J. Membrane Biol. 6, 329–352 (1971)

Ionic Diffusion in Membranes

I. A Kinetic Model for the Squid Axon Conductances

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Received 26 April 1971

Summary. A fundamental approach to calculate the diffusion of ions through membranes is introduced. The membran eis considered as a heterogeneous structure with molecules that can have a selective affinity for a certain class of diffusing ions. To diffuse through a membrane an ion must become associated with, or dissolved into, at least one component of that membrane. Diffusion is produced by thermal jumps from one molecular site to another. It is assumed that the electric field can change the binding properties between ions and membrane molecules. The kinetics of the conductances are calculated from the chemical kinetic theory. The calculations are compared to the squid axon data and the unknown parameters are adjusted to fit the data curves. The results are very satisfactory. The calculated activation energies correspond to the measured Q_{10} in the squid axon. The calculated and measured action potentials are quite similar.

Biological membranes appear to be exceedingly complex and non-homogeneous. Their exact composition and structure is not known and there is evidence that intricate differences exist among their various types. It will be assumed that the membranes are made up of proteins and lipids. Proteins are present at the surface as well as inside the membranes. Some proteins are quite specific with respect to their affinities to specific ions; they may serve as channels for conduction in biological membranes. The presence of macromolecules with special properties can give the membrane very complex and selective diffusion properties.

We shall consider the case of an infinite two-dimensional membrane separating two aqueous phases and having molecular groups among which some have a special affinity for the diffusing species. Diffusion would proceed by jumps from one site to another. We shall calculate the mobility

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and the concentration of diffusing species in the membrane. The general expressions thus obtained will be compared with the observed voltage-current relationships in the squid axon.

Ionic Diffusion in a Lattice

The basic equation to describe ionic diffusion in liquids and solids is the well known Nernst-Planck electrodiffusion equation

$$J = CU\left(\frac{d\mu}{dx} + zF\frac{d\psi}{dx}\right) \tag{1}$$

where C represents the concentration of diffusing ions, U their mobility, $d\mu/dx$ the chemical potential, $d\psi/dx$ the electrical potential, z the valence and F the Faraday constant.

The theory of lattice diffusion in solid state physics and in the physics of liquids has been well developed by Jost (1952), Frenkel (1955) and Shewmon (1963) to name a few. Because of their thermal energy, atoms are always in movement in their lattice sites with a distribution of velocities. There is a fraction that have sufficient energy to overcome the lattice energy barrier and move to the surface of the crystal or alternatively into an interstitial site or into a lattice vacancy. Thermal diffusion and ionic flow in response to external force fields is a sequence of atomic jumps from filled lattice sites to vacant ones. Although we realize that the molecular structure of cell membranes is heterogenous and anisotropic we shall use expressions from statistical mechanics developed for homogenous isotropic crystals. In these, the atom trapped in a lattice site must overcome the free energy barrier of the lattice, ΔF_d, to jump to another site. With the frequency of lattice vibrations v and the distance d between vacancies it is possible to calculate the mobility (Jost, 1952):

$$U = \frac{d^2 v}{RT} e^{-\Delta F_d/RT} \tag{2}$$

$$\Delta F_d = \Delta E_d - T\Delta S_d .$$

ΔS_d is the entropy difference and ΔE_d is the barrier energy. We realize that ΔF_d could be different at the interface and inside the membrane; the largest value of ΔF_d should be used to calculate the mobility. ΔE_d can be determined by appropriate temperature measurements.

Eq. (1) is an approximation of a more general expression calculated with the lattice diffusion theory

$$J = CU\left[\frac{d\mu}{dx} + \frac{2RT}{d}\sinh\left(\frac{z}{2}\frac{Fd}{RT}\frac{d\psi}{dx}\right)\right]. \tag{3}$$

If $d\psi/dx < RT/z\,Fd$, Eq. (3) is reduced to Eq. (1).

Interaction of Ions with Membrane Molecules

To apply the above theory to describe ionic diffusion in membranes additional specifications must be introduced. We must take into account that ions are present in two phases and that their concentrations in each phase can be quite different. The parameter C in Eqs. (1) and (3) represents the concentration of ions in the membrane phase. Since the value of C is not easily measured experimentally, some reasonable formulation must be introduced to calculate it from the known concentrations in the water phase.

We know that the solubility of ions in nonpolar lipid phases of the membrane is exceedingly low. We thus assume that among the non-lipid components there are channel molecules or molecular groups M. These may be distinguished by their affinity for diffusing ions Q. The molecule M can interact with Q, either in a solvation process or by forming a chemical bond with it.

This interaction between Q and M could involve many steps if M is a large molecule. It is possible that many ions have to interact with M before a diffusing channel is formed. The general stochiometric equations will be the following:

$$\begin{aligned} QW + M &= Q_1 M + W, \\ QW + Q_1 M &= Q_2 M + W \end{aligned} \tag{4}$$

$$\vdots \qquad\qquad \vdots$$

$$\begin{aligned} QW + Q_{n-2} M &= Q_{n-1} M + Q, \\ QW + Q_{n-1} M &= Q_n M + W. \end{aligned} \tag{5}$$

Steady state concentrations are given by

$$\begin{aligned} K_1 &= \frac{C_{Q1M} \cdot C_W}{C_M \cdot C_{QW}} \\ &\vdots \\ K_n &= \frac{C_{QnM} \cdot C_W}{C_{Qn-1M}\, C_{QW}}. \end{aligned} \tag{6}$$

$K_1 \ldots K_n$ are the equilibrium constants.

$$K_i = e^{-\Delta F_{ci}/RT} \qquad i = 1, 2, \ldots n. \tag{7}$$

ΔF_{ci} is the free energy difference between the two states of ions Q. Since the quantity of M is limited, it is necessary to introduce its total concentration, C_{MT}

$$C_{MT} = C_M + C_{Q1M} + C_{Q2M} + \cdots C_{QnM}. \tag{8}$$

With the system of Eqs. (6) and (8), the value of C_{QnM} is calculated:

$$C_{QnM} = \frac{C_{MT}}{1 + K_n^{-1}\frac{C_W}{C_{QW}} + K_n^{-1}K_{n-1}^{-1}\left(\frac{C_W}{C_{QW}}\right)^2 + \cdots K_n^{-1}K_{n-1}^{-1}\ldots K_1^{-1}\left(\frac{C_W}{C_{QW}}\right)^n}. \tag{9}$$

It is important to note that the equilibrium constants, K_1, $K_2 \ldots K_n$ are probably all different from each other. If M is a large molecule, there can be many binding sites and each can have a different binding constant. The molecules M may also deform as they accept Q. The possibility of allosteric changes in membranes has been postulated by several authors, particularly by Blumenthal, Changeux and Lefever (1970). Our treatment is not in disagreement with such models. It is possible that many types of ions Q can compete for the same molecule M. The framework proposed here to evaluate the concentration of a diffusing species in a membrane is fairly general and could be adapted to many different situations. We shall introduce other types of interactions between Q and M as demanded by the experimental evidence.

We postulated the existence of at least one type of membrane molecule M, that participates in development of ionic diffusion across the membrane. Two types of roles may be postulated for M. One may visualize that the molecule may literally participate in transport by "carrying" specific molecular groups from one side of the membrane to the other. Alternatively, the molecules may act as specific receptors for specific ions that diffuse across the membrane. In this latter case, the molecule M itself does not move. Instead it may participate alone or in company of other M molecules in the formation of a suitable molecular lattice. Diffusion may take place by movement of ions from one lattice position to the next and at the interface between a membrane lattice site and another lattice site in a suitable adjacent medium, e.g., water. We shall choose to explore the second alternative: one in which the membrane molecules themselves do not move. Instead they form a more or less regular, and possibly highly organized, localized lattice.

Integration of the Electrodiffusion Equation

Eq. (9) gives a method of calculating the boundary values of C appearing in Eqs. (1) and (3). To integrate the electrodiffusion Eq. (1) across the membrane, some approximations are usually introduced, as shown in Cole (1968), the most usual one being the constant field assumption. The result for a single ion is the following:

$$I = z^2 F^2 U \frac{E_m}{l} \left[\frac{C_0 - C_l e^{zFE_m/RT}}{1 - e^{zFE_m/RT}} \right] \tag{10}$$

where $E_m = \Delta_\psi$ and l is the membrane thickness; C_0 and C_l represent the ionic concentrations at the inside surfaces for $x=0$ and $x=l$, respectively. These values are obtained from Eq. (9) in which $C_{QW\,0}$ and $C_{QW\,l}$ are inserted, representing the ionic concentration in the aqueous medium for $x<0$ and $x>l$. For example, if M is a membrane bond water molecule, $n=1$, $K \simeq 1.0$ and the values of C_0 and C_l are given by

$$C_{Q1\,M} = \frac{C_{MT}}{C_W} C_{QW}$$

where C_{MT} is the concentration of bound water in the membrane. This would represent diffusion through water filled pores. In all cases where $n=1$ and $C_W/K_1 C_{QW} \gg 1$, the values of C_0 and C_l are directly proportional to $C_{QW\,0}$ and $C_{QW\,l}$. In other cases, Eq. (10) becomes more complicated.

There will be cases where Eq. (10) is not valid. If the concentrations C_0 and C_l both reach their saturation value, C_{MT}, the chemical potential is not taken into account any more. In such cases it is necessary to integrate Eq. (1) without the constant field assumption and obtain an expression for the conductance (Cole, 1968).

$$I = g \left(\frac{\Delta \mu}{zF} + \Delta_\psi \right) \tag{11}$$

$$g^{-1} = \frac{1}{z^2 F^2} \int_0^l \frac{dx}{CU}$$

where I is the membrane current per unit of surface and g is the membrane conductance per unit of surface. To evaluate g it is necessary to determine the profile of C. In cases where C has reached its saturation value, the profile is horizontal inside the membrane and discontinuities appear at the boundaries. Such conditions make the calculation of g rather easy:

$$g = \frac{z^2 F^2 CU}{l}. \tag{12}$$

This calculation can be used as a valid approximation if C is always much less than either of the ionic concentrations, C_{QW0} and C_{QWl}. This will happen if there are very few molecules M in the membrane, meaning that $C_{MT} \ll C_{QW0}$ and C_{QWl}.

Polarization Effect of the Electrical Field

Besides producing a current, an electrical field may also act on the molecules of the membrane by polarizing them; that is, by changing their orientation. We expect that in an electrical field, due to changed orientation, the equilibrium constants K_i characterized by free energy ΔF_{ci} [Eq. (5)] will be modified, as well as the mobility characterized by the lattice barrier energy ΔF_d [Eq. (2)]. Not having detailed knowledge about the actual physical properties of the membrane molecules M, we shall formally assume that these variations are, in first approximations, proportional to the membrane potential E_m, so that

$$\Delta F_{ci}(E_m) = \Delta F_{ci} + \alpha_{ci} F E_m + \cdots \tag{13}$$

$$\Delta F_d(E_m) = \Delta F_d + \alpha_d F E_m + \cdots \tag{14}$$

where $\Delta F_{ci} = \Delta E_{ci} - T\Delta S_{ci}$ and $\Delta F_d = \Delta E_d - T\Delta S_d$. The values of ΔE_{ci} and ΔE_d are determined experimentally from the temperature dependence of ionic flows; the entropies ΔS_d and ΔS_{ci} and the coefficients α_{ci} and α_d can be gained from data at various values of applied potential on the membrane.

With these additional specifications, Eq. (10) becomes more strongly dependent on the membrane potential. Eq. (12) acquires a potential dependence it did not have before. The fact that K_i can be changed, brings the possibility for C to vary from its saturation point to lower values, meaning that neither Eq. (10) nor (12) is really valid. In such situations it seems more appropriate to use Eq. (12) and introduce average values for C_{QW} in Eq. (9). This approximation removes the potential dependent profile of C but keeps the voltage dependence of the conductance. When using Eq. (10), the chemical gradient would disappear when K_i is such that C_0 and C_l are equal. It must be realized that both calculations of the membrane current have their range of validity and should be used according to experimental conditions. It should be expected that any analytical formulation of the electrodiffusion problem is valid only for special cases because there is no general formulation available.

Application to Steady State Current Voltage Relationships Measured on the Squid Axon

The lattice diffusion model described above may have applications to various membrane flow phenomena. We shall make an application here

to the voltage current relationships in the squid axon. There are extensive and precise data available on currents caused by specific ions beginning with the development of the voltage clamp by Cole (1949) and from the detailed investigations of Hodgkin and Huxley (1952*a–d*). They found that the currents were mostly produced by sodium and potassium ions; and that these two currents were essentially independent of one another. Since then, it has been repeatedly suggested that separate channels are available in the membrane for the two kinds of ions. Each current could be described by the following expressions:

$$I_{\mathrm{K}} = g_{\mathrm{K}}(E_{\mathrm{K}} + E_m), \tag{15}$$

$$I_{\mathrm{Na}} = g_{\mathrm{Na}}(E_{\mathrm{Na}} + E_m) \tag{16}$$

where g_{K} and g_{Na} are the potassium and sodium conductances of the membrane, E_m the electrical potential differences across the membrane, and E_{K} and E_{Na} the chemical potential differences of the potassium and sodium ions.

$$E_{\mathrm{Na}} = \frac{RT}{F} \ln C_{\mathrm{Na}\,W0}/C_{\mathrm{Na}\,W\,l}$$

$$E_{\mathrm{K}} = \frac{RT}{F} \ln C_{\mathrm{K}\,W0}/C_{\mathrm{K}\,W\,l}$$

where subscript 0 stands for outside of the axon and l for the inside.

In the usual notation, when the membrane is at rest, the membrane potential is actually about -60 mv $= E_{\mathrm{rest}}$. In this paper the resting potential $E_r = +60$ mv and is equal to the membrane potential E_m when the applied electrical potential V is zero. Generally $E_m = E_r - V$, where V is positive for a depolarization. This is why Eqs. (14) and (15) do not have the usual form.

In accordance with these experimental results, we decided to use Eqs. (11) and (12) to calculate the membrane ionic currents. It seems justified to suppose that C_{MT} is much smaller than the surrounding ionic concentrations. This is supported by the experiments with the drug tetrodotoxin (TTX) whose effects are obtained with concentrations as low as 10^{-9} M. Also from a primary analysis of the potassium conductance measured with increasing external potassium ion concentration by Ehrenstein and Gilbert (1966), it seems that the conductance maximum amplitude is independent of the potassium ion concentration. This supports the condition, that C_{MT} is much smaller than C_{QW}; it will be seen later that our calculation of C_{MT} is in accordance with this initial approximation.

Steady State Calculation of the Conductances

The model proposed above has many parameters and their values will be determined by the experimental data. To give an explicit formulation for the conductances, it is necessary to evaluate numerically the mobility U and the concentration C.

The mobility is given by Eq. (2). Some of the parameters can be given on approximate value: $\nu \simeq 10^{12}$–10^{13} cps, $d \simeq 5$–10 Å, $T = 280$ °K. There remains only $\Delta F_d(E_m)$; it can be calculated from Eq. (14) in which there are two parameters ΔF_d and α_d; the first one can be estimated from the temperature dependence of the steady amplitude of the conductances. It was found by Hodgkin, Huxley and Katz (1952) to have a $Q_{10} \leqq 1.5$. This gives an upper value of 7.5 kcal to ΔF_d and the mobility at $E_m = 0$ is approximately $zFU \simeq 10^{-5}$ cm^2/v-sec.

There remains only α_d. Experiments on the squid axon have shown that the conductances of the membrane become nearly independent of the membrane potential when the applied potential V is such that the axon is highly depolarized. In order to obtain agreement with this it was necessary that the coefficient α_d is so small that it can be neglected. This means that the potential barrier to lattice diffusion is a constant; it is not modified by the membrane potential. This is also supported by the fact that the instantaneous change of current with voltage is linear in the squid axon. If the mobility was voltage dependent, we should observe a non-linear relation between the instantaneous current and the membrane potential, because the kinetics of the polarization effect of the electric field are usually quite rapid in liquids (10^{-6} sec or less). It is not denied here that the mobility cannot, in general, be modified by the membrane potential; but it does not seem so here, according to the equations that we have established and the data we have chosen.

The calculation of C is obtained from Eq. (9). It is necessary to decide the value of n. In a previous work (Roy, 1969), it was thought that $n = 4$ was a reasonable value. Since then it was found that $n = 2$, was the minimum value in order to have a satisfying fit with the Hodgkin and Huxley (1952) data. Eq. (9) becomes,

$$C_{Q2M} = \frac{C_{MT}}{1 + C_W/K_2 C_{QW} + C_W^2/K_2 K_1 C_{QW}^2} \tag{17}$$

where C_{QW} is obtained from the average ionic concentration in the water phases surrounding the membrane; K_1 and K_2 are given by Eq. (7) and $\Delta F_c(E_m)$ is given by Eq. (13). The value of C_{MT} is estimated from the

maximum amplitude of $g \simeq 30$ mmho/cm^2

$$g_{\max} = \frac{z^2 F^2}{l} C_{MT} U$$

with $z = 1$, $l = 10^{-6}$ cm, $zFU = 10^{-5}$ cm^2/v-sec, it gives $C_{MT} \approx 30$ μmoles/liter.

There remains α_{c1} and α_{c2}, ΔF_{c1} and ΔF_{c2} which will be determined from the curve fitting of the voltage dependent conductances.

The amplitudes of the potassium and sodium conductances are given by the following expressions,

$$g_{\mathrm{K}} = \frac{F^2 C_{\mathrm{K}2M} U_{\mathrm{K}}}{l}.$$

Using Eq. (17) for $C_{\mathrm{K}2M}$ and Eq. (2) for U_{K}, and after the potential dependence of K_1 and K_2 have been introduced with Eq. (13), g_{K} becomes

$$g_{\mathrm{K}} = \frac{g_{m\mathrm{K}}}{1 + \dfrac{C_W \exp(\alpha_{2\mathrm{K}} F E_m/RT)}{C_{\mathrm{K}W} K_{20\mathrm{K}}} + \left(\dfrac{C_W}{C_{\mathrm{K}W}}\right)^2 \dfrac{\exp[(\alpha_{1\mathrm{K}} + \alpha_{2\mathrm{K}}) F E_m/RT]}{K_{10\mathrm{K}} K_{20\mathrm{K}}}} \tag{18}$$

where

$$\begin{aligned} g_{m\mathrm{K}} &= \frac{F^2 d^2 \nu C_{MT\mathrm{K}} e^{-\Delta F_{d\mathrm{K}}/RT}}{lRT} \\ K_{10\mathrm{K}} &= e^{-\Delta F_{c1\mathrm{K}}/RT} \\ K_{20\mathrm{K}} &= e^{-\Delta F_{c2\mathrm{K}}/RT}. \end{aligned} \tag{19}$$

Using the same equations with the index Na instead of K,

$$g_{\mathrm{Na}} = \frac{F^2 C_{\mathrm{Na}\,2M} U_{\mathrm{Na}}}{d}$$

$$g_{\mathrm{Na}} = \frac{g_{m\mathrm{Na}}}{1 + \dfrac{C_W \exp(\alpha_{2\mathrm{Na}} F E_m/RT)}{C_{\mathrm{Na}\,W} K_{20\mathrm{Na}}} + \left(\dfrac{C_W}{C_{\mathrm{Na}\,W}}\right)^2 \dfrac{\exp[(\alpha_{1\mathrm{Na}} + \alpha_{2\mathrm{Na}}) F E_m/RT]}{K_{10\mathrm{Na}} K_{20\mathrm{Na}}}} \tag{20}$$

where

$$\begin{aligned} g_{m\mathrm{Na}} &= \frac{F^2 d^2 \nu C_{MT\mathrm{Na}} e^{-\Delta F_{d\mathrm{Na}}/RT}}{RTl} \\ K_{10\mathrm{Na}} &= e^{-\Delta F_{c1\,\mathrm{Na}}/RT} \\ K_{20\mathrm{Na}} &= e^{-\Delta F_{c2\,\mathrm{Na}}/RT}. \end{aligned} \tag{21}$$

$C_{\mathrm{K}W}$ and $C_{\mathrm{Na}\,W}$ are the average concentrations.

The data of Hodgkin and Huxley (1952*d*, pp. 508 and 514) is compared with the calculated conductances, given by Eqs. (18) and (20). The results are very satisfactory, as seen on Fig. 1a and b.

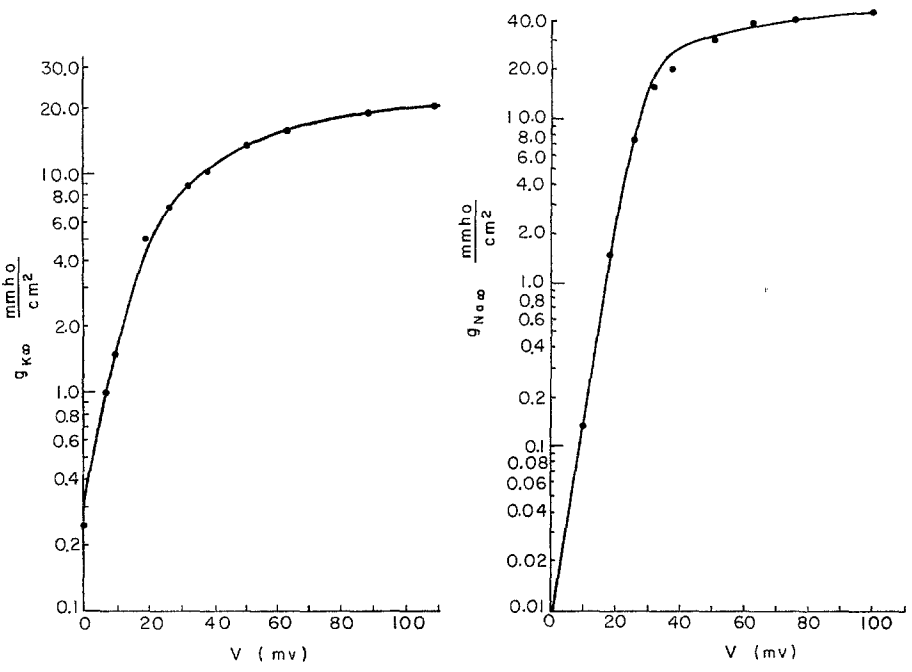


Fig. 1 a and b. Amplitudes of the potassium (a) and sodium (b) conductances vs. the applied potential. · Data taken from Hodgkin and Huxley (1952*d*). Continuous curve calculated from Eq. (18) in (a) and Eq. (20) in (b). Vertical logarithmic scale

Table 1. *Values of the parameters obtained by fitting the steady state equations to the Hodgkin and Huxley* (1952*d*) *data*

l	Na^+			K^+		
	α_l	K_{l0}	ΔF_{cl}	α_l	K_{l0}	ΔF_{cl}
1	5.1	9.0×10^4	−6.4 kcal	3.9	1.7×10^5	−6.7 kcal
2	1.4	8.7×10^2	−3.8 kcal	0.86	3.6×10^2	−3.3 kcal

$C_W = 55$ moles/liter. $C_{NaW} = C_{KW} = 0.25$ moles/liter.

The curve fitting procedure has given values to the parameters α_{cl} and K_{l0}; they are all recorded in Table 1, with the values of ΔF_{cl} calculated from K_{l0}.

A first observation to be made is the value of ΔF_{cl}: it is negative, meaning that the interaction between QW and M has a high tendancy to be performed toward making QM. The equilibrium constant is large; the ions Q have much more affinity for the molecules M than for water when

the membrane potential is zero. The reason that there is a small concentration of QM is that the concentration of molecules M is small. This high affinity of Q for some membrane components might seem curious especially for ions, because the membrane is supposed to be made of hydrophobic components. But it must be remembered that proteins have hydrophobic and hydrophilic regions and some parts of the proteins could have high dipole moments.

Kinetic Equations for the Conductances

Since the only potential dependent factors appearing in Eqs. (18) and (20) are those related to the interaction between the ions and the membrane molecules M, the kinetics of the conductances will be given by that of C_{Q2M}.

The fundamental equations of the chemical kinetic theory (Glasstone, Laidler & Eyring, 1941), will describe these time dependent changes. To calculate the rate of formation of the products from the reactants, it is supposed that an activated complex is formed and then this activated complex is transformed into the products. The concentration of the activated complex is calculated on the basis of the equilibrium theory, using an activated complex equilibrium constant K^*, which is dependent on the free energy change between the reactants and the activated complex and can be calculated in principle if the partition functions of the components are known. The rate of formation of the products is given by the rate of dissociation of that activated complex; the latter is calculated from the frequency of vibration ν of the particle in the activated complex, where $\nu = kT/h$, k is the Boltzmann constant and h is Planck's constant. The rate of formation and dissociation of the products are,

$$R_{lj} = \frac{kT}{h} K^*_{lj} \tag{22}$$

where $l = 1$ or 2 and $j = 1$ or 2.

When it is not possible to calculate in detail the value of K^*_{lj}, it is the usual practice to express it in the same form as an ordinary equilibrium constant,

$$\begin{aligned} K^*_{lj} &= e^{-\Delta F^*_{clj}/RT} \\ \Delta F^*_{clj} &= \Delta E^*_{clj} - T\Delta S^*_{clj} \end{aligned} \tag{23}$$

where ΔF^*_{clj} is the change in free energy between the substrates and the activated complex; ΔE^*_{clj} is the activation energy and ΔS^*_{clj} is the activation entropy.

The ratio of the rate constants for forward and backward reaction gives the equilibrium constant. Since the latter is potential dependent, as introduced with Eq. (13), it is expected that ΔF^*_{clj} is also potential dependent in a similar approximate manner

$$\Delta F^*_{clj}(E_m)=\Delta F^*_{clj}+\alpha^*_{clj}FE_m$$

and

$$R_{lj}=\frac{kT}{h}K^*_{lj0}\,e^{-\alpha^*_{clj}FE_m/RT} \tag{24}$$

$$K^*_{lj0}=e^{-\Delta F^*_{clj}/RT}$$

$$R_{lj0}=\frac{kT}{h}K^*_{lj0}. \tag{25}$$

From the equilibrium constants K_1 and K_2 already determined in the steady state treatment, a relation between α_{cl} and α^*_{clj} is established as

$$\alpha_{cl}=\alpha^*_{cl1}-\alpha^*_{cl2}, \tag{26}$$

and a relation between K_{l0} and K^*_{lj0} is established as

$$K_{l0}=K^*_{l10}/K^*_{l20}. \tag{27}$$

The stochiometric equations are

$$QW+M \underset{R_{12}}{\overset{R_{11}}{\rightleftharpoons}} Q_1M+W$$

$$Q_1M+QW \underset{R_{22}}{\overset{R_{21}}{\rightleftharpoons}} Q_2M+W.$$

The kinetic equations are:

$$\frac{dC_{Q1M}}{dt}=R_{11}\frac{C_{QW}C_M}{C_W}-C_{Q1M}\left(R_{12}+R_{21}\frac{C_{QW}}{C_W}\right)+R_{22}C_{Q2M}, \tag{28}$$

$$\frac{dC_{Q2M}}{dt}=R_{21}\frac{C_{QW}C_{Q1M}}{C_W}-R_{22}C_{Q2M}. \tag{29}$$

And since the availability of M is limited, we have

$$C_{MT}=C_M+C_{Q1M}+C_{Q2M}. \tag{30}$$

Solving for C_{Q2M}

$$\frac{d^2C_{Q2M}}{dt^2}+2\gamma\frac{dC_{Q2M}}{dt}+\omega^2C_{Q2M}=\omega^2C_{Q2M\infty} \tag{31}$$

where

$$2\gamma = R_{12} + R_{22} + \frac{C_{QW}}{C_W}(R_{11} + R_{21}) \tag{32}$$

$$\omega^2 = R_{11}R_{22}\frac{C_{QW}}{C_W} + R_{21}R_{11}\frac{C_{QW}^2}{C_W^2} + R_{12}R_{22} \tag{33}$$

$$C_{Q2M\infty} = \frac{C_{MT}}{\frac{R_{22}C_W}{R_{21}C_{QW}} + \frac{R_{12}R_{22}C_W^2}{R_{11}R_{21}C_{QW}^2} + 1}. \tag{34}$$

Eq. (34) is the same as the steady state Eq. (17) if

$$K_2 = \frac{R_{21}}{R_{22}} \quad \text{and} \quad K_1 = \frac{R_{11}}{R_{12}}.$$

The rate constants R_{ij} are given by Eq. (24) as functions of the potential E_m.

The solution of Eq. (31) is not oscillatory if $\gamma^2 > \omega^2$.

In that case, the solution is a sum of two exponentials and the steady state term:

$$C_{Q2M} = Ae^{-\gamma_1 t} + Be^{-\gamma_2 t} + C_{Q2M\infty}$$

$$\gamma_{1,2} = \gamma \pm (\gamma^2 - \omega^2)^{\frac{1}{2}}.$$

The constants A and B are determined from the initial value C_{Q2M0} and the initial rate of increase of C_{Q2M} which is taken to be zero. The final result becomes

$$C_{Q2M} = C_{Q2M\infty} - (C_{Q2M\infty} - C_{Q2M0})\left(\frac{\gamma_1 e^{-\gamma_2 t} - \gamma_2 e^{-\gamma_1 t}}{\gamma_1 - \gamma_2}\right). \tag{35}$$

It should be noted that the initial rate is not always zero, particularly when the membrane is returned from a depolarized state to its resting state. In that case, the initial rate could be large; it can be calculated from Eq. (29) in which the rates R_{ij} change instantaneously.

Eq. (35) can now be compared with the data on the kinetics of the g_K conductances, because g_K is directly proportional to C_{K2M} (Q is replaced by K). Hodgkin and Huxley (1952*d*) give a family of curves for g_K; each is a function of time and applied potential. When Eq. (35) is compared with these curves, values are obtained for the parameters. As seen on Fig. 2a the fit is very good; Fig. 3a gives the applied potential dependence of γ_1 and γ_2. The value of the constants are given in Table 2. It should be mentioned here that it would have been impossible to reproduce the initial delayed rise in g_K with only a first-order differential equation. This is a justification for the introduction of two sequential interactions between Q and M.

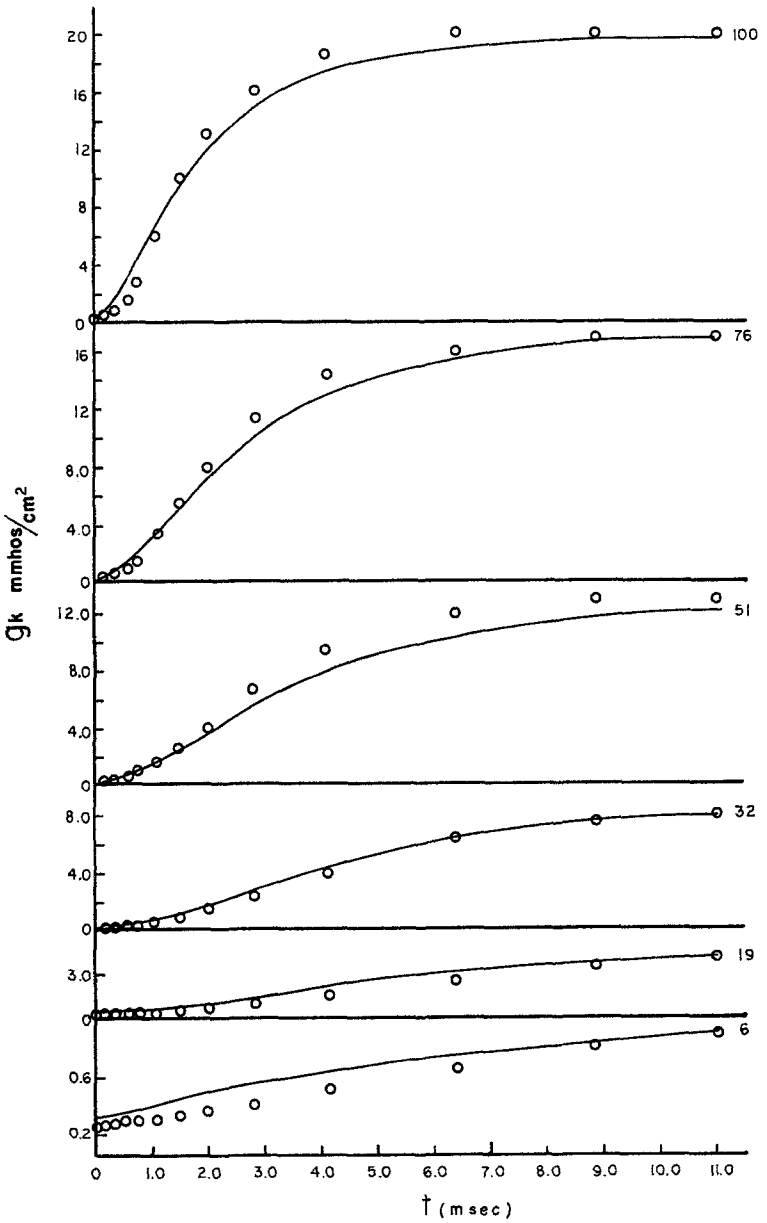
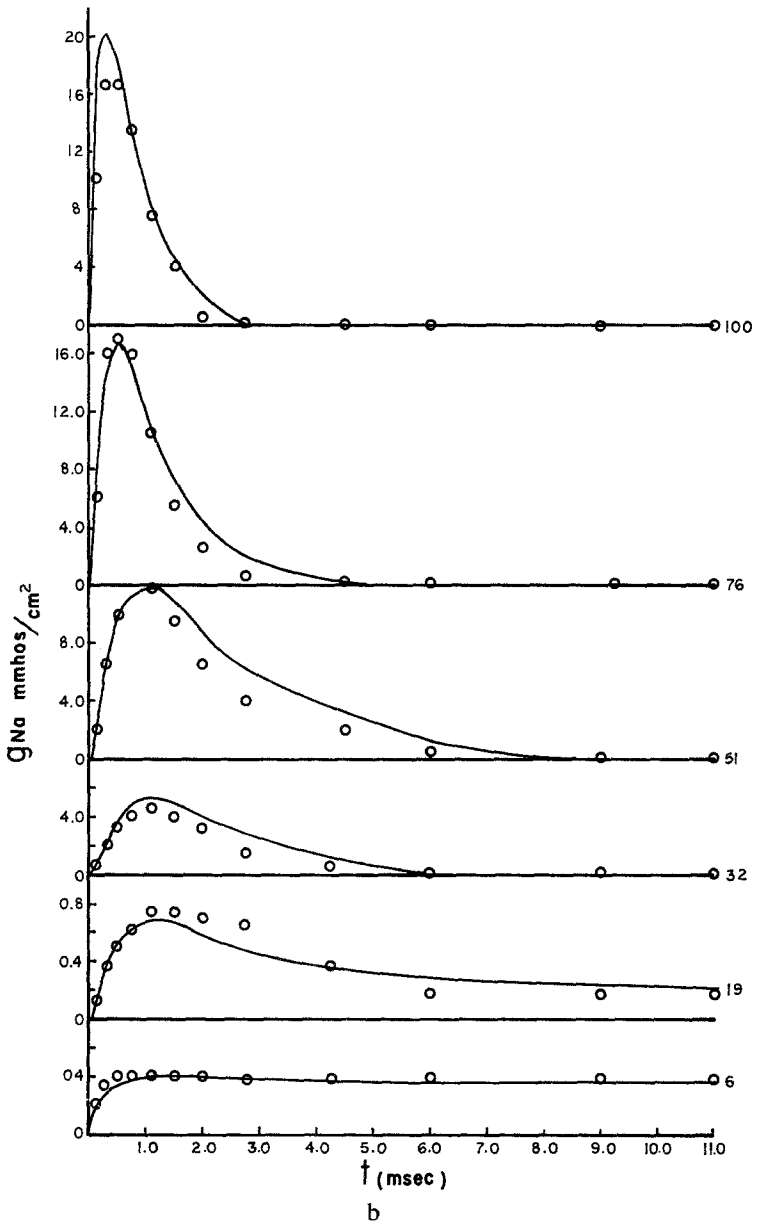


Fig. 2 a and b. Time dependence of the potassium (a) and sodium (b) conductances at
from Eq. (35) in (a) and



6 °C. ∘ Data taken from Hodgkin and Huxley (1952*d*). Continuous curves calculated Eqs. (35) and (48) in (b)

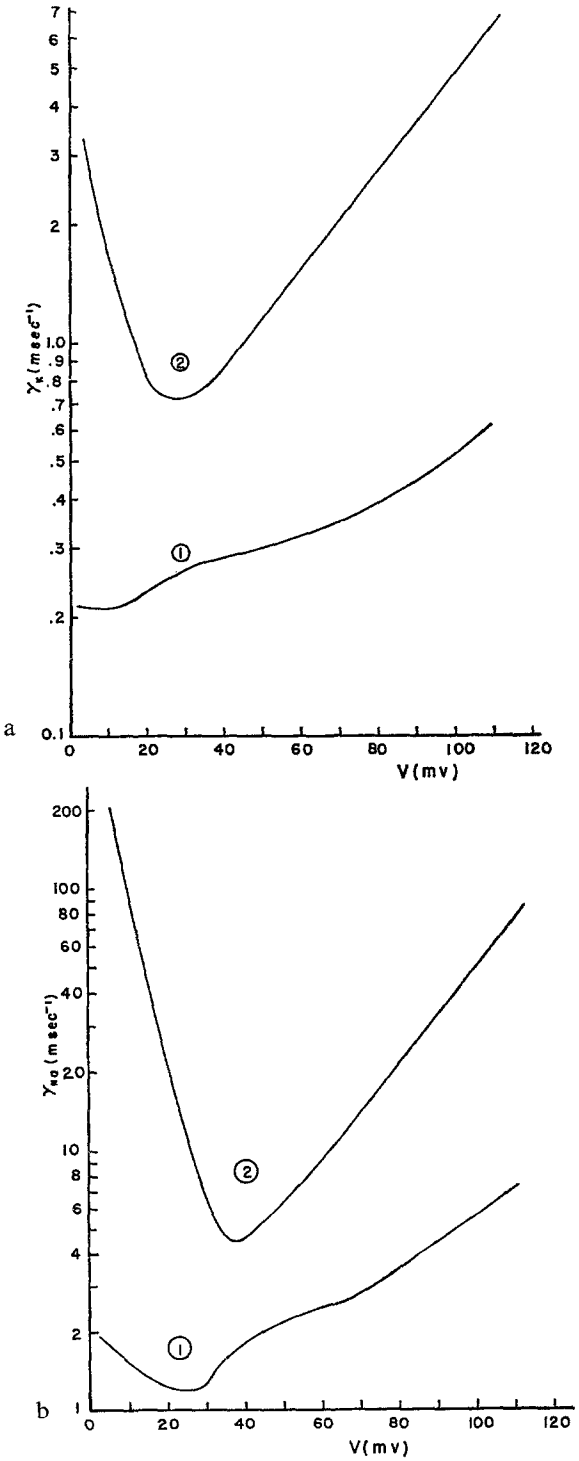


Fig. 3 a and b. Applied potential dependence of the rate constants appearing in Eq. (35) (a) for the potassium conductance and (b) for the sodium conductance

Table 2. *Values of the parameters obtained by fitting the kinetic equations to the Hodgkin and Huxley* (1952*d*) *data*

l	j	Na^+			K^+		
		α_{lj}^*	R_{lj0} (sec^{-1})	ΔF_{clj}^* (kcal)	α_{lj}^*	R_{lj0}	ΔF_{clj}^*
1	1	1.0	2.2×10^{3}	12.1	0.63	2.8×10^{2}	13.3
1	2	−4.1	2.6×10^{-2}	18.5	−3.3	1.6×10^{-3}	20.0
2	1	0.7	3.2×10^{2}	13.2	0.54	3.7×10^{1}	14.4
2	2	−0.67	4.0×10^{-1}	17.0	−0.32	1.0×10^{-1}	17.7
3	1	0.34	1.8×10^{2}	13.5	—	—	—
3	2	−3.06	3.5×10^{-4}	21.0	—	—	—

$C_{KW}=C_{NaW}=0.25$ moles/liter. $C_{XW}=0.25$ moles/liter. $C_W=55$ moles/liter.

Inactivation of the Conductances

Although Eq. (35) can fit the g_K curves very well, it cannot reproduce the g_{Na} curves. The sodium conductance has only a transitory increase when an external voltage is applied and it returns to its resting value (or almost) even when the potential is maintained. This phenomena has been called inactivation of g_{Na}.

The problem of the inactivation of the sodium conductance is still an unresolved question experimentally. Of course, mechanisms can be proposed, but they remain hypothetical until more experimental data is available. A hypothesis will be introduced to show that this theory can explain the sodium conductance data. It must be remembered that the proposed mechanisms for inactivation could be wrong without necessarily invalidating the basis of the whole theory. We would like to propose two possible mechanisms of inactivation which give similar theoretical formulations.

It is possible that the complex Na_2M has two configurations, or two allosteric states as it is usually called now. In one state the free energy of diffusion ΔF_d is small so that Na^+ can jump from one M to the other. In the other state the value of ΔF_d is much larger and Na^+ is retained in M more strongly, so that almost no diffusion occurs.

The second possibility is that the complex NaM could be involved in a second interaction with another ion. It was not specified how many different types of molecules Q could interact with M. It was mentioned before that the calculation of the interaction between Q and M was very simplified and could be much more complicated, especially if there are many types of diffusing molecules available outside the membrane. There could be competi-

tion for the molecules M or inhibition of QM by another ion or molecule. In the case of the sodium ions, it seems that there could be an inhibition of Na_2M by another ion X. The ion X can displace Na and remove it from the membrane or it could form another component $NaXM$ which binds the ion Na much more strongly, thus preventing its diffusion across the membrane. The ion X can be called an inhibitor. We will introduce equations for that last mechanism; they can be transformed easily to represent other types of interactions.

$$\begin{gathered} Na\,W+M \underset{R_{12}}{\overset{R_{11}}{\rightleftharpoons}} Na_1 M+W \\ Na_1 M+Na\,W \underset{R_{22}}{\overset{R_{21}}{\rightleftharpoons}} Na_2 M+W \\ Na_2 M+XW \underset{R_{32}}{\overset{31}{\rightleftharpoons}} Na_2 XM+W. \end{gathered} \tag{36}$$

The kinetic equations are

$$\frac{dC_{Na1M}}{dt}=R_{11}\frac{C_{Na\,W}C_M}{C_W}-C_{Na1M}\left(R_{12}+R_{21}\frac{C_{Na\,W}}{C_W}\right)+R_{22}C_{Na\,2\,M} \tag{37}$$

$$\frac{dC_{Na\,2\,M}}{dt}=R_{21}C_{Na1M}\frac{C_{Na\,W}}{C_W}-C_{Na\,2\,M}\left(R_{22}+R_{31}\frac{C_{XW}}{C_W}\right)+R_{32}C_{Na\,2\,XM} \tag{38}$$

$$\frac{dC_{Na\,2\,XM}}{dt}=R_{31}C_{Na\,2\,M}\frac{C_{XW}}{C_W}-R_{32}C_{Na\,2\,XM} \tag{39}$$

and the total concentration of M is

$$C_{MT}=C_M+C_{Na1M}+C_{Na\,2\,M}+C_{Na\,2\,XM}. \tag{40}$$

The above equations can be used with little modification to represent the first proposed mechanism of inactivation. Instead of Na_2M interacting with X, it will be

$$Na_2 M^{I} \underset{R_{32}}{\overset{R_{31}}{\rightleftharpoons}} Na_2 M^{II}. \tag{41}$$

Where M^{I} and M^{II} represent the two allosteric states of M. The symbol for the concentrations can be replaced in such a way that $C_{Na\,2\,M}$ becomes $C_{Na\,2\,M\,I}$ and $C_{Na\,2XM}$ becomes $C_{Na\,2\,M\,II}$. Since there are zero XW and zero W involved in the third reaction $C^0_{XW}=1$ and $C^0_W=1$ in Eq. (39). All the calculations can be made using Eqs. (37) to (40); the results can be easily transformed to satisfy the hypothesis of the allosteric mechanism if it is found to be the right one.

Eqs. (37) to (40) could be solved for $C_{Na\,2\,M}$. It would give a third-order linear differential equation and the time constants will be obtained by solving a cubic polynomial. Such a solution is rather complicated mathematically.

Because the experimental data do not show any oscillatory behavior, the calculation of $C_{\mathrm{Na}\,2\,M}$ can be approximated in the following way. It is supposed that at the beginning of the interactions, only $C_{\mathrm{Na}\,2\,M}$ increases and $C_{\mathrm{Na}\,2\,XM}$ has a negligible increase; when $C_{\mathrm{Na}\,2\,XM}$ becomes important, $C_{\mathrm{Na}\,2\,M}$ is not increasing appreciably any more. It means that the interactions between Na and M, and Na_2M and X go on almost independently of one another. With this approximation, the calculation of $C_{\mathrm{Na}\,2\,M}$ is greatly simplified. To do this, an intermediate concentration $C'_{\mathrm{Na}\,2\,M}$ is introduced; this is the concentration of $C_{\mathrm{Na}\,2\,M}$ if there were no inactivation.

Eq. (38) becomes

$$\frac{dC'_{\mathrm{Na}\,2\,M}}{dt}=R_{21}\,C_{\mathrm{Na}\,1\,M}\,\frac{C_{\mathrm{Na}\,W}}{C_W}-R_{22}\,C'_{\mathrm{Na}\,2\,M} \tag{42}$$

and Eq. (40) becomes

$$C_{MT}=C_M+C_{\mathrm{Na}\,1\,M}+C'_{\mathrm{Na}\,2\,M} \tag{43}$$

and Eq. (37) stays the same, except that $C_{\mathrm{Na}\,2\,M}$ becomes $C'_{\mathrm{Na}\,2\,M}$.

The three equations are solved to give $C'_{\mathrm{Na}\,2\,M}(t)$. The solution is given by Eq. (35) when E_m is constant. Then the real $C_{\mathrm{Na}\,2\,M}$ is calculated taking into account the inactivation

$$\frac{dC_{\mathrm{Na}\,2\,M}}{dt}=R_{32}\,C_{\mathrm{Na}\,2\,XM}-R_{31}\,C_{\mathrm{Na}\,2\,M}\,\frac{C_{XW}}{C_W} \tag{44}$$

and

$$C'_{\mathrm{Na}\,2\,M}=C_{\mathrm{Na}\,2\,XM}+C_{\mathrm{Na}\,2\,M}\,. \tag{45}$$

Introducing Eq. (45) into Eq. (44), gives

$$\frac{dC_{\mathrm{Na}\,2\,M}}{dt}+C_{\mathrm{Na}\,2\,M}\left(R_{32}+R_{31}\,\frac{C_{XW}}{C_W}\right)=R_{32}\,C'_{\mathrm{Na}\,2\,M}\,. \tag{46}$$

Eq. (46) is transformed into a non-dimensional form by introducing the ratio

$$\frac{C_{\mathrm{Na}\,2\,M}}{C'_{\mathrm{Na}\,2\,M}}=h\,.$$

This formulation corresponds to the inactivation factor h introduced by Hodgkin and Huxley (1952*c*)

$$\frac{dh}{dt}+h\left(R_{32}+R_{31}\,\frac{C_{XW}}{C_W}\right)=R_{32}\,. \tag{47}$$

For constant potentials E_m the solution is simple

$$h=h_\infty-(h_\infty-h_0)\,e^{-\gamma_3 t} \tag{48}$$

where

$$h_\infty = \frac{1}{1 + K_3 C_{XW}/C_W} \tag{49}$$

$$K_3 = R_{31}/R_{32}$$

and h_0 is the initial value of h obtained from Eq. (49) where K_3 is calculated before the potential is applied.

$$\gamma_3 = R_{32} + R_{31} \frac{C_{XW}}{C_W}. \tag{50}$$

The value of $C_{\mathrm{Na}\,2\,M}$ is given by the product,

$$C_{\mathrm{Na}\,2\,M} = hC'_{\mathrm{Na}\,2\,M} \tag{51}$$

where both h and $C'_{\mathrm{Na}\,2\,M}$ are time and potential dependent. The rate constants R_{31} and R_{32} have the same type of potential dependence as the one given by Eq. (24). With this result, the calculated $C_{\mathrm{Na}\,2\,M}$ can be compared with g_{Na} and values can be obtained for the unknown parameters.

The procedure is more complicated than in the case of the potassium conductance kinetics. First, the inactivation factor h is fitted with the data on inactivation in Hodgkin and Huxley (1952*c*); that determines the values of K^*_{310}, K^*_{320}, α^*_{c31} and α^*_{c32}. The ratio K^*_{310}/K^*_{320} and the difference $\alpha^*_{c31} - \alpha^*_{c32}$ are determined from the steady state data on inactivation and the absolute values are determined from the kinetic data on inactivation. Fig. 4a and b give $h\,\infty$ vs. V, the applied potential, and γ_3 vs. V. With the values of the steady state constant of $C'_{\mathrm{Na}\,2\,M\infty}$ taken from Table 1, it leaves only four parameters to be determined from the fitting of $C_{\mathrm{Na}\,2\,M}$ with the kinetic data on g_{Na}. The results giving the calculated curves and the experimental points are shown on Fig. 2b. Fig. 3b gives the applied potential dependence of γ_1 and γ_2. The values of the parameters are given in Table 2.

The calculated values of ΔF^*_{clj} contain the activation energy, ΔE^*_{clj} and the entropy ΔS^*_{clj}. If the temperature dependence of the kinetics is measured for a set of voltage steps, it is possible to calculate the values of ΔF^*_{clj} for each temperature and from their temperature dependence, we can calculate the entropy change ΔS^*_{clj} and the barrier energy ΔE^*_{clj}. An approximate correspondance can already be found between the calculated ΔF^*_{clj} and the average Q_{10} measured by Hodgkin, Huxley and Katz (1952). They found that the rate of change of the conductances has a Q_{10} between 2.7 and 3.5. If the entropy ΔS^* is small compared to the energy ΔE^*, we find from Table 2 that many of the values would give Q_{10}'s corresponding to the measured ones.

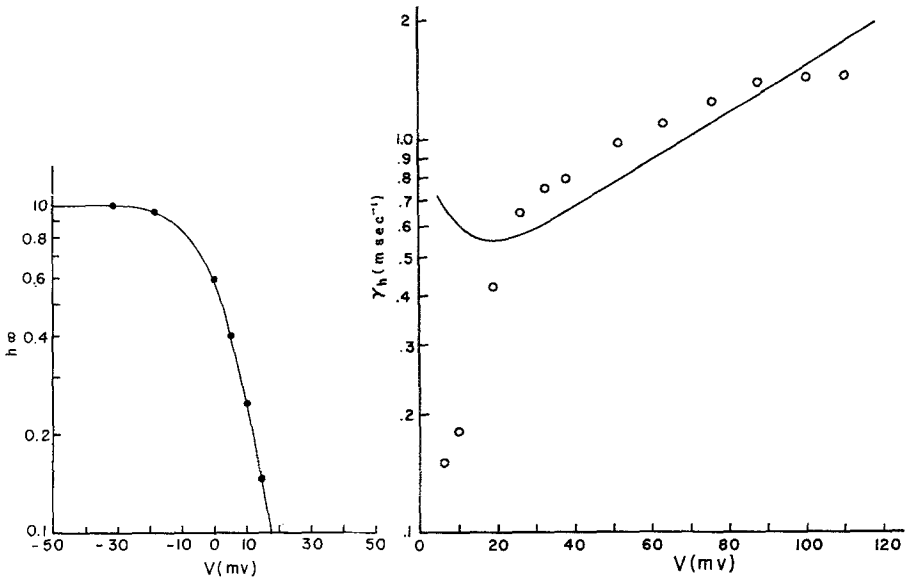


Fig. 4a and b. Applied potential dependence of inactivation. (a) Steady state amplitude of inactivation (vertical logarithmic scale); (b) rate of inactivation. ◦ Data taken from Hodgkin and Huxley (1952*c*)

But it must be realized that more accurate measurements of the temperature dependence of the conductance kinetics are needed in order to determine the activation energies and the entropies. Such experiments are actually under way in our laboratory.

With all the equations describing the conductances, it is now possible to calculate an action potential to test their validity. The cable equation used in Hodgkin and Huxley (1952*d*) can be introduced for such a purpose. We will calculate the space-clamped action potential where $\frac{d^2V}{dx^2}=0$:

$$C_m\frac{dV}{dt}=g_K(V_K-V)+g_{Na}(V_{Na}-V)+g_L(V_L-V),$$

where C_m is the membrane capacitance; g_L is a constant leakage conductance, V_L the leakage potential and $V_K=E_K+E_r$, $V_{Na}=E_{Na}+E_r$. With the time and potential dependence of g_K and g_{Na}, $V(t)$ is obtained through a numerical integration. The space-clamped action potential so obtained is shown on Fig. 5, and it is compared with a measured action potential. There is a small difference in shape, mostly at the bottom part, where the calculated action potential returns more slowly than the measured one. It has all the well known properties of experimental action potentials, like threshold and

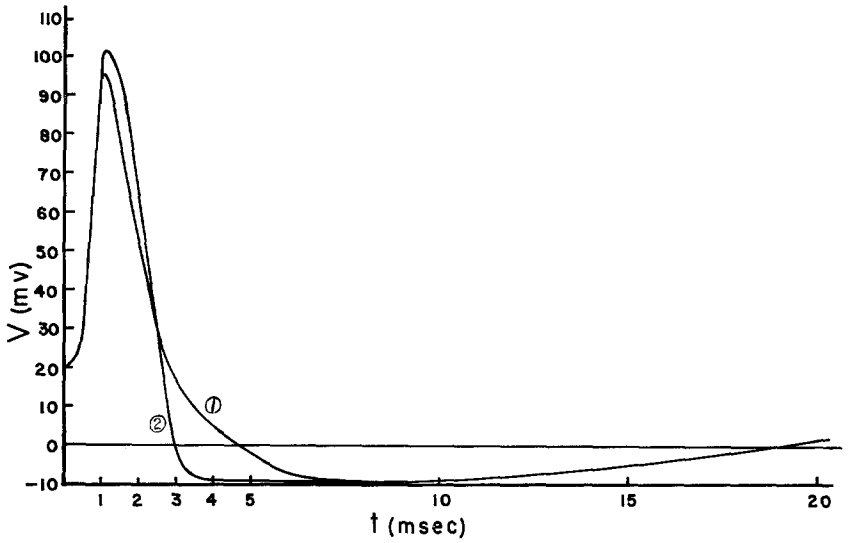


Fig. 5. Calculated (1) and measured (2) space-clamped action potential. Data taken from Hodgkin and Huxley (1952*d*)

refractory period. It is now well admitted that most properties of axons can be reproduced from the voltage-clamp data. It is not necessary to demonstrate that the action potential calculated with this theory has all the required properties, because the voltage clamp data is reproduced quite satisfactorily.

Conclusion

The purpose of this work is to describe the electrical conductivity properties of membranes. We have introduced some basic concepts and given fundamental relationships that can be used to calculate diffusion of ions across membranes. The membrane is a heterogeneous structure and diffusion across it depends very much on the affinity of the diffusing molecules for certain membrane molecules. The concentration of diffusing molecules that can be present in the membrane is a factor of primary importance to determine the flow across the membrane. The mechanism of diffusion is based on the theory of diffusion in solids and liquids; molecules and ions diffuse through a medium by jumping from one lattice site to another. Introducing the fact that the electric field on the membrane can change the binding properties of the membrane molecules, potential dependent boundary conditions are obtained and introduced into the electrodiffusion equation. Potential dependent conductances are also calculated.

The interaction of ions with membrane molecules can be calculated with the chemical kinetic theory; this gives the time dependent changes of the conductances. The theoretical calculations are compared with the Hodgkin and Huxley (1952*d*) data and the results are quite satisfying. The unknown parameters are determined by curve fitting. Among them are the activation energies which were found to be in agreement with the temperature measurements. Also an action potential is calculated using the cable theory and it compares closely with the experimental one.

A recent paper by Tsien and Noble (1969) is suggesting an approach similar to the one we have introduced; they suggest that the transition state theory could be a fairly general approach to the study of membrane conductance kinetics. The wide variation in the rate coefficients of different types of membranes is always followed by similar variation in the Q_{10}. Although it is an interesting support for this approach, it must be remembered that close quantitative comparisons could present difficulties, especially regarding the values of the entropy of activation. Nevertheless, the transition state theory is quite general and flexible, and it would be surprising that additional refinements combined with a general theory of diffusion could not take care of most of the problems. Of course more knowledge of the membrane structure is needed before most of the experimental details are explained.

It is hoped that this theoretical analysis will provide a useful basis to calculate the flow of other types of ions or molecules in membranes and also that the equations developed for the squid axon membrane will stimulate experiments to check if this model is valid. Experiments are now being conducted to find additional supports for the hypothesis introduced into this model.

I would like to express my most grateful appreciation to Prof. C.A. Tobias for his continuous support and useful criticism during the development of this project.

This work was supported at Berkeley by the Biophysics Training Grant from the National Institutes of Health and by a grant of the NASA-AEC. At Montreal, the work was supported by a grant of the National Research Council of Canada.

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