperpolarized. Then the potential θ_1 across the surface layer accounts for nearly all of the membrane potential since most of the sites are blocked. Then $\theta_1 \cong \theta_m$ and

$$n_K = n_T \frac{(qK_e + q'K')m_6}{(1 + pM)m_5} e^{2\gamma\theta_m} \tag{25}$$

While K′ depends on θ_m, it approaches limiting values at the extremes of the potential range and thus the asympotic value of n_K just given depends on θ_m only through the exponential term.

The factor S can be estimated using the constant field approximation (Goldman, 1943) which appears useful for intense fields with appreciable charge separation. It is easily shown that with

$$\theta = \theta_1 + \frac{\theta_m - \theta_1}{a} x,$$

$$S = \frac{e^{\theta_m} - e^{\theta_1}}{\theta_m - \theta_1} \quad \text{which for} \quad \theta_1 \cong \theta_m$$

is approximately e^{θ_m} and becomes negligible. Since β is appreciably less than one and γ is greater than one,

$$I_K = \frac{en_T\lambda_K(qK_e + q'K')m_6\zeta_K K_e}{(1 + pM)m_5} e^{(2\gamma-\beta)\theta_m}\left(\frac{K_i}{K_e} e^{\theta_m} - 1\right) \tag{26}$$

This expression can be compared with conductance data on the squid axon (Hodgkin and Huxley, 1952). The chord conductance, in our terminology, is

$$g_K = \frac{e}{kT} \frac{I_K}{\ln \frac{K_i}{K_o} e^{\theta_m}}$$

so if we multiply the experimental values by

$$u = \frac{\ln \frac{K_i}{K_o} e^{\theta_m}}{\frac{K_i}{K_o} e^{\theta_m} - 1}$$

and plot against membrane potential (Fig. 4), the curve should follow the relation $e^{(2\gamma-\beta)\theta_m}$. From the graph, we obtain $2\gamma - \beta = 3.8$ and since γ is about 2 and β is a few tenths, the agreement is fairly good.

Another comparison can be made using existing data on the effect of calcium concentration on the position of the current-potential curve. Since

$$g_K \propto \frac{e^{(2\gamma-\beta)\theta_m}}{1 + pM} \cdot \frac{1}{u}$$

and since it can be shown that u is roughly equal to

$$\left(\frac{K_i}{K_o} e^{\theta_m}\right)^{-0.4}$$

in the range $-4 \leq \theta_m \leq +2$ for normal potassium values, we can write

$$g_K \propto \frac{1}{M} e^{(2\gamma-\beta+0.4)\theta_m}$$

when $pM \gg 1$. Thus, an e-fold change in calcium concentration should displace the curve along the potential axis by an amount $\Delta\theta = 1/4.2$ or about 6 mv if the calcium concentration is not too small. The experimental values are about 9 mv for squid (Frankenhaeuser and Hodgkin, 1957) and 6 mv for a myelinated axon (Frankenhaeuser, 1957).

When the axon is depolarized, the state of affairs is quite different. The gates are mostly open and the potential drop across the surface layer is reduced to some fraction, μ, of the total membrane potential. At this end of the range, n_K becomes independent of potential (see equation 21). Also, with the constant field approximation S varies nearly with e^{θ_m}/θ_m. Thus, I_K becomes proportional to θ_m (see equations 21 and 24). Also,

$$\frac{I_K}{\theta_m(K_i e^{\theta_m} - K_o)} \propto e^{-\theta_m}$$

and again, reference to Fig. 4 shows that this is asymptotically correct although the

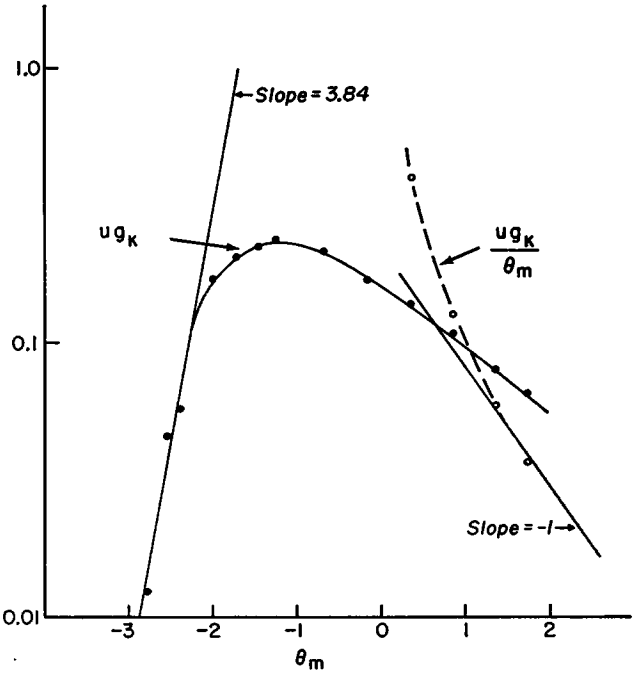


FIGURE 4 Modified steady-state potassium conductance of squid axon (calculated from Hodgkin and Huxley, 1952). Values have been divided by the limiting conductance at large positive θ_m. Numerical slopes given in terms of natural logarithms. See text.

data are not extensive in this range. This seems also to occur in lobster (Julian, Moore, and Goldman, 1962) and in myelinated axons (Frankenhaeuser, 1962).

It is worth noting that the peak sodium current also represents a stationary state and that much of the above analysis can be expected to apply to it as well. However, both the peak value and time delay depend on other factors and the situation is therefore more complicated.

Some comments can be made concerning the initial current occurring at the moment of a step change of potential. In practice, this means following an interval of up to 50 microseconds or more after the step change since the time required for adjusting the charge on the membrane, as well as limitations of amplifier pass bands, restricts short time measurements. The total current contains potassium, sodium, and leakage components. Equation (24) with n_K at its pre-step value and θ_m at its post-step value gives the potassium component of the initial current. The sodium component has the same general form but the leakage component probably derives largely from the flow equation of the membrane interior. No more can now be said than that it is unlikely that the initial current will be linear in the potential (see Adelman and Taylor, 1961).

Tracer Fluxes in the Steady-State. The analysis outlined by Hodgkin and Keynes (1955*b*) can be carried out explicitly here. In the presence of labeled potassium the exchange between it and the unlabeled ion follows the relations:

$$\frac{dn_p^*}{dt} = \sigma_K e^{-W_K/kT}(K_e^* n_p - K_e n_p^*) + \sigma_K' e^{-W_{K'}/kT}(K'^* n_p - K' n_p^*) \quad (27)$$

$$n_p + n_p^* = n_K \quad (28)$$

The flux of labeled ion is

$$\overrightarrow{r_K^*} = \sigma_K e^{-W_K/kT} n_p^*(K_e + K_e^*) \quad (29)$$

and

$$\overleftarrow{r_K^*} = \sigma_K' e^{-W_{K'}/kT} n_p^*(K' + K'^*) \quad (30)$$

where the asterisk refers to the labeled ion and n_p and n_p^* are the numbers of sites occupied by unlabeled and labeled potassium, respectively. For influx only, $K'^* = 0$ and for efflux only, $K_e^* = 0$. Also, let $K_e^* = c_1 K_e$ and $K'^* = c_2 K'$ where c_1 and c_2 are usually small. Thus, the ratio of influx to efflux is

$$\frac{c_1}{c_2}\left(\frac{\sigma_K}{\sigma_K'} e^{-(W_K - W_{K'})/kT} \frac{K_e}{K'}\right)^2$$

or

$$\frac{c_1}{c_2}\left(\zeta_K \frac{K_e}{K'}\right)^2 e^{-2\theta_1} \quad (31)$$

Since θ_1 is very nearly equal to θ_m in the hyperpolarized membrane and some small fraction of θ_m when the membrane is depolarized, we expect that a plot of the flux ratio against the potential will show a variation with $e^{-2\theta_m}$ at one end but will vary less rapidly as the membrane becomes depolarized. In Fig. 5 the product of the flux ratio and the concentration ratio K_i/K_e is plotted against θ_m. Actually we should use K'/K_e but we know only that K' approaches proportionality with K_e at extreme hyperpolarization. Since K' is usually greater than $\zeta_K K_e$, the apparent slope of the curve is increased slightly. In view of the fact that no great precision is claimed for either calculation or experiment, the agreement appears satisfactory.

Transients. For an analysis of transient currents following a step change in potential, one must return to the basic equations. Instead of equation (23), for example, we have

$$\int_0^a I_K(x, t) e^{\theta(x,t)} \, dx = e\alpha_K(\zeta_K K_i e^{\theta_m} - K'(t) e^{\theta_1(t)}) \quad (32)$$

and, for equation (12),

$$I_K(0, t) = e\lambda_K n_K(t) e^{-\beta_1 \theta(t)}(K'(t) e^{\theta_1(t)} - \zeta_K K_e) \quad (33)$$

Hence

$$\frac{1}{\alpha_K}\int_0^a I_K(x, t) e^{\theta(x,t)} \, dx + \frac{e^{\beta\theta_1(t)}}{\lambda_K n_K(t)} I_K(0, t) = e\zeta_K(K_i e^{\theta_m} - K_e) \quad (34)$$

with a similar expression for I_N. For I_C, the second term on the left is omitted. The

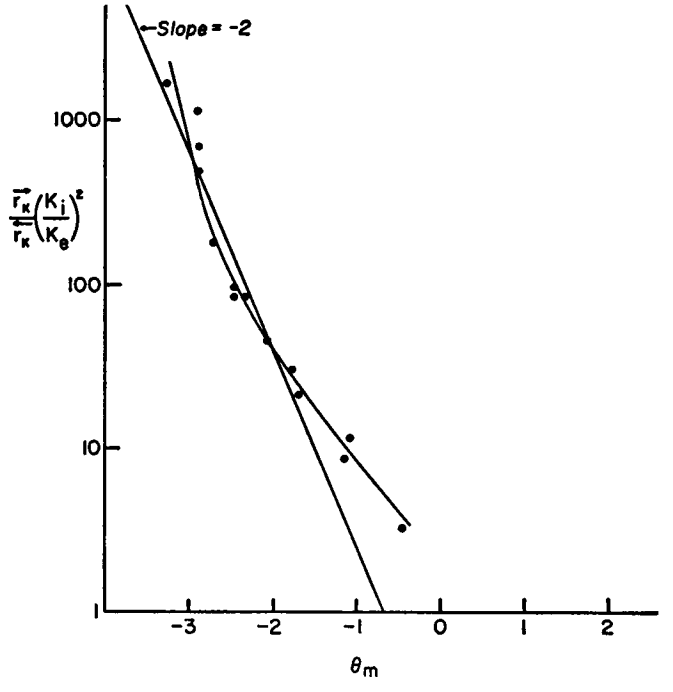


FIGURE 5 Modified steady-state flux ratios in *Sepia* axon (calculated from Hodgkin and Keynes, 1955*b*). Numerical slope given in terms of natural logarithms.

total current is, of course, the sum of the three components and is what is actually measured. In general, the current changes are governed by both the kinetics of the surface configurational changes and by diffusion delay in the membrane interior. The relative importance of these two factors depends on the potential before the step as well as on that after the step. It also changes during the time course of the transient. Details must await a more complete solution by machine methods.

The solutions of the equations (2) through (7) (Fig. 6) contain two terms exponential in time. They have initial values which are functions of the potential before the step and final values which are the same function of the potential after the step (see equations 21 and 22). The exponentials contain the reciprocal time constants

$$2\lambda_{1,2} = H \pm (H^2 - 4L)^{1/2} \tag{35}$$

where

$$H = (m_1 + m_6)e^{\gamma\theta_1} + (1 + qK_e + q'K')k_3 + k_4 + [(1 + pM)m_2 + m_5]e^{-\gamma\theta_1} \tag{36}$$

and

$$L = [(1 + qK_e + q'K')k_3 + k_4](m_1 + m_6)e^{\gamma\theta_1} + m_1m_5 + (1 + qK_e + q'K')m_2m_6 + (1 + pM)(m_2k_4 + k_3m_5)e^{-\gamma\theta_1} + (1 + pM)m_2m_5e^{-2\gamma\theta_1} \tag{37}$$

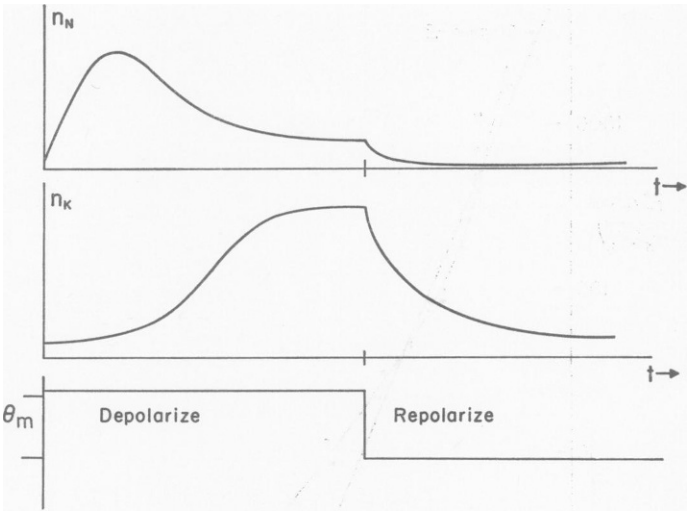


FIGURE 6 Schematic plot of linearized approximation to the number of sites available to sodium and to potassium as functions of time after the application of a step depolarization and repolarization (see equations 35 through 37).

The above is not strictly true since θ_1 and K′ are actually time-dependent and the linear treatment is an approximation. Nevertheless, it is clear that the time constants controlling the rates of rise and fall of the apparent membrane conductance components vary with potential more or less as indicated above. They have a maximum at some potential and decrease as the potential deviates from this in either direction. Qualitatively this is entirely consistent with experimental data on squid (Hodgkin and Huxley, 1952), and probably in lobster (Julian, Moore, and Goldman, 1962), and myelinated axon (Dodge and Frankenhaeuser, 1958) although detailed calculations have not been made.

The observations of Cole and Moore (1960) that prehyperpolarization of the squid axon results in an increased delay in the rise of potassium current upon depolarization, although little effect on sodium current is seen, can be explained roughly on the basis of the formulation given here. As the axon is further and further polarized (short of breakdown), the effective resistance to potassium current increases partly because of closure of the gates and partly because the steady-state potassium concentration in the membrane is reduced. Thus on subsequent depolarization, the apparent diffusion coefficient is initially very low and more time is needed for the membrane to attain its final state than if the membrane had not been hyperpolarized. This argument is not applicable to sodium current since the optimum configuration is reached relatively quickly. However, one would except the peak current to be reduced from its value in the absence of the prehyperpolarization.

Another aspect of the current-time relations for which an approximation can be

made is the initial rate of change of the current elements of the transient. At the first instant after a step potential change, there will have been no time for adjustments of the surface layer or of the internal concentrations. Thus, the number of gates in any configuration has its pre-step value as also does the ratio of θ_1 to θ_m.

For a depolarizing step, it turns out that for t very small,

$$\frac{dn_K}{dt} = n_T \frac{qK_e + q'K'}{1 + pM} m_6 e^{\gamma\theta_m}$$

$$\frac{dn_N}{dt} = n_T\left[m_1 + m_6 - \frac{1 + qK_e + q'K'}{1 + pM} \frac{(1 + qK_e + q'K')k_3 m_6 + m_1 k_4}{(1 + qK_e + q'K')k_3 + k_4}\right] e^{\gamma\theta_m}$$

and if we use these values to determine the rate of change of

$$\frac{I_K}{K_i e^{\theta_m} - K_e}$$

we get

$$e\zeta_K n_T \lambda_K \frac{qK_e + q'K'}{1 + pM} m_6 e^{(\gamma-\beta)\theta_m}$$

with an analogous expression for

$$\frac{I_N}{N_i e^{\theta_m} - N_e}$$

For a repolarizing step,

$$\frac{dn_K}{dt} = -n_T(qK_e + q'K') \frac{k_3 m_5}{(1 + qK_e + q'K')k_3 + k_4} e^{-\gamma\theta_m}$$

$$\frac{dn_N}{dt} = -n_T \frac{(pM - qK_e - q'K')k_3 m_5 - (1 + pM)m_2 k_4}{(1 + qK_e + q'K')k_3 + k_4} e^{-\gamma\theta_m}$$

and

$$\frac{d}{dt}\left[\frac{I_K}{K_i e^{\theta_m} - K_e}\right] = -e\zeta_K n_T \lambda_K \frac{(qK_e + q'K')k_3}{(1 + qK_e + q'K')k_3 + k_4} m_5 e^{-(\gamma+\beta)\theta_m}$$

On depolarization the rate is thus positive, and with suitable values of the parameters, may be quite small. In any case, the rate on repolarization is negative and much larger. The above and corresponding calculations for sodium current agree qualitatively with experimental results on squid, lobster, and myelinated axon.

Membrane Resistance. The electrical impedance of the membrane can, in principle, be calculated from the basic equations but this will not be done here as it demands a more complete treatment than is provided by the approximations used. In the hyperpolarized state, both potassium and sodium slope conductances are propor-

tional to $e^{(2\gamma-\beta)\theta_m}$ and are constant in the depolarized state. In the latter case we have

$$I_K = e\zeta_K K_i \frac{\alpha_K}{a}\theta_m \qquad \text{as a limiting value.}$$

Since ζ_K is roughly 10^{-4}, a is about 10^{-6} cm, and K_i is normally about 0.3 molar, we find that a potassium conductance of 30 to 50 mmho/cm^2 corresponds to a diffusion constant of the order of 10^{-7} cm^2/sec. The diffusion constant for sodium is roughly the same since both the internal sodium concentration and limiting sodium current are smaller in about the same proportion. These diffusion constants are, of course, much longer than would be calculated from a resting membrane resistance since the ion flow in a polarized axon is controlled primarily by the surface layer.

An important criterion for electrical activity in an axon is that there be a region of negative slope in the current-potential curve so that the necessary reverse (active) current can be produced during a shift from the resting to the depolarized state. In the normal axon, this phenomenon occurs with sodium because there is a region in which the slope of the conductance curve is larger and of opposite sign to that of the driving force curve. If the axon is placed in isotonic KCl, a similar phenomenon occurs with potassium (Moore, 1959; Julian, Moore, and Goldman, 1962). It is clear from the analysis given earlier that the model behaves in the same way. It is also evident that the denominator of equation (24) becomes very large at both extremes of the potential scale although it rises much more steeply on the negative potential side than on the positive. The numerator is negative with a small slope below the crossover and the slope increases exponentially as θ_m becomes large and positive. It is therefore possible that under certain conditions, a negative slope region may occur at positive membrane potentials as well as on the negative side.

The effects of temperature on the current-voltage curves are also calculable by machine methods provided that the parameters do not turn out to be significantly temperature-dependent. This is a problem for future consideration.

We have also encountered a number of situations in which ion concentrations have an important effect on the electrical response characteristics of the membrane. Some of these should be testable experimentally and in this way it may also be possible to clarify and extend our understanding of the reaction network of the ions and phosphate groups.

DISCUSSION

It is worth while at this point to restate the main features of the membrane model:

1. The flow of ions, primarily sodium and potassium, is controlled by the action of the membrane potential on an oriented double layer of lipid and phospholipid molecules.
2. The phosphate groups of certain of the phospholipids act as ion exchange sites

whose affinity for cations depends markedly on the configuration of the dipolar complexes which in turn is dependent both on the electric field strength and on the presence of appropriate cations.

3. Ion transfer across the surface layer of the membrane occurs primarily through these sites. Ion transfer through the membrane interior is governed by electrical and diffusion gradients.

With the aid of this model we have been able to develop expressions for membrane current components as functions of pre- and post-step potentials, time, and environmental concentrations. The formulation involves a number of factors which have been made as explicit as appeared possible in the present state of knowledge. The interaction of these factors has produced a degree of complexity which requires sophisticated computational methods for solution. The results obtained by approximate treatment are consistent with what is now known experimentally but many matters remain to be cleared up if the path laid out here is to be followed much further. Much can be learned from a study of the effects of varied ionic environments on voltage-clamped axons. From the quantitative point of view, what is also needed is a determination of the parameters so that machine computations can be carried out. A few methods for this have been outlined and there are evidently more.

The analysis made of the dipole configurations and their ion-binding properties is consistent with current notions on the effects of electric fields on the probable molecular structure. However, a number of questions arise concerning the geometrical structure of the important configurations and their combining properties. The particular phospholipids concerned have not yet been identified. It is not known whether diphosphoglyceroinositide is present in the membrane. This molecule is not a dipole but may serve as an ion exchange site. The study of the electrical and chemical properties of phospholipids is therefore a matter of great importance. Relatively little seems to have been done in this field but, in view of the key role which they seem to play in ion transfer, more work is urgently needed. Analysis of interaction with various cations including those not normally found in or near axons could profitably be extended. Recent developments in techniques for producing phospholipid films in aqueous media are encouraging (Monnier and Monnier, 1959; Mueller *et al.*, 1962).

A major problem not dealt with in this study is that of the resting potential in relation not only to the passive aspects of the structure but also to metabolic activity. If, for example, the mechanism for maintaining the axoplasmic concentrations moves sodium or potassium in ionic form, there may be additional, though probably small, contributions to current flow which can influence the resting potential. Electroosmosis can also affect the driving force on ions in passage through the membrane interior.

The importance of unstirred fluid boundary layers is difficult to assess accurately. The presence of narrow channels between the axolemma and Schwann cell seen in

squid axons may play a role (Geren and Schmitt, 1954), though probably a secondary one.

Finally, it should be noted that the model has highly specific properties. To the extent that it may be valid for one type of axon, it should be valid for other types without radical modification. For muscle and other non-nervous excitable cells, considerable modification may be required; for still other types of cell the phospholipids present in their membranes probably have a different structural relationship and functional behavior.

The opinions or assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the Naval service at large.

Received for publication, May 15, 1963.

REFERENCES

ADELMAN, W. J., and TAYLOR, R. E., *Nature*, 1961, **190**, 883.
BAKER, P. F., HODGKIN, A. L., and SHAW, T. I., *Nature*, 1961, **190**, 885.
BÖTTCHER, C. J. F., Theory of Electric Polarization, Amsterdam, Elsvieer Publishing Company, Inc., 1952.
COLE, K. S., and MOORE, J. W., *Biophysic. J.*, 1960, **1**, 1.
DEBYE, P., Polar Molecules, New York, The Chemical Catalog Company, Inc., 1929.
DODGE, F. A., and FRANKENHAEUSER, B., *J. Physiol.*, 1958, **143**, 76.
FOLCH-PI, J., and LEBARON, F. N., *in* The Metabolism of the Nervous System, (D. Richter, editor), New York, Pergamon Press, Inc., 1957, 67.
FRANKENHAEUSER, B., *J. Physiol.*, 1957, **137**, 245.
FRANKENHAEUSER, B., *J. Physiol.*, 1962, **160**, 40.
FRANKENHAEUSER, B., and HODGKIN, A. L., *J. Physiol.*, 1957, **137**, 218.
GEREN, B. B., and SCHMITT, F. O., *Proc. Nat. Acad. Sc.*, 1954, **40**, 863.
GLASSTONE, S., LAIDLER, K. J., and EYRING, H., The Theory of Rate Processes, New York, McGraw-Hill Book Company, 1941.
GOLDMAN, D. E., *J. Gen. Physiol.*, 1943, **27**, 37.
GOLDMAN, D. E., *Proc. Internat Biophysic. Cong.*, Stockholm, 1961, 236.
GOLDMAN, D. E., *Proc. 22nd Internat. Cong. Physiol. Sc.*, Leiden, 1962, **1**, 583.
HODGKIN, A. L., and HUXLEY, A. F., *J. Physiol.*, 1952, **117**, 500.
HODGKIN, A. L., and KEYNES, R. D., *J. Physiol.*, 1955*a*, **128**, 28.
HODGKIN, A. L., and KEYNES, R. D., *J. Physiol.*, 1955*b*, **128**, 61.
HODGKIN, A. L., and KEYNES, R. D., *J. Physiol.*, 1957, **138**, 253.
JULIAN, F. J., MOORE, J. W., and GOLDMAN, D. E., *J. Gen. Physiol.*, 1962, **45**, 1217.
KARREMAN, G., *Bull. Math. Biophysics*, 1951, **13**, 189.
MONNIER, A. M., and MONNIER, A., *Activities Neurophysiol.* 1959, **1**, 71.
MOORE, J. W., *Nature*, 1959, **183**, 265.
MUELLER, P., RUDIN, D. O., TIEN, H. T., and WESCOTT, W. C., *Nature*, 1962, **194**, 979.
MULLINS, L. J., *J. Gen. Physiol.*, 1959, **42**, 1013.
ROBERTSON, J. D., *Progr. Biophysics and Biophysic, Chem.*, 1960, **10**, 343.
SHANES, A. M., *Proc. 22nd Internat. Cong. Physiol. Sc.*, Leiden, 1962, **1**, 93.
TEORELL, T., *Progr. Biophysics and Biophysic. Chem.*, 1953, **3**, 305.
TOBIAS, J. M., *J. Cell and Comp. Physiol.*, 1955, **46**, 183.
TOBIAS, J. M., AGIN, D. P., and PAWLOWSKI, R., *J. Gen. Physiol.*, 1962, **45**, 989.
VILLEGAS, R., and VILLEGAS, G. M., *J. Gen. Physiol.*, 1960, **43**, No. 5, suppl., 73.