THE EXCITABLE MEMBRANE

A PHYSIOCHEMICAL MODEL

FRANKLIN F. OFFNER

From the Technological Institute, Northwestern University, Evanston, Illinois 60201

ABSTRACT The model of the excitable membrane assumes common channels for Na^+ and K^+; the two ion species interact within the pores through their electrostatic forces. The electric field varies across the membrane and with time, as a result of ionic redistribution. Ionic flow is primarily controlled by energy barriers at the two interfaces and by Ca^{++} adsorption at the external interface. When the membrane is polarized, the high electric field at the external interface acting on the membrane fixed charge keeps the effective channel diameter small, so that only dihydrated ions can cross the interface. The higher energy required to partially dehydrate Na^+ accounts for its lower permeability when polarized. Depolarized, the channel entrance can expand, permitting quadrihydrated ions to pass; the large initial Na^+ flow is the result of the large concentration ratio across the interface. The effect at the internal interface is symmetric; Na^+ crosses with greater difficulty when the membrane is depolarized. Na^+ inactivation occurs when the ion distribution within the membrane has assumed its new steady-state value. Calculations based on parameters consistent with physicochemical data agree generally with a wide range of experiments. The model does not obey the two fundamental Hodgkin-Huxley (HH) postulates (independence principle, ion flow proportional to thermodynamic potential). In several instances the model predicts experimental results which are not predicted by the HH equations.

INTRODUCTION

The excitability process in the axonal membrane, resulting from a local increase in membrane permeability (Bernstein, 1912), has frequently been described in terms of an equivalent electrical circuit. Offner (1939) and Offner et al. (1940) used such a representation (Fig. 1) in deriving the permeability change necessary for sustained conduction. Hodgkin and Huxley (1952 *a*, *b*, *c*, *d*) showed that to represent the observed phenomena such a simple circuit is inadequate, and they employed a circuit (Fig. 2) including the Na^+ electromotive force (emf).

By making the K^+ and Na^+ conductances in this circuit continuous functions of time and membrane voltage, Hodgkin and Huxley were able to describe with good accuracy the axonal current-voltage relationship. While Hodgkin and Huxley specifically stated that their equations were only intended to be an empirical description of

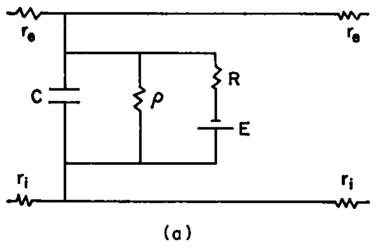

FIGURE 1

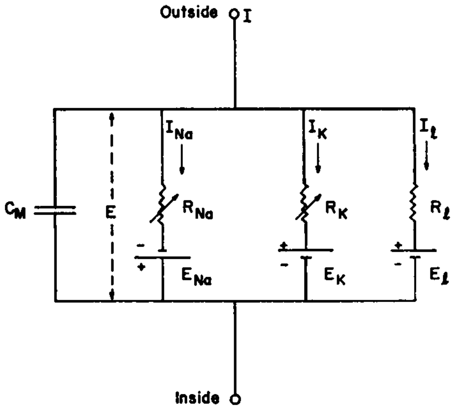

FIGURE 2

FIGURE 1 Simple equivalent circuit for the excitable membrane, based on the Bernstein hypothesis, as used by Offner et al. (1940).

FIGURE 2 Equivalent membrane circuit proposed by Hodgkin and Huxley (1952 *d*).

the ionic currents (Hodgkin and Huxley, 1952 *d*, p. 541), there are certain theoretical implications inherent in the use of an electrical circuit representation of the membrane. Thus they stated, "The driving force for a particular ion species is clearly zero at the equilibrium potential for that ion" (Hodgkin and Huxley, 1952 *a*, p. 461). This is evidently implied by their circuit (Fig. 2), since if, for example, the potential applied between the "inside" and "outside" terminals is equal to E_{Na}, I_{Na} must be equal to zero, independent of the value of R_{Na}. The difficulty with this argument is that it assumes that there is only a single component of current associated with, e.g. I_{Na}, and thus that the ion current I_{Na} flowing into the membrane from the external solution is always instantaneously equal to the current I_{Na} flowing out of the membrane into the internal solution, that is, that there can be no storage of ions within the membrane.

In fact, not only are these two components of the current not necessarily equal, they may be actually in opposing senses. That this effect is not trivial is immediately obvious from a simple calculation.

The total net influx of Na^+ during an action potential is approximately 4×10^{-12} moles/cm^2 (Hodgkin and Huxley, 1952 *d*). If all the ions flow in through the external interface of a membrane 60 A thick but none leave through the internal interface during the depolarization time, the concentration of Na^+ ions within the membrane would only increase 0.0067 M.

In general, any net flow of one cation species into the membrane will be accompanied by a simultaneous outflow of another cation species (or inflow of anions). In a 60 A membrane a change of membrane potential of 35 mv would be produced by a change of total univalent ion concentration within the membrane of 5×10^{-13} moles/cm^2, assuming uniform charge distribution and a relative dielectric constant of 5. Thus the net charge on the membrane can depart only slightly from electroneutrality.

It will further be shown that there are in fact four components to be considered in the total membrane current flow. These are the flow of the ions across each membrane interface, as discussed above; the "capacitative transient," due to charging of the double layers as a result of the electrostatic capacitance of the membrane; and finally a redistribution of ions between the double layers, due to a progressive change of the interface potentials Ψ_i after a voltage step. This last component appears to account for the nonideal nature of the membrane capacitance and also is partially responsible for the "sodium tail" currents.

The "independence principle" as assumed by Hodgkin and Huxley is stated as "The chance that any individual ion will cross the membrane in a specified interval of time is independent of the other ions which are present" (Hodgkin and Huxley, 1952 *a*, p. 467).

This principle would be a reasonable deduction from their equivalent circuit, although it is not a necessary consequence; R_{Na} and R_K could each be functions of the sum of the ionic concentrations within the membrane. In fact, if the diffusion of each ion species at each point is due to not only its own local concentration gradient but also to the local electric field, it would be expected that the independence principle would not in fact hold.

A difficulty in interpreting current flow in voltage clamp experiments is that it is not possible to distinguish unequivocally the Na^+ and K^+ components of the total current. Thus, as will be shown, a portion of the apparent Na^+ current inflow may be actually due to K^+ flow; the total flow may result in approximately the same externally measured current as would be expected if the independence principle were valid.

In the end, a better physical understanding of membrane function is obtained by abandoning the circuit representation ot the membrane and examining it directly from the standpoint of diffusion theory. To compute properly the diffusion of ions through a thin membrane, it is necessary to consider the variation of both the ionic concentrations and the electric field at each point in the membrane; thus the Goldman equation (Goldman, 1943) may not be employed. The actual computation of diffusion under such conditions involves rather complex analytical problems, which, however, are amenable to resolution by numerical methods (Offner, 1971 *a*, 1972), but the problem is not *conceptually* particularly complex.

On the contrary, this more exact approach to the membrane resolves certain apparent anomalies, as already discussed (Offner, 1970 *b*), and shows that a considerble proportion of the available experimental data can be "explained" by a rather simple model. Such data include not only the conventional voltage clamp current-voltage relationships but also such other diverse phenomena as birefringence and membrane noise variations. The model does not require that there be separate Na^+ and K^+ channels.

The equations of Hodgkin and Huxley (1952 *d*) give the membrane current as a function of membrane "holding" potential, voltage step, and time. In the present model, the dynamic performance of the membrane is given by having the permeability of the membrane at the interfaces as only a function of the interface potential Ψ_i, and at the external interface, the Ca^{++} concentration. That is, at the external interface, the permeability of the interface for Na^+ ions is given by

$$P_{0Na} = P_{0Na}(\Psi_0, [Ca^{++}]), \tag{1}$$

and similarly for K^+. The external interface is found to be primarily concerned with the depolarization process.

The variation in permeability of the internal interface is principally of importance in the recovery process; the relationship is similar to equation 1 except that no effect of $[Ca^{++}]$ is considered. Given the appropriate P functions, the flux of the individual ion species is then given by classical physiochemical theory, with a suitable membrane configuration; the time variation of the interface potentials results from the redistribution of ions within the membrane after an applied voltage step.

The problems then attacked in this paper are twofold: the establishment of a membrane configuration (fixed charge distribution and ion mobility contour) which will result in the observed membrane properties with a suitable P function; and the formulation of a reasonable physiochemical model which will result in the necessary P function, that is, the variation of the interface permeability with the boundary electric field and Ca^{++} concentration.

It should be emphasized that the principal point of this presentation is not the specific boundary processes postulated, although they appear reasonable and have the merit of simplicity, it is rather to show that the several apparently independent processes described by the HH equations are in reality the result of ionic interactions under normal physiochemical forces, granted the existence of the boundary permeability functions. Neither is it our object to provide a precise numerical fit to any specific membrane data, at least not at this time. The HH equations provide such a "quantitative description" of the membrane current, as their authors intended, granted qualitative discrepancies in certain regions; some of these are better described by the present formulation. Our present objective is rather to show that the model points in the correct direction, that there are no departures from quantitative results which are inherent in the theory, and that a more exact fit to any specific set of experimental data can be obtained by a suitable selection of parameters.

The general form of the model, in which the Na^+-K^+ cycle is the result of boundary permeability change with Na^+ "inactivation" the result of ion storage within the membrane, has been presented in several previous brief communications (Offner, 1969, 1970 *a*, 1971 *a*, *b*). In this paper the physiochemical basis of the model is more fully developed, and the consequences of the model are applied to a variety of experimental conditions. Recently Hoyt and Streib (1971) have presented a somewhat similar model of Na^+ inactivation, but with "gating" at the interior interface.

ACTIVATION ENERGY THEORY OF DIFFUSION[1]

According to the activation theory of diffusion, an ion diffusing through a medium (solute, pore, etc.) is assumed to remain normally at a potential minimum and to jump to an adjacent minimum, separated a distance δ, only when it has attained an energy greater than α, the height of the barrier separating the two minima (Fig. 3). The ion may be thought of as attaining its instantaneous energy from the ambient blackbody radiation. Assuming Boltzmann statistics apply, the mean energy of quanta of this radiation will be kT, k being Boltzmann's constant, and the mean fraction of all quanta having an energy greater than α will be $e^{-\alpha/kT}$.

By the quantum theory, $h\nu = kT$, where ν is the frequency of the quantum of radiation having an energy kT. Thus an ion at a temperature T will receive quanta of radiation of mean energy kT at a frequency ν. The mean frequency at which it attains a translational energy greater than α will thus be

$$f_\alpha = \nu e^{-\alpha/kT}, \tag{2}$$

with $\nu = kT/h = 6.3 \times 10^{12}$ at 25°C. Thus f_α is the frequency with which any ion at the potential minimum will pass over the barrier of height α. That is, an ion will remain at the minimum for a mean dwell time equal to $1/f_\alpha$. For $\alpha/kT = 12, f_\alpha = 3.7 \times 10^7$, so that the mean dwell time of an ion will be 0.27×10^{-7} sec.

It is then assumed that each ion achieving sufficient energy to pass the energy barrier instantaneously passes to the next minimum, a distance δ away. The neglect of the transit time is justified by the velocity of the ion and the short distance it moves in each jump, of the order of 10 A or less. Thus a K^+ ion having an energy $12kT$ (at 25°C) will have a velocity of the order of 10^{13} A/sec, moving with its primary hydration shell. The time to make a 10 A jump is thus less than 10^{-12} sec, and the dwell time of the ion between jumps is over 10^4 times the transit time of the ion.

It is thus seen that the flow of ions from position 1 to position 2, due to the osmotic gradient, will be

$$J_{01} = c_1\delta \cdot \nu e^{-\alpha/kT}, \tag{3}$$

where c_1 is the ionic concentration at position 1. Similarly, the osmotic flow in the counter sense will be

$$J_{02} = c_2\delta \cdot \nu e^{-\alpha/kT}, \tag{4}$$

and the net osmotic flow is

$$J_0 = J_{01} - J_{02} = (c_1 - c_2)\delta\nu e^{-\alpha/kT}. \tag{5}$$

---

[1] A more rigorous treatment of activation theory is given in Glasstone, Laidler, and Eyring, 1941, Theory of Rate Processes, McGraw-Hill Book Company, New York. A more complete discussion of its application to diffusion through membranes is given in N. Lakshminarayanaiah, 1969, Transport Phenomena in Membranes, Academic Press, Inc., New York.

On a macroscopic basis, $(c_1 - c_2)/\delta = dc/dx$. Fick's law of diffusion is

$$J_0 = -D\,dc/dx, \tag{6}$$

where D is the diffusion coefficient. Comparing equations 5 and 6,

$$D = \delta^2 \nu e^{-\alpha/kT}. \tag{7}$$

Electrodiffusion

The effect of an electric field is readily included in the activation formulation of diffusion. A constant electric field E over the interval between positions 1 and 2 produces a potential difference $-E \cdot \delta$ across the interval. If the crest of the potential barrier is assumed to be halfway between 1 and 2, half the potential difference aids the ion in passing one sense and hinders it in passing the opposite sense. Thus an ion of valence z requires an energy $\alpha - zqE\delta/2$ to overcome the potential barrier in passing from 1 to 2, while it will require $\alpha + zqE\delta/2$ to pass from 2 to 1 (q is the electron charge). If E is positive, a cation will be aided in passing over the barrier from 1 to 2 and will be hindered in passing in the opposite sense. For simplicity, the height of the barrier may be measured in units of kT, and potential in units of kT/q (i.e., in units of RT/F, 25 mv at 25°C). Then the flow from 1 to 2 becomes,

$$J_1 = c_1 \delta \nu e^{-(\alpha + \Psi_\delta/2)} \tag{8}$$

and from 2 to 1,

$$J_2 = c_2 \delta \nu e^{-(\alpha - \Psi_\delta/2)}, \tag{9}$$

$$J = J_1 - J_2 = \nu\delta(c_1 e^{-\Psi_\delta/2} - c_2 e^{\Psi_\delta/2})e^{-\alpha}, \tag{10}$$

where

$$\Psi_\delta = -E\delta. \tag{11}$$

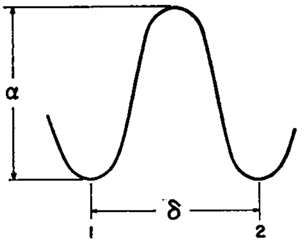

FIGURE 3

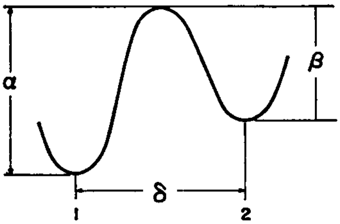

FIGURE 4

FIGURE 3 Symmetrical potential barrier.
FIGURE 4 Unsymmetrical potential barrier.

If the exponentials are approximated by the first two terms of their power series expansion, and c_1 and c_2 are replaced by their average value over the interval c_{12}, equation 10 becomes

$$J = \nu\delta^2 c_{12} E e^{-\alpha}, \tag{12}$$

where Ψ_δ has been replaced by E using equation 11. Comparing this with equation 7, (and remembering that α is now measured in units of kT), it is seen that this treatment gives the same results as the Nernst-Planck equation, provided that the electric field is sufficiently low that higher terms may be neglected and that the concentration gradient is also sufficiently low. Neither condition is met in the transition regions of the excitable membrane, so that the more precise activation treatment is preferable. It is also simpler where solution is by numerical methods.

Unsymmetrical Potential Barriers

In general, the potential barriers to ion flow will not be symmetrical; the ion will have a higher potential energy on one side of the barrier than on the other. Such a barrier is shown in Fig. 4. Here, an energy α is required for the ion to pass from left to right, but an energy β to pass in the opposite sense. Making this substitution in the expression for J_2 gives, in place of equation 10,

$$J = \nu\delta[c_1 e^{-(\alpha+\Psi_\delta/2)} - c_2 e^{-(\beta-\Psi_\delta/2)}]. \tag{13}$$

The asymmetry of the potential barrier is probably of particular significance at phase interfaces, such as at the external and internal surfaces of the membrane.

Thermodynamic Consistency

Examination of equation 13 indicates that, even in the absence of any electric field, J would not be zero, even though $c_0 = c_2$, when $\alpha \neq \beta$. This is obviously inconsistent with thermodynamics. To meet the requirement that no flow occur in the absence of osmotic and electric grandients, it is sufficient to have the sum of all the activation energies across the regime equal to zero (terms passing from right to left being taken with opposite sign). Rather than to explicitly so define the individual terms, a quasi-force may be added to the electric field acting on each ion species, given by

$$f = \sum_i (\beta_i - \alpha_i)/d, \tag{14}$$

the summation being taken over the i barriers across the regime of total thickness d. This may be viewed as having the physical significance of a relaxation force acting across the regime. For example, it might represent the force exerted by the relaxation of configurational distortion of the hydration shell of an ion. In electrochemical thermodynamic terms f would be then a term in the gradient of the electrochemical potential.

ADSORPTION

The importance of adsorbed Ca^{++} (or other divalent cations) has been previously discussed (Offner, 1970 *b*). Activation energy theory provides a convenient formulation of the problem.

The theory is based on the premise that univalent cations cannot pass a region of the external membrane surface in the immediate vicinity of an adsorbed Ca^{++} ion. The Ca^{++} is unable to enter because of its tightly bound hydration shell, held with approximately four times the energy of that of a univalent cation. The probability that any given region of the surface will be so blocked will now be calculated. The Ca^{++} ion will be bound to the membrane by two forces: electrostatic and specific chemadsorption.

The electrostatic contribution to the free energy of adsorption[2] is $-2\Psi_0$ (neglecting such small corrections as electrostriction), where Ψ_0 is the potential of the plane passing through the center of the adsorbed ions with respect to a remote point in the solution, potentials being measured in units of RT/F. The free energy of specific chemadsorption, independent of Ψ_0, is G. The total binding energy of the Ca^{++} is then $G - 2\Psi_0$.

Activation theory may now be applied to the adsorbed ion, in just the same manner as it was applied to a diffusible ion. The ion will receive energy sufficient to remove it from the boundary at a frequency $\nu e^{-(G-2\Psi_0)}$, so that τ_d, the mean dwell time of an adsorbed Ca^{++} ion, will be

$$\tau_d = \frac{1}{\nu} e^{G-2\Psi_0}. \tag{15}$$

When a patch of the surface has become free of an adsorbed ion, τ_0, the mean time before it again adsorbs an ion, will be the reciprocal of the frequency f_0 with which Ca^{++} ions impinge on this region of the surface, with such an energy that they will be adsorbed. This frequency is similarly given by

$$f_0 = k' c_{Ca} \nu e^{-G'}, \tag{16}$$

where c_{Ca} is the concentration of Ca^{++} ions in the bathing solution, G' is the free energy required for a Ca^{++} ion to leave the solution phase, and k' is a constant dependent upon the area of surface involved and the proability of capture of an impinging ion.

Except for possible second-order effects, such as the effect of the concentration of other cations on the Ca^{++} activity coefficient, all factors in equation 16 except c_{Ca} should be constant, so that equation 16 may be written

$$f_0 = k'' \nu c_{Ca}, \tag{17}$$

---

[2] The convention used is: positive free energy change results in increased adsorption.

with k'' being another constant. The time τ_0 the patch of membrane is free of an adsorbed Ca++ ion is the reciprocal of f_0 :

$$\begin{aligned} \tau_0 &= 1/k''\nu c_{\mathrm{Ca}}, \\ &= k_0'/\nu c_{\mathrm{Ca}}. \end{aligned} \tag{18}$$

T_0 , the fractional time the patch of membrane surface is free of a Ca++ ion (and therefore permeable to univalent cations), is then

$$T_0 = \tau_0/(\tau_0 + \tau_d) = \frac{k_0'}{k_0' + c_{\mathrm{Ca}} e^{G-2\Psi_0}}, \tag{19}$$

$$T_0 = \frac{k_0/c_{\mathrm{Ca}}}{k_0/c_{\mathrm{Ca}} + e^{-2\Psi_0}}, \tag{20}$$

which is the Langmuir isotherm of adsorption. Equation 20 is of significance in showing how the fractional time the membrane is permeable varies with the Ca++ concentration and with the boundary potential Ψ_0 , and that all the remaining factors can be absorbed into a single parameter k_0 , whose value is, to a first-order approximation, constant.

Including the effect of blocking, equation 13 then becomes, as applied to the external interface, for ion species i,

$$J_i = T_0\nu\delta[c_{ie}e^{-(\alpha+\Psi_\delta/2)} - c_{ii}e^{-(\beta-\Psi_\delta/2)}], \tag{21}$$

where c_{ie} and c_{ii} are, respectively, the concentrations of the ion just outside the membrane and at the potential minimum just inside, α and β refer to the height of the first potential barrier in the two senses, and δ is the distance of the first minimum from the external surface.

In the calculations, the approximation is made that Ψ_0 is equal to the boundary potential, that is, that the center of the adsorbed Ca++ ion lies at the surface plane of membrane. This may be approximately correct if the ion can enter partially into the mouth of the pore.

BARRIER HEIGHT

For an ion to enter the membrane from the bathing solution, several processes will be involved, each of which involves an energy interchange. (*a*) A hole large enough to accommodate the entering ion must be formed just within the interface. (*b*) The ion may lose part of its primary hydration shell. (*c*) The ion in leaving the solution phase will leave a hole which will disappear.

The energy of hole formation is estimated from the estimated volume of the ion, carrying as much of its hydration shell as appropriate to the conditions. The process of hole formation is assumed to differ from that classically considered for diffusion, in that the hole must be large enough to permit the ion to be displaced completely across the interface in one jump. Thus it must be large enough to admit the ion with its adhering hydration shell. The approximation of the energy of hole formation, based on the assumption that the ion passes into an essentially aqueous medium within the membrane, is given in Appendix I.

It is further assumed that the pore size is not large enough to pass a fully hydrated ion without expansion. Since the membrane carries a fixed negative charge at its surface, in the presence of an external electric field, there will be energy required to displace the membrane charges through the field. This energy is given by

$$U_e = nFE_x x/\epsilon, \tag{22}$$

where n is the number of elementary charges displaced an average distance x by the entry of one ion, E_x is the component of the electric field in the direction of the displacement, ϵ is the relative dielectric constant, and F is the Faraday constant. In the meter-kilogram-second system, U_e is given in joules per mole. If x is 3 A, $\epsilon = 3$ and $E_x = 10^6$ v/cm, $U_e = 10^8\, n$.

Dzidic and Kabarle (1970) have measured the free energy of hydration of ions as a function of the hydration number. Their experiments were made in the gas phase so that their results cannot be used to calculate directly the energy required to free an ion from successive hydrating molecules in the solution phase. The *difference* between hydration energies which they measured for K^+ and Na^+ ions should, however, be significant. They find 45 kJ/mole required to go from quadrihydrated K^+ to the dihydrated ion; the corresponding figure for Na^+ is 65 kJ/mole. A very rough estimate of the possible correction for the dehydration occurring *while* the ion is passing into the membrane diminishes the dehydration energies of each species by perhaps 20 kJ/mole; this value is, it must be emphasized, only an order-of-magnitude value, but should be approximately the same for each species.

Table I shows how the various energy terms may combine to explain the variation of the activation energy for K^+ and Na^+ with the boundary electric field. It is assumed that either ion species, when carrying two water molecules, can enter the membrane without distorting the lipid. Thus there will be no electrostatic energy term in the activation energy, even when the membrane is polarized to its resting boundary field, whose effective component is probably in excess of 10^6 v/cm. A dihydrated ion will have the two water molecules situated symmetrically across the ion. The effective diameter of the hydrated ion, moving along this axis, is therefore the diameter of the water molecule.

With four bound water molecules, they will be situated in a tetrahedral structure. This will require a larger hole diameter for entry into the membrane; it is assumed that the necessary compression of the polarized membrane to form such a hole will require of the order of 30 kJ/mole for Na^+ and 32 kJ/mole for K^+, proportional to

TABLE I

EXPLANATION OF THE VARIATION OF THE ACTIVATION ENERGY FOR K^+ AND Na^+ WITH THE BOUNDARY ELECTRIC FIELD

	Na^+ Hydration No.		K^+ Hydration No.	
	2	4	2	4
Dehydration energy	45	0	25	0
Hole formation	30	41	32	45
Electrostatic	0	30	0	30
Total, polarized	75	71	57	75
Total, depolarized	75	41	57	45

Energies are in kilojoules per mole.

the hydrated radius. The value assumed for this term is not critical to the argument; the assumed value for Na^+ is the minimum based on the other assumed terms.

It is assumed also that, in the absence of any electric field, the lipid pore has a high mechanical compliance, so that negligible energy is required to open the pore to admit a quadrihydrated ion, in the absence of a polarizing field. Again, this assumption is not essential to the model but is made for simplicity; energy of deformation would increase the activation energy for all ion species in the quadrihydrated state.

Based on these values, it is seen that K^+ ions will enter the polarized membrane in the dihydrated form, since the total activation energy is about 23 kJ/mole less than for quadrihydrated. With Na^+, however, the dihydrate and quadrihydrate require 14 and 18 kJ/mole greater energy to enter the membrane than does the dihydrate of K^+. In the depolarized membrane, Na^+ should enter with slightly lower energy than K^+; a difference of 4 kJ/mole is estimated, based on the difference of energy of hole formation.

It must be emphasized that the values used in Table I are not presented as being other than rough approximations based on physicochemical principles. The table demonstrates, however, that simple classical processes can account for the differential change in activation energies, as between K^+ and Na^+, as a function of electric field.

In the present model, the net effect of the change in the boundary electric field on the activation energy is assumed to be quadratic; that is, the combined effect of the variation in hole formation energy, dehydration energy, and electrostatic energy is such as to make the activation energy increase as the square of the boundary field. This assumption is made solely for convenience, in that it results in voltage clamp currents resembling those found experimentally. Since the precise form of the current flow is influenced by a number of parameters, however, other functional relationships could be used with approximately the same results.

In addition to the pressure developed in the lipid by the interaction of the electric field with the fixed charge, there will be other forces of much smaller magnitude, such as the dielectric force proportional to E^2 (Offner, 1970 *b*). The total effect of all these field-dependent forces will be referred to herein as "electrostriction."

STEADY-STATE SOLUTION

The steady state of the membrane is calculated by solving the equations for the flow of the various ion species as a boundary value problem. These equations are in the form 13, but where the stations are the successive potential minima across the regime. The steady state requires that the flow be the same at all stations and at the interfaces. The problem, and the method of solution, is essentially the same as when numerically solving the diffusion problem by use of the Nernst-Planck differential equation by finite differences, but here the differencing is between the assumed potential minima, rather than being an approximation of an infinitesimal. The equations can be solved as an initial value problem (shooting method, Offner, 1970 *b*), or by the difference equation method (Offner, 1971 *a*); the latter is generally preferable. In solving the equations, the electric field is calculated at each station (potential minimum), the local cation and fixed charge concentration being used in Poisson's equation. The ions are assumed to be concentrated at the potential minima, and for simplicity, the fixed charge is also assumed to be so situated.

It is convenient to employ a dimensionless system of parameters (Offner, 1970 *b*). The length parameter ξ is measured in units of the Debye length λ in the membrane in osmotic equilibrium with the external solution where $\lambda = \sqrt{\epsilon RT/2F^2c_s}$. Hence c_s is the total univalent cation concentration; it is assumed that the solution is essentially a 1:1 electrolyte, the divalent ion concentration being small. Then the dimensionless electric field φ is given by $\varphi = -\mathrm{d}\Psi/\mathrm{d}\xi$. C_i, the concentration of each cation species within the membrane, is measured in terms of c_s : $C_i = c_i/c_s$. The fixed negative charge density Q is similarly measured: $Q = q/c_0$, where q is the molar fixed charge density at any point within the membrane.[8]

In this system of units, Poisson's equation for the electric field becomes

$$\mathrm{d}\varphi/\mathrm{d}\xi = \tfrac{1}{2}\,(\sum_i C_i - Q). \tag{23}$$

The charge being localized, equation 23 reduces to a simple algebraic equation:

$$\varphi_j = \varphi_{j-1} + \tfrac{1}{2}\,(\sum_i C_{ij} - Q_j)\delta, \tag{24}$$

where φ_j is the electric field to the right of station j, c_{ij} is the ion concentration of species i at station j, and Q_j is the local fixed charge density.

BOUNDARY POTENTIAL AND FIELD

The boundary potential at the external interface Ψ_0 is related directly to the electric field φ_1 in the solution just outside. The exact relationship depends upon the nature

---

[8] Since the ions and the fixed charge are assumed located at the potential minima, the concentration is taken as the quantity so situated divided by the distance between minima.

of the interface surface and the ions present in the solution. In our previous work (Offner, 1970 *b*) the Gouy-Chapman relation

$$\varphi_0 = 2 \sinh \Psi_0/2 \tag{25}$$

was employed. This equation is only an approximation, since it assumes the ions are point charges. The result of this assumption is that a considerable quantity of charge is assumed to be stored very close to the interface, closer in fact than a hydrated ion can approach. The initial current flow, on the application of a voltage step across the membrane, is almost exclusively due to the partial discharge of the double layers at the two interfaces. Thus an incorrect estimation of the charge stored in the double layer, i.e. the capacitance of the layers, will result in an overestimation of the magnitude of the initial "capacitive" current flow. While the discrepancy is of less importance in treating the steady-state condition, as in Offner (1970 *b*), it is of importance in the transient solution.

A more accurate representation of the double layer is provided by the Stern model (Stern, 1924). In this, the double layer is represented as consisting of two parts: a flat double layer, as originally proposed by Helmholtz (1853), in which the ions are adsorbed at their distance of closest approach, and beyond this, a diffuse double layer, calculated according to the Gouy-Chapman theory.

While the Stern model would give a more accurate representation of the double layer, there are theoretical complexities in computing the relative contributions of the diffuse Gouy-Chapman and Helmholtz components. As pointed out by Bockris and Reddy (1970, p. 737), however, the diffuse double layer makes relatively little contribution to the over-all properties in electrolytes of over about 0.1 M, that is, as concentrated as exist in the axon, and the simple Helmholtz model can be used as a reasonably good approximation. This is therefore used in the present work.

The Helmholtz double layer may be looked upon as a parallel plate capacitor. The boundary field φ_0 is thus related to the boundary potential Ψ_0 by

$$\varphi_0 = \Psi_0/x, \tag{26}$$

where x is the distance of closest approach of ions in the double layer.

A layer of adsorbed ions, e.g. Ca^{++}, will increment the external electric field φ_0 by an amount

$$\Delta\varphi_0 = \tfrac{1}{2}\sigma_{Ca} \tag{27}$$

in accordance with Poisson's equation (equation 23).

TRANSIENT SOLUTION

The calculation of the activity and recovery cycles requires the solution of the time-dependent partial differential equation of the system or, more properly here, the partial derivative-difference equation. The classical method of solution of the time-dependent diffusion problem, as illustrated by, for example, the work of Cohen and Cooley (1965), uses the continuity equation

$$\partial c_i/\partial t = -\partial J_i/\partial \xi, \tag{28}$$

where the right-hand side is obtained by differentiating the Nernst-Planck equation for J_i.

In the present treatment, the ion flow is assumed to be constant between stations and to change abruptly in passing through the station (potential energy minimum). The expression equivalent to equation 28 for this case is

$$\partial c_i/\partial t = -(J_{i,j} - J_{i,j-i})/\delta. \tag{29}$$

That is, ions accumulate at a potential minimum at a rate proportional to the difference between their inflow and outflow.

The complications of the present problem are several. It is necessary to recalculate the electric field at each station after each time step. Calculations are made for voltage clamp conditions; therefore the electric field must be adjusted to hold the total potential across the membrane constant at the clamped voltage. In addition, each change in the electric field changes the adsorbed Ca^{++}. An iterative solution is required at each time step to find the ion flow which is consistent with the electric fields at the end of each time step.

This calculation procedure is quite involved; the development of the solution program has been a major problem of the present investigation. The method will be published elsewhere.

ION CURRENTS

The concept of separate ion currents appears simple. It has generally been treated as the component of the total current due to the flow of a given ion species *through* the membrane. This, however, loses sight of an important consideration; ions are stored *in* the membrane, so that the quantity of a given ion species entering one interface is not necessarily equal to that leaving the opposite side, except in the steady state. Nor will the total electrical charge inflow through one interface (obtained by summing the flow of all ion species, cation and anion) be necessarily equal to that leaving the opposite interface. There will thus in general be a net change in the electrical charge of the membrane during the activity cycle.

Since the bulk solutions remain electrically neutral, any gain or loss of charge within the membrane must be accompanied by a corresponding loss or gain in the charge stored in the electrical double layers at the two interfaces. The externally measured current (that which is observed in, for example, the voltage clamp experiment) is that existing outside the double layer region at either interface.

This system is diagramed in Fig. 5. The sum of the ion current densities into the external interface of the membrane is I_0, that out of the internal, I_d. The net electrical charge of the membrane per square centimeter is q_m, that of the external and internal double layers q_e and q_i. Then because of over-all electroneutrality, $q_e + q_i + q_m = 0$, and

$$\mathrm{d}q_e/\mathrm{d}t + \mathrm{d}q_i/\mathrm{d}t = -\mathrm{d}q_m/\mathrm{d}t. \tag{30}$$

Since $\mathrm{d}q_m/\mathrm{d}t = I_0 - I_d$,

$$\mathrm{d}q_e/\mathrm{d}t + \mathrm{d}q_i/\mathrm{d}t = I_d - I_0, \tag{31}$$

which expresses the total charge neutrality of the system.

Charge conservation at the external region gives I_e, the external current:

$$I_e = I_0 + \mathrm{d}q_e/\mathrm{d}t, \tag{32}$$

and similarly at the internal region, the external current can similarly be computed:

$$I_e = I_d - \mathrm{d}q_i/\mathrm{d}t. \tag{33}$$

The ion fluxes, and thus I_0 and I_d, are calculated at the two interfaces, using equations 21 and 13, respectively. The external currents could then be found, using either expression, by the use of equation 32 or 33.

There are difficulties in calculating either $\mathrm{d}q_e/\mathrm{d}t$ or $\mathrm{d}q_i/\mathrm{d}t$. As a first approximation, the two double layers may be considered as homogeneous capacitors. If $I_d = 0$ and the external circuit is open, then by equations 32 and 33 $\mathrm{d}q_e/\mathrm{d}t = -I_0$, $\mathrm{d}q_i/\mathrm{d}t = 0$. The change in charge produces a change in the electric field, using Poisson's equation in the form

$$\mathrm{d}\varphi_0'/\mathrm{d}t = \tfrac{1}{2}\,\mathrm{d}q_e/\mathrm{d}t = -\tfrac{1}{2}\,I_0, \tag{34}$$

where φ_0' is the boundary field over the homogeneous membrane surface, that is, over a region not including diffusion. The potential change $\mathrm{d}\Psi_0'/\mathrm{d}t$ is obtained by using the applicable equation, equations 25 and 26 being two approximations; equation 26 will be used herein.

Thus, in the system as so far described, there would be a change in the total potential across the regime at a rate given by

$$\mathrm{d}\Psi_0'/\mathrm{d}t = \tfrac{1}{2}xI_0, \tag{35}$$

using the Helmholtz approximation; but in the voltage clamp experiment the total voltage is constant after the voltage clamp step has been applied. This constancy is obtained by passing a countercurrent through the system, equal to $-\frac{1}{2} I_0$. At the external double layer then

$$d\Psi_0'/dt = \tfrac{1}{4}xI_0, \tag{36}$$

while at the internal,

$$d\Psi_d'/dt = -\tfrac{1}{4}xI_0, \tag{37}$$

so that the total voltage drop is constant. The external observed current is thus equal to $\frac{1}{2} I_0$.

A similar treatment can be applied to I_d, giving for the observed external current in the presence of flow across both interfaces,

$$I_e = \tfrac{1}{2}(I_0 + I_d). \tag{38}$$

So far, the treatment has considered only the portion of the membrane surface not responsible for diffusion, that is, where the membrane itself can be treated as a capacitor, so that the potential divides in a constant ratio between the two double layers and the membrane itself. This is not true where diffusion is concerned since, as already discussed above and in Offner (1970 *b*), the change in the ionic distribution within the membrane will change both the boundary potentials Ψ_0 and Ψ_d, and thus in general the potential across the membrane itself.

At the two interfaces we similarly have, in the region of a pore,

$$dq_e/dt = 2d\varphi_0/dt, \tag{39 a}$$

$$dq_i/dt = 2d\varphi_d/dt. \tag{39 b}$$

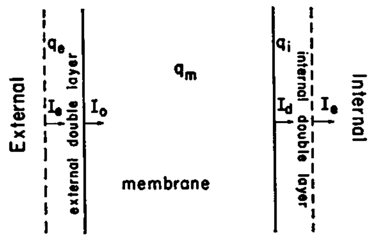

FIGURE 5

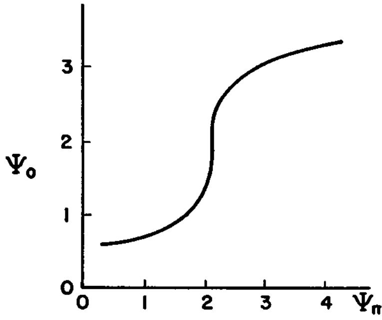

FIGURE 6

FIGURE 5 Membrane with boundary layers, showing the four components of membrane current.

FIGURE 6 Illustrative relationship between membrane boundary potential Ψ_0 and total potential Ψ_m.

Or, in terms of the two boundary potentials,

$$dq_e/dt = -\frac{2}{x}\, d\Psi_0/dt, \tag{40 a}$$

$$dq_i/dt = -\frac{2}{x}\, d\Psi_d/dt. \tag{40 b}$$

The rate of change of boundary layer charge in the region of the pores, as obtained from equations 39 or 40 is then used in equations 32 and 33. The two equations are then added, giving,

$$I_e' = \tfrac{1}{2}\,(I_0 + I_d + dq_e/dt - dq_i/dt), \tag{41}$$

where I_e' is the value of the external current as calculated in the region of a pore.

The correction terms, given by the difference between the rate of change of boundary layer charge, and thus of boundary potentials, are of particular importance in the early phase of the transient, when the potential is changing relatively rapidly.

The fraction of the external current given by equation 41 depends upon the fraction of the membrane surface responsible for diffusion, that is, occupied by pores. This is probably less than 0.05, so that the correction due to equation 41 is of minor importance, except immediately after an abrupt change in membrane potential. Thus this term is of significance in modifying the form of the capacitive transient, and also contributes to the Na^+ tail on repolarization (see below).

These calculations have been made on a one-dimensional basis, that is, considering a "smeared" charge layer. Since the pores should constitute more nearly a point charge concentration, the pore diameter surely being small compared with the membrane thickness, the calculation of I_e' by equation 41 can be considered at best only an approximation.

The recognition of the difference between the ion flux across the two interfaces is of fundamental importance to the understanding of the transient ion fluxes and especially that of Na^+. As already stated, if all the Na^+ inflow during a voltage clamp step enters the membrane through the external interface and none leaves through the internal, the Na^+ concentration of a 60 A thick membrane would increase only approximately 0.067 M. As discussed later, the present calculations show that this can be the mechanism of Na^+ current turnoff: the current ceases when the membrane is "charged" with Na^+. The internal Na^+ current does increase during recovery, that is, when the membrane is repolarized.

In the present calculations, ion currents for each species are obtained at the external interface by equation 21, and at the internal, by the appropriate application of equation 13. As a check on the accuracy of calculation, the internal flow is also computed by adding to the external flow, the rate of depletion of ions stored in the membrane.

DEPOLARIZATION

An essential factor in obtaining the abrupt change in membrane conductance, typical of axonal excitation, appears to be the existence of "voltage amplification" at the external interface (Offner, 1970 *b*). That is, the boundary potential Ψ_0 changes more rapidly than the total membrane potential Ψ_m, over some region of Ψ_m, as shown in Fig. 6, or at least changes more rapidly than would be predicted if the redistribution of ions within the membrane were not considered.

It was shown in the previous treatment (Offner, 1971 *a*) that such a relationship between Ψ_0 and Ψ_m could be obtained in the case of equimolar K^+ on the two sides of the membrane, because of the desorption of Ca^{++}. As the external K^+ concentration is reduced, however, the rate at which Ψ_0 changes with Ψ_m diminishes.

The admission of Na^+ into the membrane from the external solution, due to the lowered potential barrier, augments the effect of K^+, producing a local increase in membrane cation concentration near the external interface, resulting in voltage amplification.

The depolarization cycle may be divided into several phases. Initially, the application of the depolarizing voltage clamp step causes a drop in the potential across the boundary double layers; this is assumed "instantaneous" in the present calculations, since the double layers are assumed to be in instantaneous equilibrium, justified by the time scale of the membrane events. The fraction of the applied step which will appear at the interface, and thus instantaneously reduce Ψ_0, will depend upon the capacitance of the double layers as compared with the membrane itself. Calculation of the former will depend upon the structure assumed for the double layer (see above), while the latter can be computed from the (assumed) dielectric constant and thickness of the membrane.

Next, there is a redistribution of the ions within the membrane, especially K^+ since this is the predominant species present. This redistribution will cause a further fall in Ψ_0. At the same time, K^+ enters through the internal interface, further lowering Ψ_0 and resulting in a corresponding relaxation of the processes at the external interface (desorption of Ca^{++}, reduction in electrostriction). There is, however, initially little flow at this interface.

The continuation of the process results in a continuation of the relaxation, permitting an inflow of Na^+ to commence. A regenerative decrease in Ψ_0 occurs; Na^+ inflow first rapidly increases (it being favored by two relaxation processes), and K^+ outflow increases somewhat more slowly, primarily because of Ca^{++} desorption.

The flow of Na^+ into the membrane continues, partially displacing K^+. Only a small portion of the Na^+ entering the membrane through the external interface, however, leaves through the internal during this time, since the barrier at the internal interface is assumed to be significantly higher than for K^+ (see below, Sodium Turnoff). Thus when the replacement process has been completed, the Na^+ flow falls to a low value.

The K^+ flow, in contrast, continues to increase, so long as Ψ_0 continues to fall, and thus desorption continues to increase. The outflow of K^+, that is the K^+ conductance, is essentially proportional to τ_0 (equation 21), since K^+ flow is almost entirely limited by the external interface processes and the activation energy for K^+ changes only a small amount with Ψ_0.

When the membrane is polarized at the K^+ equilibrium potential, there is no K^+ flow. Thus the K^+ ion distribution through the membrane would not be changed by raising τ_0 (equation 20) by the desorption of Ca^{++}, or by the symmetrical lowering of any of the potential barriers to K^+ flow, except to the extent that such changes are accompanied by a change in the electric field, due to Na^+ flow.

The situation is very different with Na^+ in the polarized state. Practially the whole thermodynamic potential difference exists across the external interface. Thus the desorption of Ca^{++} and any lowering in the potential barrier to Na^+ at this point will immediately result in a large increase in Na^+ flow.

SODIUM TURNOFF

The depolarization of the membrane results, as already discussed, in a reduction in the barrier to passage of ions at the external interface. Desorption of Ca^{++} facilitates the passage of both K^+ and Na^+, but the reduction of the potential barrier for Na^+ gives a relatively greater change in Na^+ flow, which is in the inward direction. If, however, the potential barrier or barriers at or near the *internal* interface now become the limiting factor, Na^+ ions will tend to accumulate in the membrane, replacing K^+. When the replacement has reached its steady-state value, the only further Na^+ inflow will be that accompanied by Na^+ flow across the internal interface. If this potential barrier is high, the final Na^+ flow will be low. This is sodium turnoff.

A critical factor in obtaining this phenomenon is the relative effects of the external and internal barriers. Examining equation 15, it is seen that log τ_0 may be added to α and β, the potential barrier heights in the two senses, at the external interface, in calculating an effective height. This height is to be compared with the height at the internal interface; it must be higher in the polarized condition and lower in the depolarized to obtain "sodium inactivation."

REPOLARIZATION

When a depolarizing step is removed, the depolarization process is normally reversed, ionic concentrations and concomitant electric fields returning to their original values. If, however, the effect of voltage amplification is such as to give a skew-shaped curve of Ψ_0 vs. Ψ_m, repolarization may not occur. Thus a membrane, originally polarized to point A (Fig. 7), may be depolarized to point B, by reducing Ψ_m from 3.0 to 1.0. If, after the new steady state has been attained, Ψ_m is again raised to 3.0, the membrane may return to its original condition, point A. It can also return, however, to a partially depolarized condition, point C. The point to

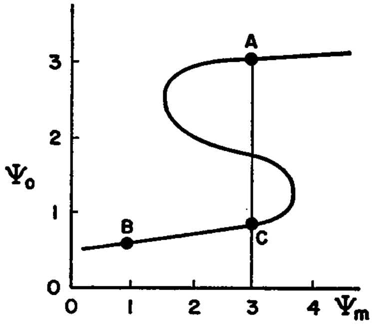

FIGURE 7

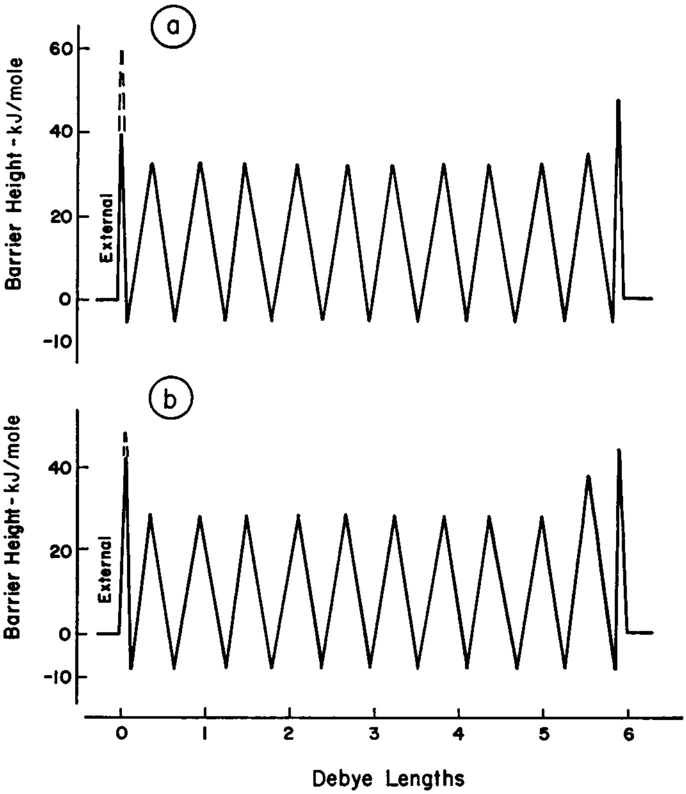

FIGURE 8

FIGURE 7 Relationship between boundary potential and total potential which could result in two stable states.

FIGURE 8 Energy barriers assumed for the membrane model. (*a*) Na^+; (*b*) K^+. External barriers shown solid for depolarized, dashed for polarized state. Internal barriers shown in polarized state.

which it actually returns when the depolarizing step is abruptly removed depends upon the dynamics of the repolarization process. The calculations indicate that it should be possible, in some cases at least, to have the membrane remain in a partially depolarized state by slowly returning the voltage clamp potential to its original value, which previously corresponded to the polarized state. It is not known whether this actually occurs in any excitable membrane. Under normal conditions it is probable that functional membranes would not have a skew characteristic, as this would make them susceptible to malfunction. It may be, however, that suitable modification of conditions, e.g. lowering of Ca^{++} concentration, could result in developing the bistable characteristic.

Although the boundary potential Ψ_0 rapidly returns to near its resting value on repolarization, and in fact may overshoot it for a period, the membrane will not return to its original state until the resting ionic distribution has been attained. This requires that Na^+ be extruded into the internal solution, across the internal potential barrier. This extrusion proceeds rather slowly; until it is complete, the membrane is in its "refractory" phase.

As above discussed (Barrier Height) the recovery process is speeded by electrostriction at the internal interface, which is the inverse of that at the external. The absolute value of the electric field at the internal interface increases (in the steady state) as the membrane is depolarized, thus increasing the electrostriction and raising the potential barrier height; conversely, it decreases on repolarization. But the transient decrease is greater than the steady-state value, because of the accumulation of ions within the membrane near the interface. This augments the fall in the local electric field, until the new steady-state ion distribution is achieved. The magnitude of this process appears to depend upon the strength of the negative-fixed charge layer near the internal interface, just as the depolarization dynamics depend upon such a layer close to the external interface.

APPROXIMATIONS OF THE MODEL

Some of the approximations of the model have already been discussed: the simplified double-layer theory and the concentration of the ions and the fixed charge on plane surfaces.

This "smeared charge" treatment permits the use of Poisson's equation in one dimension, simplifying the mathematical treatment. Some error is undoubtedly introduced thereby in calculating the double-layer currents (see above, Ion Currents).

In equation 3 the concentration factor appears in a form implying continuous divisibility of the ions. This, however, produces no error, even if the maximum concentration at a potential minimum should be only a single ion; the concentration factor is to be interpreted as the probability of finding an ion at the point, and it multiplies the exponential energy factor. Diffusion due to concentration gradients is thus ergodic, and the instantaneous spacial average is equal to the time average.

The discrete nature of the charge does, however, introduce some approximation in considering electrodiffusion (equation 8). Consider that there are only two ions in a channel, each at the potential minimum. The presence of the second ion will produce an increment in the electric field at the first and thus in the potential difference Ψ_δ. If the second ion is present only a fraction of the time, the potential increment will be effective for this fraction. To the extent that the exponential may be approximated as varying lineally, that is, if $\Delta\Psi_\delta$ is small compared with unity, the error is negligible. The interaction of ions present at various minima, as well as between those present in neighboring pores, will further reduce the error. Thus while the calculation of electrodiffusion on a continuum basis is not exact, the error due to this approximation probably does not significantly change the results.

CALCULATED MODELS

The choice of parameter values for the models calculated has been dictated by several considerations. Originally parameter values were selected purely empirically, so that the calculated voltage clamp current-voltage time relations would resemble experimental values. It has been encouraging to find that those values which have now been determinable by approximate physiochemical calculation or experiment in each case fall close to the previously assumed values. One example is the boundary activation energy (Table I). Another example is the relative energies of Na^+ and K^+ within the membrane. The calculations indicated that K^+ should be bound with an energy of the order of 5 kJ/mole more strongly than Na^+; this is now supported by experimental evidence (Davies and Rideal, 1961).

The parameters used in the present calculations give a peak inward current about 1/10 its experimental value. As a result, "capacitive" components are disproportionately large. Improved calculations will be presented in a future communication, giving results in closer quantitative agreement with experiment. However, the present calculations can be interpreted by considering the membrane capacitive currents (i.e., those representing a change in net membrane charge) to be essentially correct, but that the current flow *through* the membrane should be increased by ratio of the actual to the assumed membrane concentration. In the following, except where otherwise stated, potentials will be in units of $RT/F = 25$ mv; energy in units of kilojoules per mole, and distances through the membrane in units of Debye length λ. Specific quantities are per square centimeter of membrane surface.

The calculation of the Debye length within the membrane requires the knowledge of ϵ, the dielectric constant, and c_s, the total cation concentration in the membrane which would be in osmotic equilibrium with that in the solutions, that is, the partition fraction must be known.

The value used for the relative ϵ is 6, as already discussed (Adsorption). If the molar concentration of the external solution is 0.64 and a partition fraction of 0.03 is assumed, then in calculating the Debye length (Steady-State Solution), $c_s = 1.9$ moles/m^3, and $\lambda = 6$ A.[4]

The order of magnitude of the barrier heights at the interfaces has been estimated from the considerations previously given; the barrier heights within the membrane have been found not to have a significant effect on the results, provided they are low enough not to limit diffusion in the depolarized state. A total of 12 potential barriers is assumed; this number is again not critical, their average spacing, approximately 5 A, appears reasonable.

A high fixed charge is assumed at the two interfaces; such a distribution is required to obtain the boundary fields necessary for excitation and recovery. A lower

---

[4] The uncertainty of these figures must be emphasized. Thus proportional changes in the assumed values of ϵ and of c_s would leave the numerical results unchanged, but would proportionately change the scale factors. Similarly, changes in assumed values which result in a change in λ will change the numerical results in quantitative details, but not in their general form.

charge density is assumed through the interior of the membrane; its value affects the decay rate of the Na^+ current, a low value favoring rapid Na^+ turnoff.

In the model, the fixed charge (as well as the stored ions) is assumed to be on planes situated at the potential minima. This would result in an unrealistically large potential drop across the first potential barrier, if the boundary fixed charge were 5 A from the interface, the spacing assumed for the interior minima. Of course the charge is actually distributed over a finite width; the discrepancy is taken care of in the calculations by assuming the first potential minimum to be situated close to the interface.

In most of the calculations, the diffusion of anions has not been explicitly included. Anion flow has, however, been found to be essential in explaining such experiments as those with the internal cation replaced with Na^+ (see below). For normal conditions, however, including anion flow does not change the fundamental phenomena but complicates the model by the need for selecting a number of additional parameters. Further, the numerical problems are increased by its inclusion.

The normal univalent cation concentrations considered are, K^+ mole fraction of univalent cations in the external solution, 0.025, and 0.95 in the internal. In each case, the remainder is considered to be Na^+.

The heights of the potential barriers assumed for Na^+ and K^+ are shown in Fig. 8; the boundary barriers are shown for the normal polarized condition (internal potential -75 mv), and for depolarized. The height of the internal potential barriers is not critical, so long as they are sufficiently less than those at the boundaries. With the present numerical methods, however, low internal energy barriers tend to make the calculation of the transient solutions very time consuming. Therefore most of the calculations have been made with internal barrier heights that affect the time-course somewhat. The principal effect is on the form of the initial quasi-capacitive transient K^+ flow. This also tends to affect the appearance of the inflow current when going to the Na^+ potential.

The present calculations also do not explicitly allow for the change in the boundary electric field produced by the adsorbed Ca^{++}. On the other hand, a 2 unit increment in the electric field is assumed at the internal interface, equivalent to a layer of positive ions at that point. This, however, is only employed here as a mathematical artifice to set the net electric field, and probably has no physical significance.

The adsorption parameter $k_0/c_{Ca} = 10$ in equation 20, for normal Ca^{++} concentrations. Because of the various approximations, simplifications, and idealizations made in the present model, a given set of parameters does not give a "best fit" to all experimental data. Therefore some of the calculations are made with small changes in parameters, as noted.

CALCULATED PERFORMANCE OF THE MODEL

The results of calculation, based on the assumed model, are given in Figs. 9–30. These results are not to be considered definitive, because of the uncertainty as to the value of the various parameters and the approximations used in the model. Rather they are presented to demonstrate the qualitative effect of various factors and to show a possible physical basis of the phenomena.

Fig. 9 shows current flow calculated for a series of depolarizing voltage steps from a hyperpolarized holding potential of 3, i.e., −75 mv. As the step is increased, the inward current rises to a maximum, then decreases, and eventually disappears with a sufficiently large step. At the same time, the duration of the inward current decreases. The delayed outward current continually increases with the size of the step.

In Fig. 10 a step is made to the same final potential as the above −3 step but from the "resting" potential, −2.4, i.e., −60 mv. The peak inward current is decreased and occurs sooner, while the final outward current is unchanged.

The time-course of the change in the boundary potential is shown in Fig. 11 for a 3 unit depolarizing step. The time delay in the boundary potential change is the principal factor in determining the time-course of the membrane process.

Two features of the calculated response as illustrated in Figs. 9 and 10 differ from those calculated by the HH equations. First, there is an initial current that has been reported by Fishman (1970) and can be considered as a quasi-capacitance current; it is due to K^+ flow into the membrane at the internal interface and to a change in

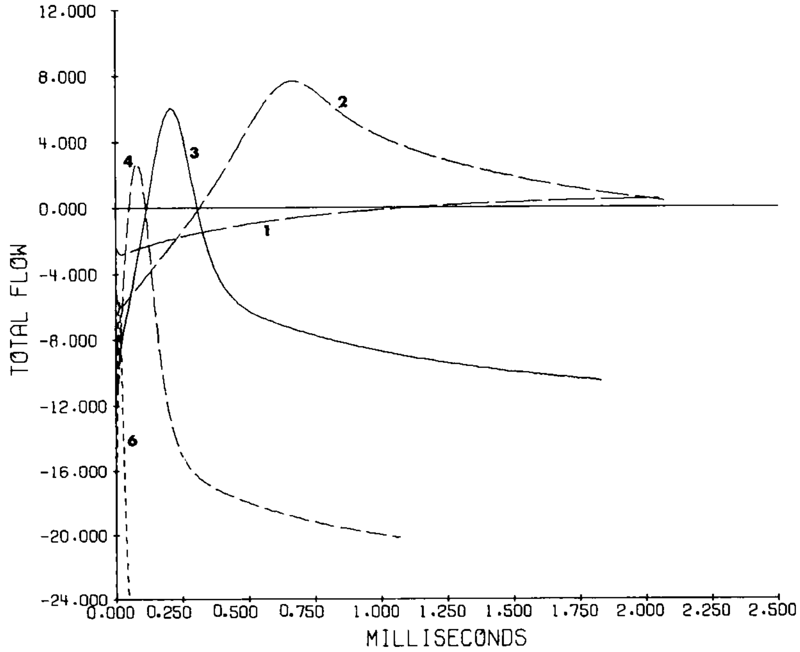

FIGURE 9 Externally measured flow resulting from depolarization steps of 1, 2, 3, 4, and 6 units from a holding potential of 3 (−75 mv internal potential). Based on assumed parameters, unit for flow in this and succeeding figures is 1.0 unit = 0.06 mamp/cm².

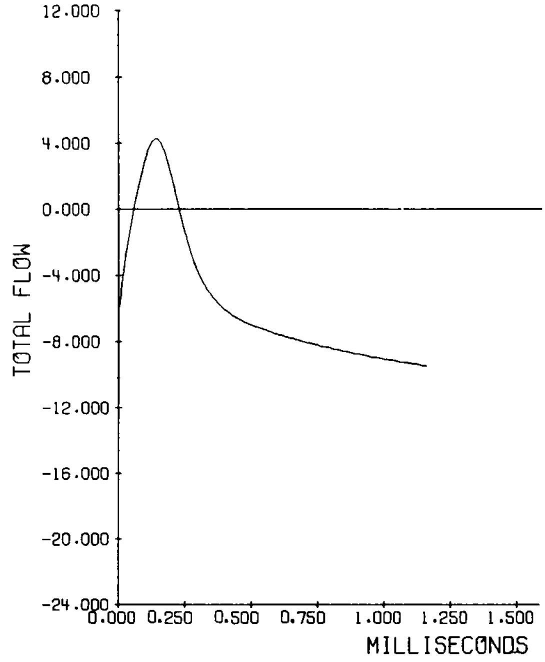

FIGURE 10 Depolarization of 2.4 units from a holding potential of 2.4; that is, to the same final potential as the 3 unit depolarization of Fig. 9.

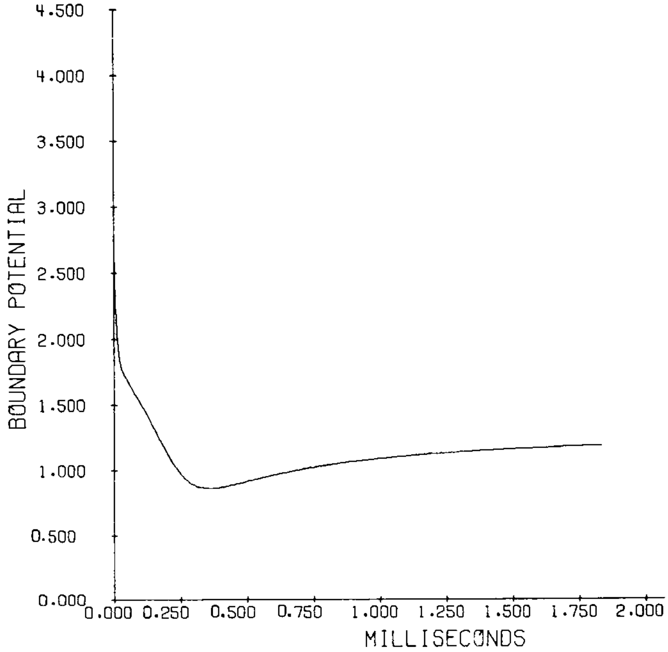

FIGURE 11 Boundary potential variation for the 3 unit step of Fig. 9.

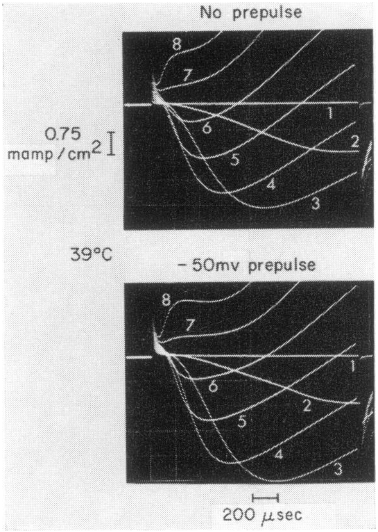

FIGURE 12 Voltage clamp data obtained from squid axon. From resting potential (upper), and with 50 mv hyperpolarization (lower). Trace 1 is for a step to 10 mv below the resting potential in each case, and successive traces are in steps of 20 mv. Records courtesy of Prof. Harvey Fishman.

ion storage in the double layers (see above, Ion Currents). The actual magnitude of this current depends upon the K^+ barrier height at the internal interface and on the fraction of the surface over which the boundary potential changes during activity.

The second difference is the small delay in the rate of rise of the inward current, produced by previous hyperpolarization, here about 50 μsec. This delay is also not shown by the HH equations, when the results are plotted for the HH standard constants. The small delay requires refined techniques for its measurement, but it is shown in the voltage clamp records, Fig. 12, for which I am indebted to Dr. Fishman.[5] These records show a delay of approximately 40 μsec produced by hyperpolarization.

Fig. 13 shows the first 0.5 msec of Fig. 9, with expanded time scale. Here another discrepancy with the HH equations is seen: an inward-directed "hump" in the current for a 6 unit depolarization to the Na^+ equilibrium potential. This is discussed below (Na^+ Potential).

If the membrane is returned to the "holding potential" during the activity cycle, there is increased Na^+ inflow, the Na^+ tail as shown by Hodgkin and Huxley (1952). A series of such calculated responses is shown in Fig. 14. In the present model, this current inflow is due to the boundary potential being at a lowered value, so that Na^+ ions flow inward across the lowered barrier, until the original boundary potential is reestablished.

---

[5] Personal communication.

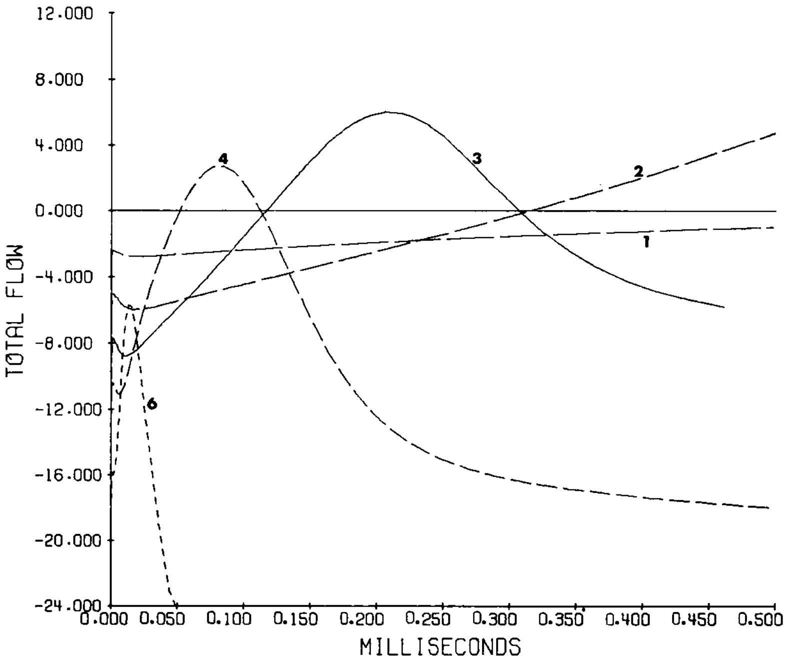

FIGURE 13 Initial portion of Fig. 9 with expanded time scale.

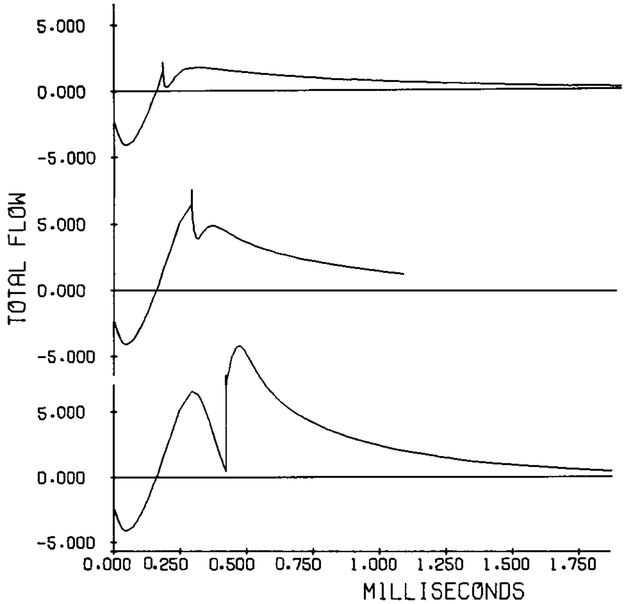

FIGURE 14 The Na^+ tail calculated for a 3 unit depolarization returning to the holding potential after successively longer times.

For brief pulses, the membrane response is substantially linear. Such responses are shown in Fig. 15 for both depolarizing and hyperpolarizing pulses. (The purely capacitive transient, theoretically of infinite amplitude and infinitesimal duration, is not shown in the figure.)

The contribution of the double-layer flow to the total current is shown in Fig. 16, based on an assumed 0.02 fraction of the surface being active. It is seen that even for this rather high assumed fraction, the double-layer component is negligibly small except immediately after a voltage step. The double-layer component is, in

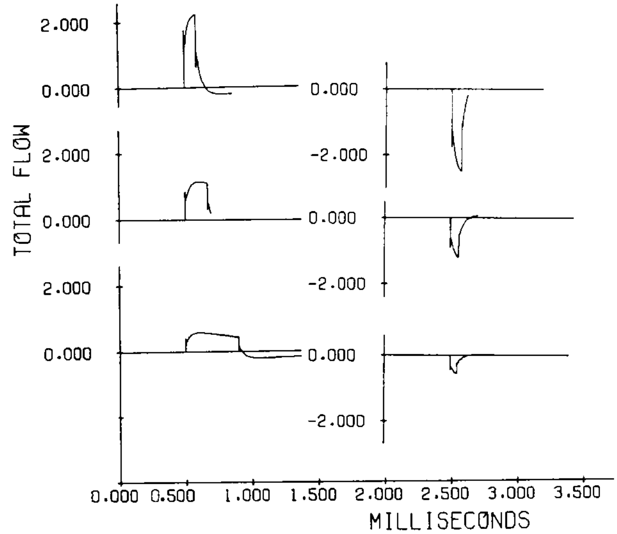

FIGURE 15 Initial currents resulting from the application of pulses of 0.4, 0.8, and 1.6 unit amplitudes. Left, hyperpolarization; right, depolarization.

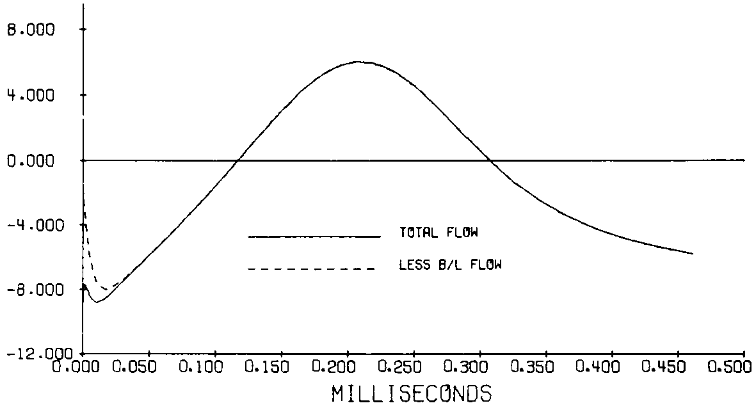

FIGURE 16 Influence of double layer (B/L) flow on the total measured current, for a 3 unit depolarization.

effect, a part of the capacitive current transient and is partially responsible for the departure of the phase angle of the capacitance from 90°.

Repolarization Currents

If the membrane is highly depolarized, as, for example, to the Na^+ potential, then, in the HH equations, the Na^+ system will be completely inactivated ($h = 0$). If, then, it is returned to the K^+ potential, there should be instantaneously zero current flow: the Na^+ system is inactivated, and there is no K^+ driving force, in HH terms.

Hodgkin and Huxley (1952 *b*) investigated this question in Na^+-free seawater and found that there was little repolarization current after the initial capacitive transient, when repolarizing after 0.63 msec.

Frankenhaeuser and Hodgkin (1956) found that with normal seawater and depolarizations of greater duration there was an inward current when repolarizing to the apparent K^+ potential. The amount of this inward transient depended on the duration and amplitude of the depolarization in such a way that they attributed it to a shift in the K^+ potential due to an accumulation of K^+ in a layer just outside the membrane, in a space constricted by Schwann cells.

While Frankenhaeuser and Hodgkin (1957) did not discuss this question, the same phenomenon is apparent in their Fig. 13. Here a large inward current is shown on repolarizing in a solution containing 112 mM Ca^{++}, but a lesser current in "Ca^{++}-free" solution. To reconcile this result with their diffusion-barrier hypothesis would require that the presence of Ca^{++} reduces the permeability of the Schwann cell layer.

In the present model, a current flow on repolarization is to be expected; the boundary potential at the external interface will rise only with a time-course, so that Na^+ will initially be free to enter, and K^+ and Na^+ will flow across the internal interface. The depolarization current depends critically on the K^+ barrier at the inner interface. The results of calculations for repolarization from steady state at the Na^+ potential are shown in Fig. 17 *a*. The three curves show the flow with the internal barrier as in the previous calculations, and with it raised 0.5 and 1 unit, that is, 1.25 and 2.5 kJ/mole. It is seen that this small change varies the repolarization inflow from a value comparable with depolarizing currents, to a very small value.

In the present model the effect of reduced Ca^{++} is shown in Fig. 17 *b*. The repolarization inflow current is reduced by reducing external Ca^{++} to $\frac{1}{100}$ its "normal" concentration.

The time-course of development of the repolarization current in the model depends, in part at least, on the flow of Na^+ across the membrane from the external interface region to the region close to the internal interface. Na^+ enters the membrane on depolarization across the external interface but leaves on repolarization by flowing into the axoplasm across the internal interface. If the depolarization is

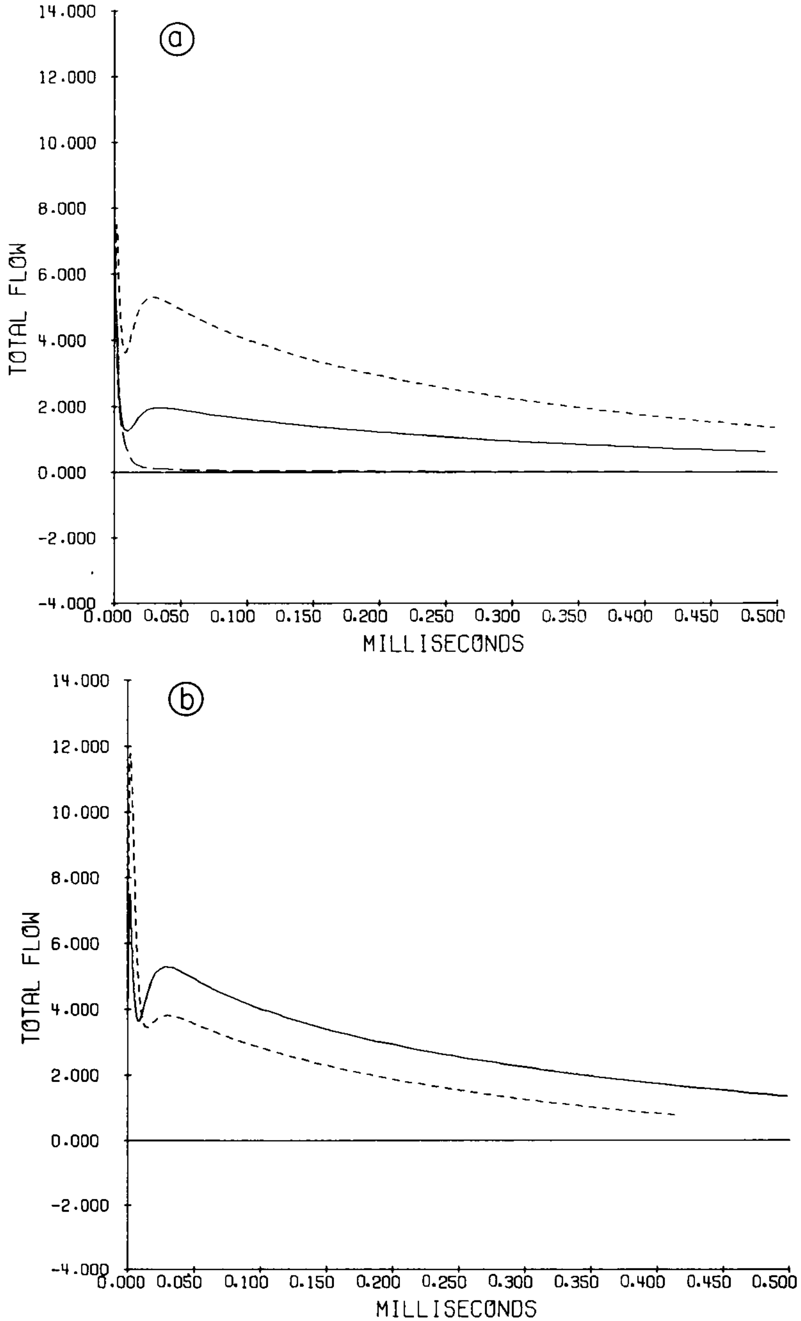

FIGURE 17 Illustrating repolarization current flow. (*a*) Repolarization from steady state at +75 mv, to −75 mv. Top curve, model described; succeeding lower curves for model with internal K^+ barrier raised 1.25 and 2.5 kJ/mole, respectively. (*b*) As preceding (top curve) but showing effect of reducing external Ca^{++} concentration. Solid curve, normal $[Ca^{++}]$; dashed curve, 1/100 normal $[Ca^{++}]$. (*c*) Repolarization to −75 mv after depolarization to zero potential; various durations of depolarization. The last curve is for repolarization after the steady state had been reached. Model as described in text.

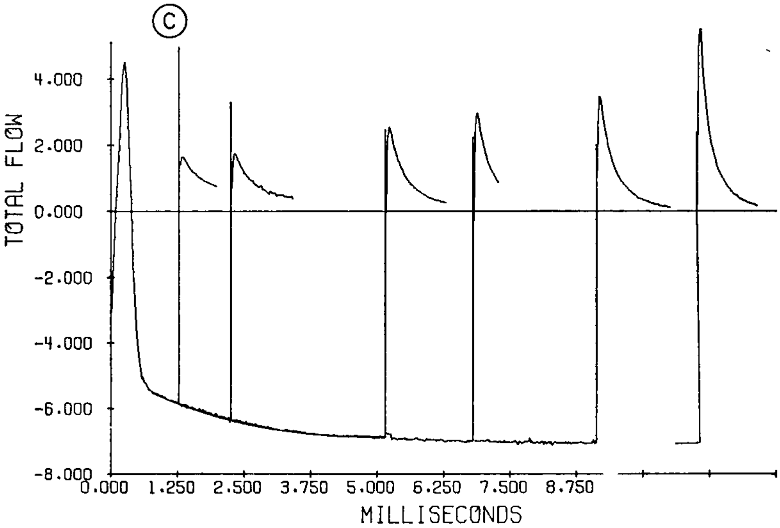

FIGURE 17 *c*

maintained for a sufficiently long time, Na^+ will have flowed across the internal diffusion barriers to accumulate at the internal interface. On repolarization, it can then rapidly commence its outflow; while with short depolarizations little Na^+ will have reached the internal interface, so that the repolarization current will be reduced.

The effect of depolarization time on repolarization currents is shown in Fig. 17 *c*. The curves are for a membrane with the internal K^+ barrier raised 1 unit, as in Fig. 17 *a*. The Na^+ barriers within the membrane are 4 units higher than in the other calculations, slowing the flow of Na^+, and thus the development of the repolarization current.

Armstrong (1971) found repolarization currents in axon treated with tetrodotoxin (TTX). This would be expected in the present model, in which TTX reduces the rate of entry of Na^+ into the membrane but does not eliminate it. Therefore Na^+ would flow to the internal interface region on a sufficiently prolonged depolarization, despite the TTX. His finding that the repolarization currents are substantially reduced by triethanolamine-like substances is consistent with the effect of raised internal K^+ barrier height, illustrated in Fig. 17 *a*. More experimental data appear necessary to characterize fully repolarization phenomena, as well as to examine the agreement of the model with experiment.

The Independence Principle and Separate Ion Currents

In actual experiments, it is not possible to measure the instantaneous values of the separate ion species currents; these are derived only from indirect evidence. They are, however, directly obtained from the calculations, which further give the component of each ion species current at the two interfaces.

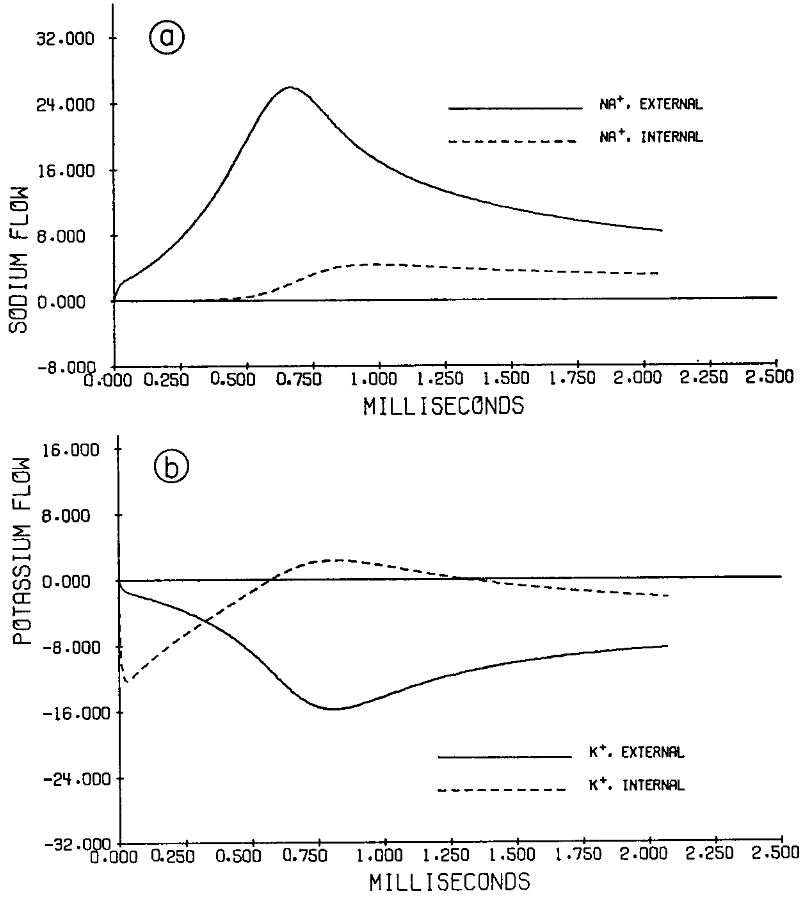

Figure 18 (*a*) Na^+ flow, and (*b*) K^+ flow at the two membrane interfaces, after a 2 unit depolarization.

Fig. 18 shows such currents for the 2 unit (50 mv) depolarization step of Fig. 9. The Na^+ flow is principally across the external interface during the depolarizing step, as already discussed. The K^+ flow shows several points of interest. There is an initial rapid increase in the K^+ outward flow at the internal interface. Averaged with the much lesser increase in flow at the external interface, this results in a rapid increase in K^+ outflow during the first 50–100 μsec.

The reduction in K^+ flow at the internal interface after the first 100 μsec is due to the inflow of Na^+, which displaces K^+ by electrostatic repulsion. With a moderately low depolarizing step, as in this example, the result is an actual K^+ inflow across the internal interface, although the net flow continues outward.

Similar calculations for a 3 unit depolarization (75 mv) are shown in Fig. 19. With the larger depolarization, the K^+ flow is outward at both interfaces. Na^+ flow is reduced at the internal interface by increased electrostriction.

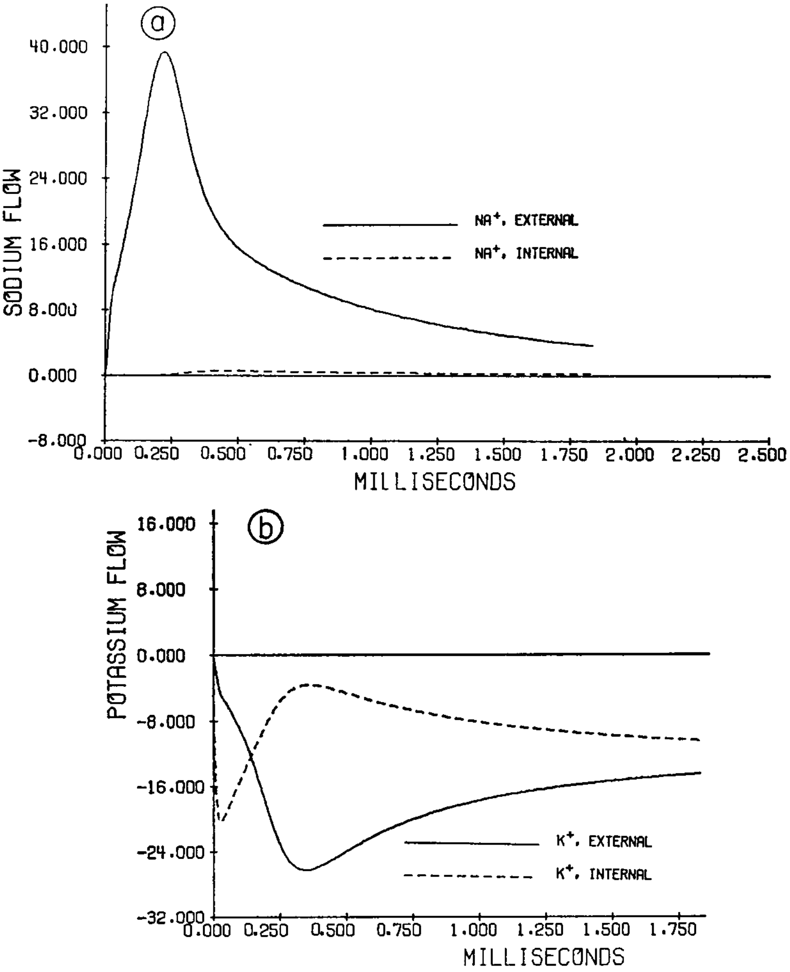

FIGURE 19 Same as Fig. 18, but for a 3 unit depolarization.

The total flow and the net K^+ and Na^+ flows through the membrane (calculated by equation 38) are shown in Figs. 20 and 21 for the 2- and 3-unit depolarizing steps, respectively. It is apparent that the flow of one ion species, in this model, profoundly affects that of the other. This is further illustrated by Fig. 22, which shows the calculated K^+ flow, the total flow, and the K^+ flow in the absence of Na^+.[6] The initial K^+ flow during the first 150 μsec is substantially independent of the presence of Na^+; this flow is due to K^+ entering at the internal interface. The K^+ flow thereafter increases more rapidly in the presence of Na^+; this is due to the Na^+

---

[6] In the calculations for this figure, the internal K^+ barrier was raised 1 unit, to correspond more nearly to experimental observations.

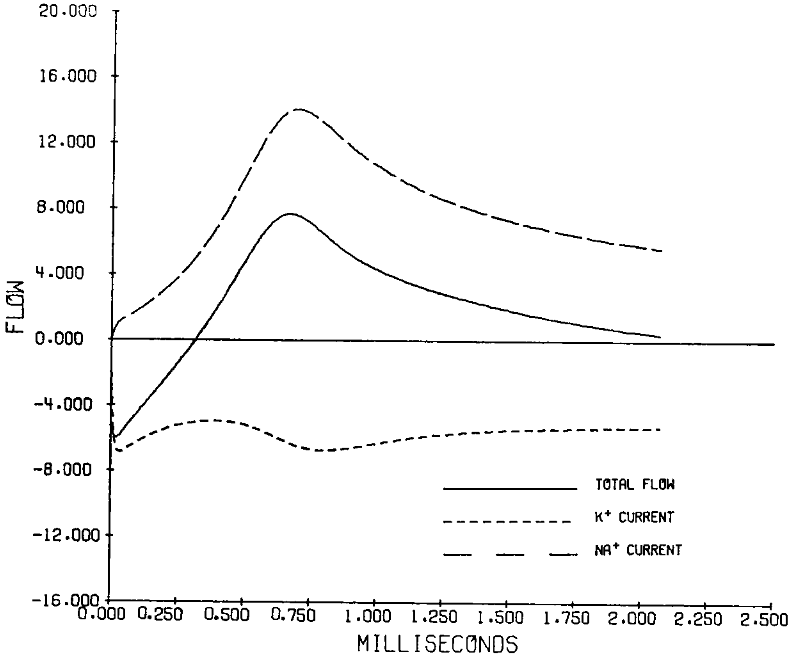

Figure 20 Total externally measured flow for a 2 unit depolarization and components of flow due to Na^+ and K^+ membrane flow.

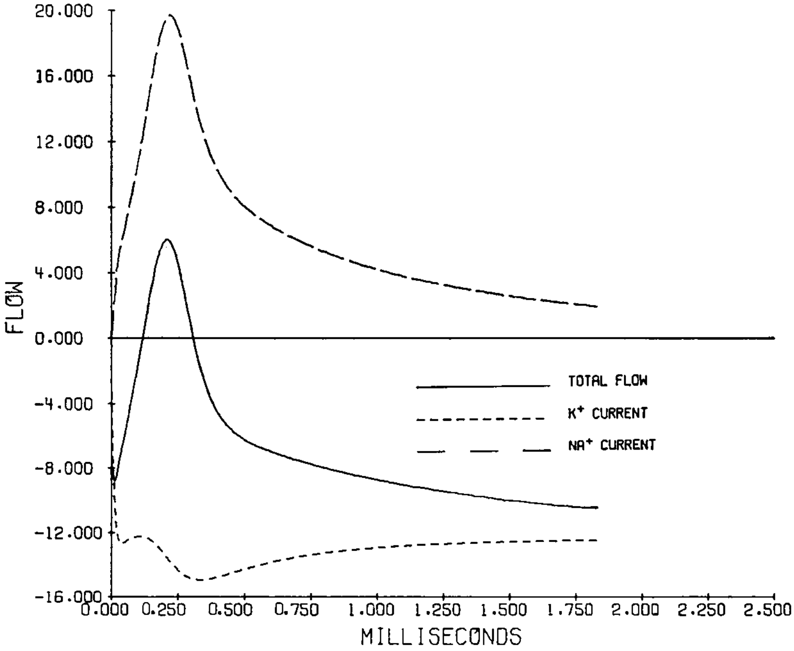

Figure 21 Same as Fig. 20, but for a 3 unit depolarization.

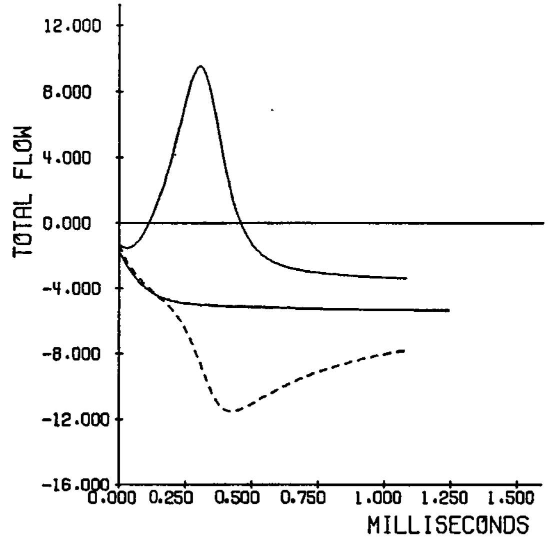

Figure 22 Illustrating departure from the independence principle. 3 unit depolarization. Upper solid curve, total flow; dashed curve, flow due to K^+; lower solid curve, K^+ flow when Na^+ is replaced by nondiffusing ion. Model has internal K^+ barrier rasied 2.5 kJ/mole.

entering through the external interface and giving rise to a rapid decrease in the boundary potential.

This last result is of interest, in view of the findings of Hodgkin and Huxley (1952 *a*). They found that their experiments seemed to indicate that the K^+ current increased more rapidly when Na^+ was present in the external solution, but in view of their having adopted the independence principle, they assumed their experimental results to be in error. If the model here presented is accepted, then the experiments of Hodgkin and Huxley can be accepted as correct.

Although there is a transient effect of Na^+ flow on K^+ flow, the steady-state current is substantially independent of the presence of Na^+, as shown by Fig. 23. Thus there is a profound departure from the independence principle in this model. It is therefore of interest to examine experiments which presumably test the validity of the principle.

One such experiment is the effect of hyperpolarization on the K^+ current. If the membrane is hyperpolarized to various voltages and then depolarized to a fixed voltage, the HH equations indicate that the K^+ current should be unchanged in form but increasingly delayed with increasing hyperpolarization.

Cole and Moore (1960) experimentally verified this prediction, under the assumption that when depolarizing to the Na^+ potential, all the current flow is due to K^+. The latter portions of their curves were superimposable, although there were variations in the initial portions.

The same results are obtained with the present model (Fig. 24), although neither

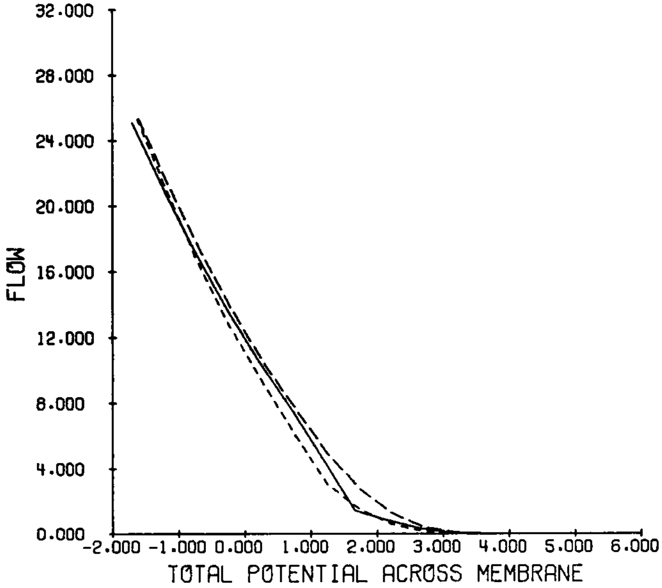

FIGURE 23 Steady-state flow across membrane as function of membrane potential (positive values are in direction of hyperpolarization). Solid line, total flow with Na^+ and K^+ present; longer dashes, without presence of Na^+. Increased flow at small depolarizations, largely due to incomplete Na^+ inactivation, so that some Na^+ flow subtracts from the K^+. Shorter dashes, for TTX model.

the independence principle nor the lack of Na^+ current applies. The results of Cole and Moore do not, therefore, confirm the validity of the HH equations. The analysis of Hoyt (1971) must be interpreted with this limitation.

The Na^+ Potential

As discussed in the Introduction, a point in the present model which differs from the HH formulation is that the model does not predict that the Na^+ current inflow will necessarily be zero when going to the Na^+ potential. This appears to be contradicted by experiment, since no inward current flow is, in general, found when depolarizing to a potential which is close to the calculated Na^+ equilibrium potential.

An example of such a calculated inward-flowing current is shown in Fig. 13, in going to the Na^+ potential. The current reversal occurs, however, in the first few microseconds of the response, and would not be resolvable from the capacitive transient, even if it existed in the form calculated. In an actual membrane, however, there would be a dispersion of membrane properties over the surface, which would shift the time of the current reversal from point to point of the membrane; the result would be a flattening of the apparent peak, so that it could probably not be observable in experiment.

An important factor in determining the amount of inflow when going to the Na^+

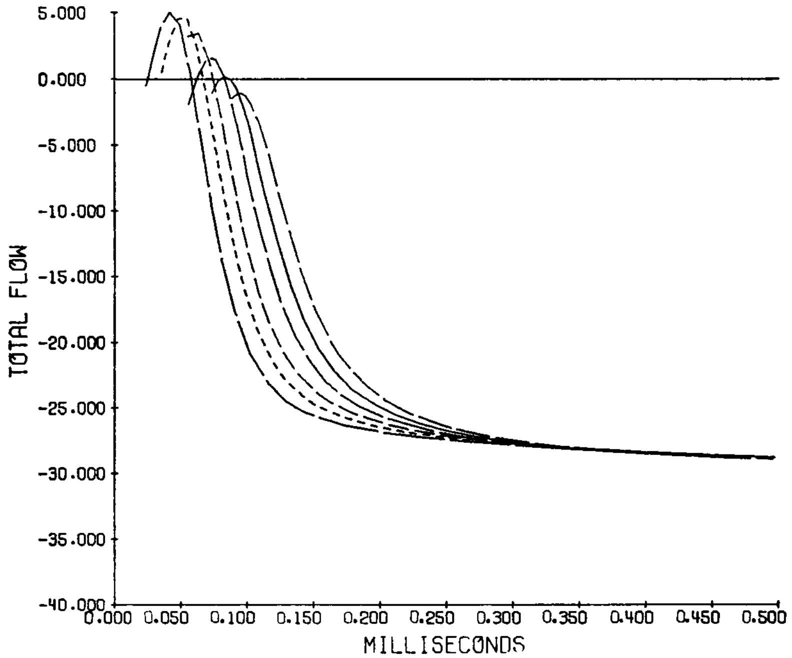

Figure 24 Effect of hyperpolarization on the K^+ process. Curves show current flow for depolarization to the Na^+ potential, from holding potentials from 70 to 110 mv (internal negative). Initial portions of curves have been omitted to avoid confusion by the Na^+ transient.

potential is the relative stored Na^+ in the membrane when at the holding potential and at the Na^+ potential; if these are the same, there will be only a small oscillation in the Na^+ current, as the new steady state is established. There will in general be cation flow into the membrane (or anion flow out) in order to reduce the boundary electric field to its new value; the change in ion concentration necessary is given by equation 23. The relative Na^+ inflow across the external interface and K^+ flow into the membrane across the internal interface (which thus appears in the externally measured current as an outward-flowing current) will depend upon the relative K^+ barrier at the internal interface and the Na^+ barrier at the external.

Chandler and Meves (1970) replaced all the internal K^+ with Na^+. Thus in their experiments any Na^+ inflow would not be obscured by the large K^+ outflow in the normal axon. They found that their replacement of the K^+ profoundly and permanently changed the membrane characteristics, but it did result in Na^+ currents which were inward when depolarizing from an internally negative holding potential to a potential more negative than the Na^+ equilibrium potential, and outward, when less negative (e.g., when internally positive). Their currents were very much slowed, the inward current lasting of the order of 10 msec.

Calculations have been made to approximate their experiment. Internal Na^+

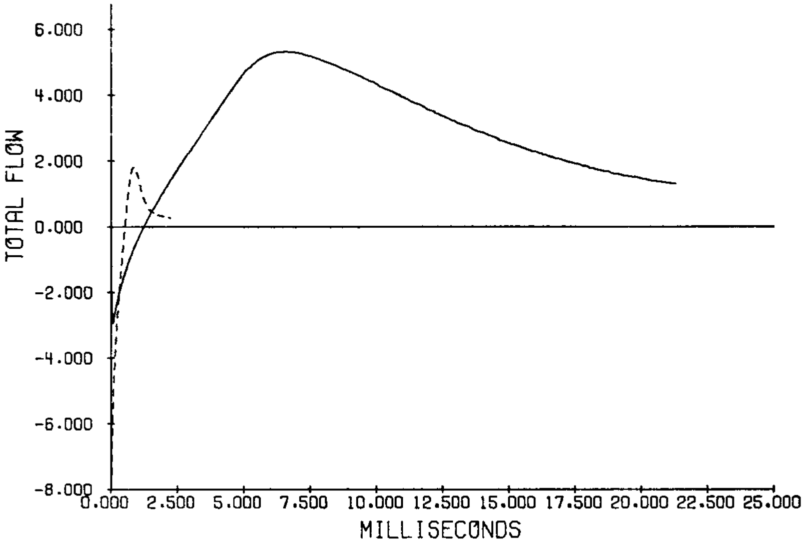

FIGURE 25 Calculated flow with internal Na$^+$ activity (concentration) 0.65 of exterior (Chadler and Meves experiment). Model includes effect of anion flow. Solid line, depolarization from −75 mv holding potential to −25 mv; dashed line, depolarization to +10.7 mv (Na$^+$ potential).

concentration was assumed 0.65 that outside the membrane, giving an equilibrium potential of 10.7 mv. In these calculations it is necessary to include the effect of anion flow, since it would not be possible to have an appreciable Na$^+$ ion accumulation in the membrane without a simultaneous anion accumulation (in the normal axon, the Na$^+$ accumulation is accompanied by a K$^+$ depletion). The relative barrier heights are such that, when depolarized, Na$^+$ tends to enter more readily from the external solution, while anions tend to enter from the internal. The results of the calculation are shown in Fig. 25.

When depolarizing to the Na$^+$ equilibrium potential, there is a small initial current inflow, but this rapidly (on the scale of these experiments) falls to close to zero. The initial inflow is that required to reduce the boundary field to its new value; it is, in effect, a capacitive transient.

The present model does not explain Chandler and Meve's findings that sustained hyperpolarization is required to "activate" the membrane with internal Na$^+$. There are, however, apparently profound and sustained changes which take place in the membrane under these conditions. A possibly significant finding in this respect is that the model has two stable states, as discussed above (Repolarization). In the model calculated, however, the second stable state is one of high conductivity, rather than of low, as found by Chandler and Meves.

Effect of Ca^{++}

The effect of varying Ca^{++} (or other divalent cation) concentration was calculated in Offner (1970 *b*) for the case of equimolar K$^+$ on the two sides of the membrane but using the Nernst-Planck treatment. Essentially similar results are found in the present model, but the calculations now are presented for both K^+ and Na^+. The Ca^{++} concentration enters into the calculations through equation 20, through which it affects T_0 ; $\Delta\varphi_0$ will also be affected, when the effect of Ca^{++} adsorption on the boundary field is included, through equation 27. This is not included, however, in the present calculations. The results of the calculations may be compared with the experimental results of Frankenhaeuser and Hodgkin (1957).

The steady-state K^+ conductance g_K is readily calculated. It is obtained from the solution of the total differential equations, g_K being defined in the same manner as in Hodgkin and Huxley (1952 *a*):

$$g_K = I_K/(\Psi_m - \Psi_K),$$

where Ψ_K is the thermodynamic equilibrium potential of K^+, approximated by

$$\Psi_K = \log_e[K_e^+]/[K_i^+],$$

$[K_e^+]$ and $[K_i^+]$ being, respectively, the external and internal concentration of K^+. The value of g_K as a function of Ψ_m is plotted in Fig. 26, for three values of relative Ca^{++} concentration: 0.2, 1, and 5 times the value used in the calculations for the previous curves. In this model, for small depolarizations a fivefold change in $[Ca^{++}]$ is approximately equivalent to a 20 mv increase in membrane potential; Frankenhaeuser and Hodgkin found values ranging from 10 to 25 mv.

Fig. 27 shows the effect of Ca^{++} concentration on the rate of rise of the Na^+ current, or more accurately, on the early component of the total current flow. It is seen that a fivefold increase in Ca^{++} concentration is compensated by a 15 mv in-

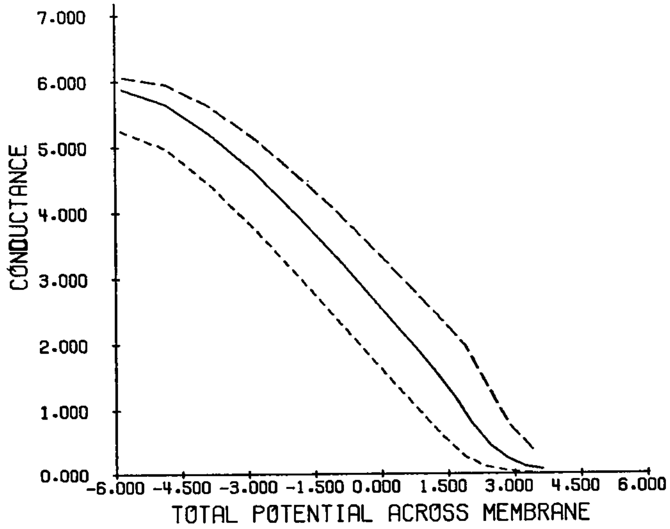

FIGURE 26 Steady-state K^+ conductance. Solid line, "normal" Ca^{++} concentration; longer dashes, 0.2; shorter dashes, 5 times normal concentration.

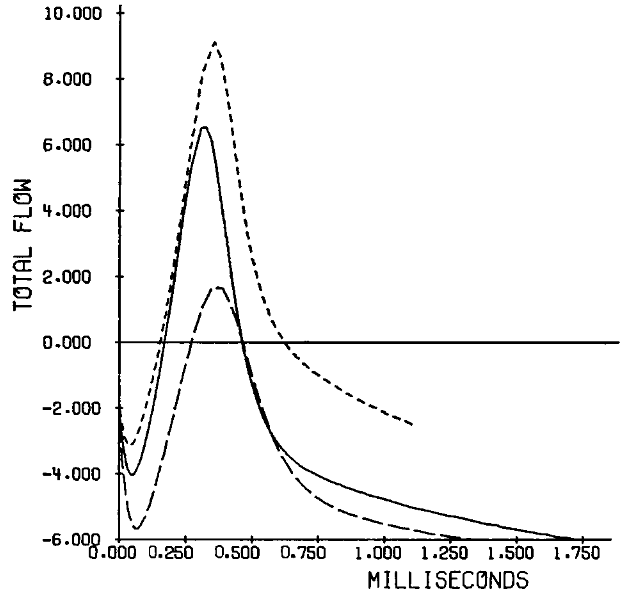

FIGURE 27 Effect of Ca^{+} concentration on rise of the Na^{+} current. Solid line, normal Ca^{++}, 75 mv depolarizing step; shorter dashes, 0.2 times normal Ca^{++}, 60 mv step; longer dashes, 5 times normal Ca^{++}, 90 mv step. All from −75 mv holding potential.

crease in depolarization, to obtain the same time to peak, in agreement with Frankenhaeuser and Hodgkins; there is more effect on the peak Na^{+} current than they find.

TTX

TTX applied externally to the membrane blocks the flow of Na^{+} (Narahashi et al., 1964) but injected internally has no effect (Narahashi et al., 1966). TTX therefore presumably acts at or near the external interface of the membrane by some presently unknown mechanism. Because of this complete lack of knowledge of its mechanism of function it is impossible to introduce explicitly its effect into the model. Since the TTX effect has been used as evidence, however, that there are separate Na^{+} and K^{+} channels, and since the present model assumes the channels are common, it appears desirable to show that there are possible mechanisms which *could* account for the effect of TTX with this model.

The hypothesis that TTX blocks the Na^{+} process by binding to a specific Na^{+} channel would, in any case, appear to be highly unlikely, and to be in conflict with other considerations. As quoted by Hille (1970), Na^{+} conductance can be blocked by as few as 13 molecules of TTX per square micron in lobster axon. If this were to correspond to the number of Na^{+} channels, the TTX molecule would have to have a remarkably specific attraction to the Na^{+} site. Activation energy considerations would require a much greater density of channels, based on barrier heights of the order of magnitude estimated herein: certainly more than 1000 channels/μ^2.

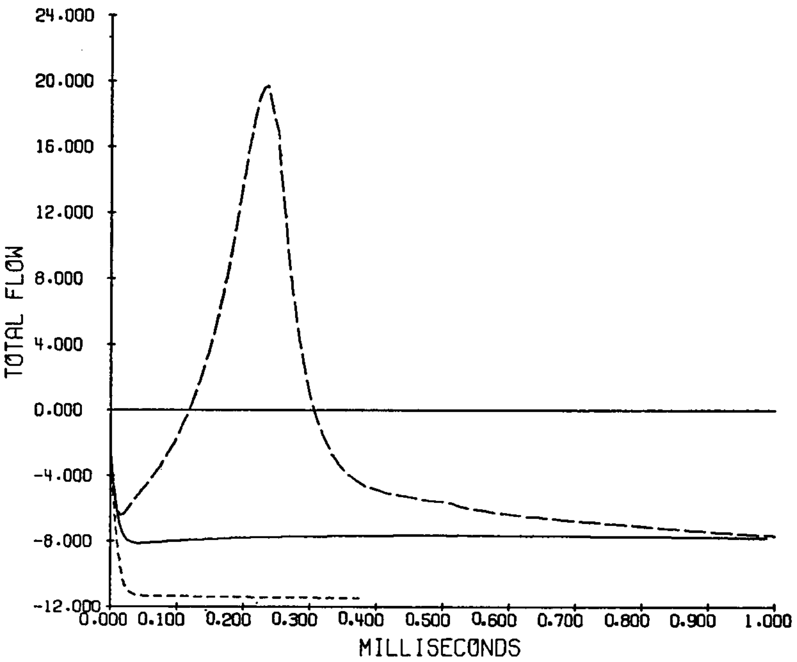

FIGURE 28 Solid line, flow after 3 unit depolarization (75 mv) with incremented boundary field (TTX model). For comparison, flow in the same model is shown without incremented field (no TTX, longer dashes), and with no Na^+ present (shorter dashes).

There are of course a number of alternative hypotheses for explaining the TTX effect. While there is presently no basis for deciding between such hypotheses, calculations have been made on one simple basis, only to show that one can obtain results approximating those obtained in experiments, with the present model, in which Na^+ and K^+ pass through common channels. This hypothesis is that TTX (and TTX-like substances) act to increase the electric field at the external interface. Calculations have been made assuming the field is increased 2 units; that is, 50 mv/Debye length.[7] Fig. 28 shows the current flow computed for a 50 mv depolarization.

Fig. 23, which showed the steady-state flow of the normal axon, and without Na^+, also shows the steady-state flow of the "TTX" model. There is little change in the net current, which is substantially all due to K^+.

Another hypothesis for the effect of TTX which has some appeal, in view of the long-lasting optical effects produced in the membrane (Cohen et al., 1968; Cohen et al., 1971) is that TTX disrupts the structure of the lipid, thereby changing the pore diameters and possibly the rigidity of the lipid surface. This could have an effect analogous to the electrostrictive effect above discussed, but could more readily account for the apparent widespread effect of a single molecule.

---

[7] The shape of the activation energy-boundary field curve is slightly different in this calculation than in the previous, the field-dependent term being decreased by 1 unit (2.5 kJ/mole).

Birefringence

A change has been found in the birefringence of the squid axon with depolarization and hyperpolarization (Cohen et al., 1968; Cohen et al., 1971). The retardation of the birefringent rays was found to vary as the square of the applied voltage step across the membrane; the change was described as occurring in two stages, the first occurring during a time of a fraction of a millisecond to several milliseconds, and the second, over a period of up to 100 msec.

A quadratic change in retardation would be expected if the effect is due to local strains produced in the membrane by the field. In this case, however, the retardation would occur within a fraction of a microsecond of the time of application of the step, if the field distribution were constant after the step, e.g., if the constant field equation were applicable.

Another prediction from a constant field hypothesis would be that the retardation should be a minimum at zero potential across the membrane. Cohen et al. (1971) find, however, that the minimum retardation occurs at 75–100 mv greater depolarization.

In the present model, the change in apparent birefringence, or retardation, can be computed under the hypothesis that it is proportional to the square of the local electric field, by integrating the field across the membrane. Since the field distribution changes as the ions diffuse, the retardation follows a time-course as the ions proceed towards their steady-state distribution.

The time-course of the retardation is plotted in Fig. 29 for depolarizing steps of

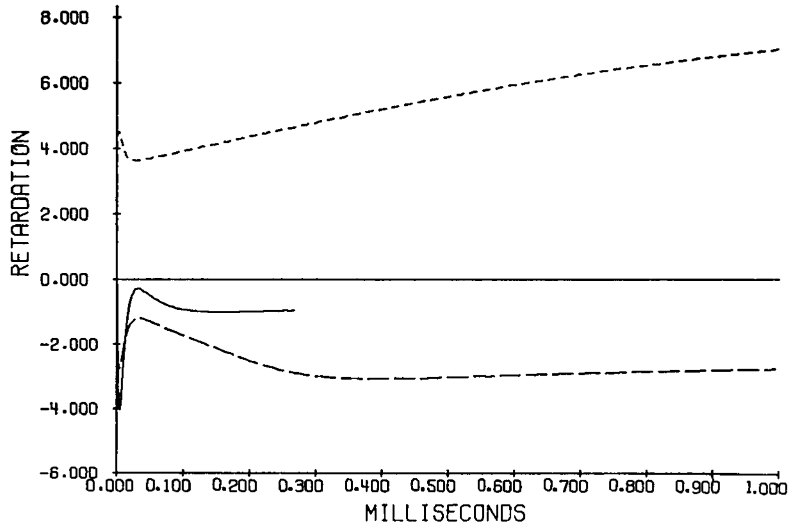

Figure 29 Calculated time-course of retardation to be expected in birefringence measurement. Solid line, 150 mv depolarization; longer dashes, 75 mv depolarization; shorter dashes, 75 mv hyperpolarization. All from −75 mv holding potential.

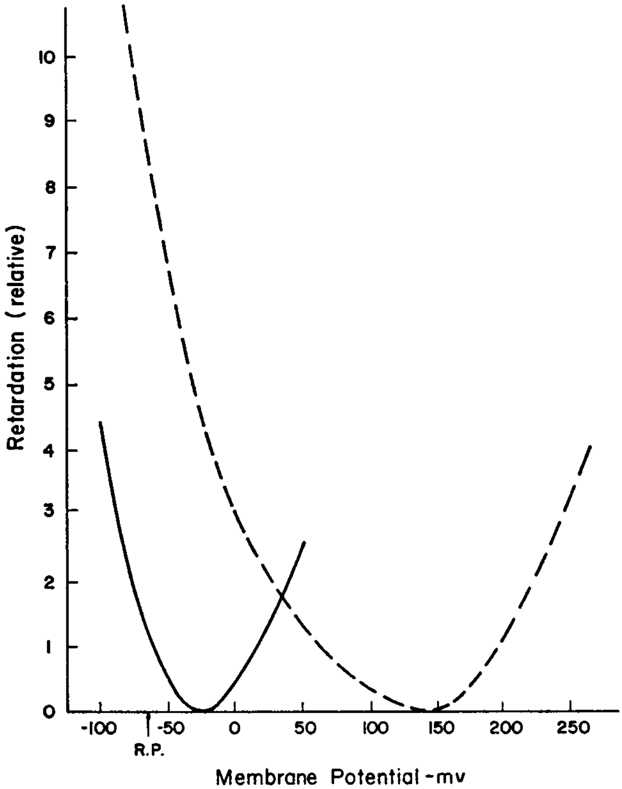

Figure 30 Steady-state retardation, as a function of total potential across membrane. Solid line, based on model described; dashed line, similar model but with boundary field incremented, as to be expected from bound Ca^{++}. R. P., resting potential. See text.

75 and 150 mv and a hyperpolarizing step of 75 mv. It is seen that with depolarization the change occurs the more rapidly, with a tendency to an overshoot. The height of this overshoot depends on the size of the step; with a depolarizing step of 150 mv, the retardation overshoots considerably beyond its steady-state value. With hyperpolarization, the change in retardation is greater than for the equal depolarizing step, but it reaches its final value more slowly.

These results appear to be generally consistent with the experimental findings of Cohen et al. (1971), but it is difficult to draw any definite conclusions, because of the variability of the experimental results, which has been taken to mean that there are several independent processes involved. While the overshoot on depolarization has been found experimentally, it is attributed by Cohen et al., at least in part, to electronic artifact (lack of complete amplifier compensation).

The steady-state values of retardation are plotted in Fig. 30. The curve of lower amplitude is for the model described; it shows a minimum retardation at −25 mv internal potential. This minimum point varies, however, with the model parameters.

The second curve is for the same model, but with the effect of bound Ca^{++} at the external interface included. It is assumed to be sufficient to raise the field 8 units; this implies 0.9×10^{-12} moles of bound Ca^{++}/cm^2 of membrane surface. These curves may be compared with Fig. 8 of Cohen et al.

DISCUSSION

The model of the excitable membrane here presented has included many simplifications, some of which have already been explicitly discussed. The attempt has been made to develop the model on the basis of purely physiochemical factors, that is, no specific chemical interactions of the ions with the membrane are considered. This is not to say that such interactions may not exist, but, at least within the range of experimental evidence so far examined, they do not appear necessary to explain observed phenomena.

Especially encouraging, in the consideration of the model, is the fact that those parameter values for which an estimate of the value is possible from physicochemical data fall reasonably close to the values which give the best fit to experimental data, when used in the calculations.

Similarly of interest is the slow response the model predicts with internal Na^+ (see above, Na^+ Potential, Fig. 24). This slowing is solely due to the tendency of the membrane-fixed negative charge to exclude anions.

No attempt has been made to do any precise "curve fitting" to match experimental data exactly. It is felt that this would neither be likely to be successful nor significant, in view of the simplifications of the model, and particularly since one model can only even approximately represent the membrane at one point, while the measured responses are surely the result of a statistical summation of varying properties.

It would be of interest to examine the effect of temperature to be predicted by the model. Temperature enters into the calculations through its explicit influence on each parameter (e.g., Debye length; potential, in units of RT/F), but it also enters implicitly into the calculation of activation energies in ways which are more difficult to calculate. This problem has not yet been attacked. The application of the model to the theory of membrane electrical noise has already been published (Offner, 1971 *c*).

I wish particularly to acknowledge the continuing valuable discussions I have had with Professor Hugh Hulburt on theory of activation and diffusion.
Doctors Barry Rubin and Sang-Hyung Kim have assisted in the physicochemical calculations.

Computations have been performed on the CDC 6400 computer at the Vogelback Computing Center, Northwestern University.
This research has been supported by U. S Public Health Service contract NS08137.

Received for publication 11 April 1972 and in revised form 12 July 1972.

APPENDIX I

We will estimate the hole formation term for the activation energy for holes in an aqueous medium, of size sufficient to accept quadrihydrated and dihydrated ions of Na^+ and K^+. Our method is based on that of Eyring (Glasstone et al., 1941, chapter IX).[1]

The hole required to accept a quadrihydrated ion will be smaller than the hole produced by removing a tetrahedron composed of five water molecules, but larger than the hole produced by removing four water molecules.

Each water molecule has four hydrogen bonds, each having a bonding energy of approximately 10 kJ/mole (Worley and Klotz, 1966; Walrafen, 1967). The total bonding energy is thus 40 kJ/mole.

If a tetrahedron containing five water molecules is removed as a group, only the three bonds at each of the four corners must be broken. Thus a total of 12 bonds are broken, as compared with 20 which must be broken if each of the molecules is removed separately. The energy of hole formation should therefore be 0.6 times the energy required to remove five water molecules, i.e., 120 kJ/mole.

To remove four water molecules a total of 10 bonds must be broken. The energy of hole formation should then be 10/16 times the energy to remove four water molecules independently or 100 kJ/mole.

The volume of the quadrihydrated ion is estimated from the volumes of dilute solutions of NaCl, KCl, and HCl. It is assumed that in dilute HCl the H^+ ion has negligible volume, so any change in volume is attributable to the Cl^- ion.[8] For dilute solutions, the molar volume of Cl^-, so calculated, is 18.2 ml.

Substracting the Cl^- ion molar volume from the total volume increase of dilute NaCl the molar volume *decrease* for the Na^+ ion is 0.9 ml. Taking the molar volume of water as 18 ml, the molar volume of the quadrihydrated Na^+ ion is 71.1 ml, or approximately the same as that of four water molecules alone; the energy of hole formation is taken as 100 kJ/mole.

For KCl, the molar volume increase due to the K^+ ion is 9.4 ml. The volume of the quadrihydrated ion is thus approximately that of 4½ water molecules; the energy of hole formation is taken as halfway between that for removing four, and for removing five water molecules, or 110 kJ/mole.[9] Energy of hole formation for the dihydrated ion is calculated in a similar manner but based on the removal of two and three water molecules.

The dihydrated ion is assumed to be linear, with the two water molecules disposed symmetrically across the ion. This results in an effective volume molar V_i given by

$$V_i = (2r_w + r_i)/r_w \cdot V_w,$$

where V_i is the molar volume of the dihydrated ion, r_w is the radius of a water molecule, taken as 1.5 A, r_i is the ionic radius of the ion, and V_w is the molar volume of water.

Six hydrogen bonds must be broken to remove two water molecules, and eight, for three molecules. The corresponding energies are 60 and 80 kJ/mole. The effective molar size of the dihydrated Na^+ ion so calculated is 2.65 times the molar volume of water, for K^+, 2.9. The corresponding energies are 73 and 78 kJ/mole.

The energy of hole formation is multiplied by a factor of 0.41, in accordance with the theory of Eyring. This correction factor was obtained from experimental data by Eyring, and his theoretical basis is not completely clear. It may, however, be related to the energy available from the residual hole which the ion leaves, in assisting the formation of the hole into which the ion goes.

These results must be considered only very approximate. Not only is Eyring's theory only

---

[8] Any error due to neglecting the volume of the H^+ ion would be constant for all species and would not change the results appreciably.

[9] The justification for interpolating the energies is that the structure of water is not rigid, so that the number of water molecules displaced is a most probable value, which would vary randomly about this value.

approximately verified by experiments, but numerous additional assumptions are made in the present calulations. The purpose of the calculations is therefore to give an order of magnitude, rather than an exact value.

APPENDIX II

An order of magnitude for the minimum number of membrane pores per unit area is here calculated on the basis of activation energy. Evaluation 2 gives the rate of occurrence of events having an energy greater than the barrier height α. If there is a potential difference Ψ_δ across the barrier, then equation 2 must be modified in a similar manner to equation 8:

$$f_\alpha = \nu e^{-(\alpha+\Psi_\delta/2)/kT}. \qquad (\text{A } 1)$$

Depolarization currents occur when the electric field is presumably reduced to a low value, so that the potential drop Ψ_δ across the interface barrier is low. Therefore only α, the barrier height itself will be used, i.e., equation 2 is used unmodified.

If for Na^+ we take the depolarized value for α from Table I as 41 kJ/mole, $\alpha/kT = 16$, and $f_\alpha = 6.3 \times 10^5$. This then indicates a maximum limit of 10^{-13} A/pore saturation current, if every "event" resulted in a Na^+ ion traversing the barrier. This is certainly not correct; if the boundary field is 3×10^6 v/cm and is the result of a Helmholtz layer 4 A thick, then the univalent cation density in the layer would be approximately 1000 A^2/ion. Thus certainly less than 1 event in 20 will result in the passage of an ion and probably more nearly 1 in 100. Using the latter value, the saturation current for a pore is 10^{-15} A. Thus to obtain a peak current of 1 mamp/cm^2 would require 10^{12} pores/cm^2 = 10^4 pores/μ^2.

Hille (1970) uses a peak Na^+ inflow of 4.5 mamp/cm^2 in his calculations, but this is for the Na^+ tail on repolarization, so that Ψ_δ in equation A 1 can not be neglected. If $\Psi_\delta = -50$ mv this would require approximately 2×10^4 pores/μ^2.

If each pore adsorbs one Ca^{++} ion, then, using the assumptions in the text, this pore density would cause an increment in the boundary electric field of 6 units, i.e., 2.4×10^6 v/cm, when all pores are so blocked.

REFERENCES

Armstrong, C. M. 1971. Interaction of tetraethylammonium ion derivatives with the potassium channels of giant axons. *J. Gen. Physiol.* **58**:413.

Bockris, J. O. M., and A. K. N. Reddy. 1970. Modern Electrochemistry. Plenum Publishing Corporation, New York.

Bernstein, J. 1912. *In* Electrobiologie. Friedrich Vieweg and Sohn, Braunschweig, W. Germany. 95.

Chandler, W. K., and H. Meves. 1970. Sodium and potassium currents in squid axons perfused with fluoride solutions. *J. Physiol.* (*Lond.*). **211**:623.

Cohen, H., and J. W. Cooley. 1965. The numerical solution of the time-dependent Nernst-Planck equations. *Biophys. J.* **5**:145.

Cohen, L. B., B. Hille, R. D. Keynes, D. Landowne, and E. Rojas. 1971. Analysis of the potential-dependent changes in the optical retardation in the squid giant axon. *J. Physiol.* (*Lond.*). **218**:205.

Cohen, L. B., R. D. Keynes, and B. Hille. 1968. Light scattering and birefringence changes during nerve activity. *Nature* (*Lond.*). **218**:438.

Cole, K. C., and J. W. Moore. 1960. Potassium current in the squid giant axon: dynamic characteristics. *Biophys. J.* **1**:1.

Davies, J. T., and E. K. Rideal. 1961. Interfacial Phenomena. Academic Press, Inc., New York.

Dzidic, I., and P. Kebarle. 1970. Hydration of the alkali ions in the gas phase. *J. Phys. Chem.* **74**: 1466.

FISHMAN, H. 1970. Leakage current in the squid axon membrane after application of TTX and TEA. *Biophys. Soc. Annu. Meet. Abstr.* **10:**109a.

FRANKENHAEUSER, B., and A. L. HODGKIN. 1956. The after-effects of impulses in the giant nerve fibers of *Loligo. J. Physiol.* (*Lond.*). **131:**341.

FRANKENHAEUSER, B., and A. L. HODGKIN. 1957. The action of calcium on the electrical properties of squid axons. *J. Physiol.* (*Lond.*). **137:**218.

GOLDMAN, D. 1943. Potential, impedance, and rectification in membranes. *J. Gen. Physiol.* **27:**37.

HELMHOLTZ, H. L. F. v. 1853. *Ann. Phys.* (*Leipzig*). **89:**211.

HILLE, B. 1970. Ionic channels in membranes. *Prog. Biophys. Mol. Biol.* **21:**1.

HODGKIN, A. L., and A. F. HUXLEY. 1952 *a.* Currents carried by sodium and potassium ions through the membrane of the giant axon of *Loligo. J. Physiol.* (*Lond.*). **116:**449.

HODGKIN, A. L., and A. F. HUXLEY. 1952 *b.* The components of membrane and conductance in the giant axon of *Loligo. J. Physiol.* (*Lond.*). **116:**473.

HODGKIN, A. L., and A. F. HUXLEY. 1952 *c.* The dual effect of membrane potential on sodium conductance in the giant axon of *Loligo. J. Physiol.* (*Lond.*). **116:**497.

HODGKIN, A. L., and A. F. HUXLEY. 1952 *d.* A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* (*Lond.*). **117:**500.

HOYT, ROSALIE. 1971. Independence of the sodium and potassium conductance channels. A kinetic argument. *Biophys. J.* **11:**110.

HOYT, ROSALIE, and J. D. STREIB. 1971. A stored charge model of the sodium channel. *Biophys. J.* **11:**868.

NARAHASHI, T., H. C. ANDERSON, and J. W. MOORE. 1966. Tetrodotoxin does not block excitation from inside the nerve membrane. *Science* (*Wash. D. C.*). **153:**765.

NARAHASHI, T., J. W. MOORE, and W. R. SCOTT. 1964. Tetrodotoxin blockage of sodium conductance in lobster giant axons. *J. Gen. Physiol.* **47:**965.

OFFNER, F. 1939. Circuit theory of nerve conduction. *Am. J. Physiol.* **126:**594.

OFFNER, F. 1969. Interface kinetics in excitable membranes. Abstracts of the 3rd International Biophysical Congress of the International Union for Pure and Applied Biophysics. Cambridge, Massachusetts.

OFFNER, F. 1970 *a.* Diffusion theory of axonal excitability. *Biophys. Soc. Annu. Meet. Abstr.* **10:**111a.

OFFNER, F. 1970 *b.* Kinetics of an excitable membrane. Voltage amplification in a diffusion regime. *J. Gen. Physiol.* **56:**272.

OFFNER, F. 1971 *a.* Nernst-Planck-Poisson diffusion equation: numerical solution of the boundary value problem. *J. Theor. Biol.* **31:**215.

OFFNER, F. 1971 *b.* Theory of ionic diffusion through excitable membranes. Annual Conference on Engineering in Medicine and Biology, 24th Meeting. 116. (Abstr.)

OFFNER, F. 1971 *c.* Quantitative measurement of $1/f$ noise and membrane theory. *Biophys. J.* **11:**969.

OFFNER, F. 1972. Numerical solution of the transient ionic diffusion equation. *Biophys. Soc. Annu. Meet. Abstr.* **12:**264a.

OFFNER, F., A. WEINBERG, and G. YOUNG. 1940. Nerve conduction theory: some mathematical consequences of Bernstein's model. *Bull. Math. Biophys.* **2:**1485.

STERN, O. 1924. *Z. Electrochem.* **30:**508.

WALRAFEN, G. E. 1968. *J. Chem. Phys.* **48:**224.

WORLEY, J. D., and I. M. KLOTZ. 1966. *J. Chem. Phys.* **45:**2868.