ON THE CHANGES IN
CONDUCTANCE
AND STABILITY PROPERTIES
OF ELECTRICALLY EXCITABLE
MEMBRANES
DURING VOLTAGE-CLAMP
EXPERIMENTS

R. LEFEVER AND J. L. DENEUBOURG

Faculté des Sciences, Université Libre de Bruxelles,
Bruxelles, Belgium

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I. INTRODUCTION

It is a most remarkable natural phenomenon that small and local depolarizations of the electric field established across electrically excitable membranes can amplify in the form of action potentials propagating in a sustained fashion, at a rate of more than 20 m/sec and without
distortion. The all-or-none character of this behavior has always been a fascinating property, suggesting the existence of some kind of "phase transition" of the membrane taking place when it switches from the resting polarized state to the excited depolarized state.

The molecular basis of this transition has not yet been elucidated, but since the work of Hodgkin and Huxley (HH) and their followers (for references see the books by Hodgkin\(^1\) and Katz\(^2\)), we have had a quite precise picture of the drastic permeability changes and the ionic events that are associated with it. These authors also proposed a quantitative, phenomenological model based on a detailed analysis of the ionic currents recorded during voltage-clamp experiments as well as during the propagation of action potentials. The properties of this model have been widely confirmed, so that any more recent attempt to describe electrical excitation on a physicochemical basis has in general been evaluated with regard to its agreement with the HH results. Let us therefore first briefly summarize the essential characteristics of electrical excitation as they have been demonstrated by Hodgkin and Huxley. The basic equation describing the potential changes across the membrane is

\[
C \frac{\partial V}{\partial t} = I(V) + \frac{a}{2R} \frac{\partial^2 V}{\partial x^2} \tag{1.1}
\]

The current density flowing through the membrane has been decomposed into a capacitive and a resistive component, respectively \(C\partial V/\partial t\) and \(I(V)\); \((a/2R)\partial^2 V/\partial x^2\) is then the current flowing along the axon in the direction of spatial coordinate \(x\). The dependence of \(I(V)\) on \(V\) is quite complicated. There are three principal separate systems carrying the current through the membrane: the so-called sodium and potassium channels, and some leakage channels associated with chloride and other ions present in the internal and external mediums. This can be expressed in the following manner:

\[
I(V) = g_{Na}(V)(V - V_{Na}) + g_{K}(V)(V - V_{K}) + g_{L}(V - V_{L}) \tag{1.2}
\]

\(g_{Na}\) and \(g_{K}\) are the sodium and potassium conductances, which depend sensitively on the voltage across the membrane; \(g_{L}\) is a constant; \(V_{Na}\), \(V_{K}\), \(V_{L}\) are the equilibrium voltages of Na, K, and the leakage current, depending on the ratios of the concentrations of these ions on both sides of the membrane.

The major characteristic properties of the sodium and potassium conductances can then be summarized as follows:
1. In response to an abrupt change of membrane potential, the instantaneous sodium and potassium currents are in first approximation linear functions of the applied voltage.

2. Sustained depolarizations are followed by an increase in sodium and potassium conductance (activation of Na and K channels). The time course of this increase in conductance obeys a high-order kinetics.

3. For depolarizations close to the threshold, the maximum conductance for sodium varies steeply as a function of potential: an e-fold increase of sodium conductance can be brought about by a change of membrane potential of the order of 5 mV.

4. The increase in $g_{Na}$ following a depolarization is not sustained, but "inactivates" spontaneously. This inactivation process follows a simple exponential decay in time.

5. After inactivation, the sodium transport system cannot be rapidly activated even with large depolarizing stimuli: there exists a refractory period. The time lag necessary for the recovery to be complete is much larger (30 msec) than the duration of the action potential (2 msec).

The activation of potassium and sodium as well as the inactivation of sodium constituting independent processes, HH introduced as set of three parameters ($m, n, h$), referring respectively to the sodium and potassium activation and to the sodium inactivation, whose value is simply related to the conductances $g_{Na}$ and $g_{K}$, and whose change in time is given by three independent first-order linear differential equations; the parameters of these equations are complicated functions of the external potential. Although this model was first derived in the case of the giant axon of Loligo, it has also proved to be a very useful tool for investigation and description, applicable to a large number of other electrically excitable membranes.

Many studies have investigated the properties of the HH equations in simulation experiments on analog or digital computers\textsuperscript{3,4,7,8} and have demonstrated the rich set of mathematical phenomena that this model may exhibit. Its complexity, however, and the various nonlinearities present in the equations make its theoretical study rather tedious and discourage analytic approaches. Several ingenious simplifications of the HH equations have been proposed, which retain the principal feature of nerve excitation: the existence of a threshold below which perturbations are rapidly damped, and beyond which they are amplified in a characteristic traveling wave. A system of this type that has been particularly well studied is the Nagumo equation,\textsuperscript{9} for which several distinct regimes of propagation are known.\textsuperscript{10,11} These results have led to a detailed understanding, but in phenomenological terms, of the type of
stability behavior that might be associated with nerve conduction and excitation.

In this chapter we attempt to provide a link between this behavior and a more precise physicochemical basis suggested by recent developments in various domains of membrane biology. Particularly, the artificial ionophores now offer a picture of the type of molecular structures that might selectively recognize ions and transport them through a membrane\textsuperscript{12}; the allosteric transitions that account for the regulation of biochemical reactions illustrate how such transport might be controlled by a change of conformation.\textsuperscript{13} On the other hand, the recent developments of nonequilibrium thermodynamics furnish a general conceptual framework\textsuperscript{14} for the interpretation in physical terms of the stability problems raised by excitable membranes.

II. GENERAL HYPOTHESES OF THE MODEL

A. Structure of the Ionophores

The transporting systems of electrically excitable membranes are constituted of elementary units or ionophores selectively involved in the translocation of the ions. The ionophores both recognize the permeant ions and transport them selectively along their electrochemical gradient. The ions play the role of both a ligand and a permeant. The capacity for selective transport of the ionophores involved in the excitation process changes upon excitation. They are "versatile"\textsuperscript{15} in the sense that they undergo a structural transition between at least two states, one for the membrane at rest (S), and the other for the membrane during excitation (R). These two states differ in their affinity for the ions and their rate of transport. By convention, the active R state is taken as the most permeable one.

B. Distribution of the Ionophores in the Membrane

Embedded in a lipid bilayer, the ionophores are organized in small-number aggregates, or in much larger ones that then may be assimilated to a continuous lattice structure. Interactions might be established between individual ionophores through a cooperative conformational coupling.

C. Effect of the Electric Field on the Conformational Equilibrium

The ionophore is a macromolecule or a macromolecular assembly that bears a number of charges or polar groups, which may be considerable and depend on the conformational state. For the time being, we
shall suppose that the essential consequence of this situation is to make the conformational equilibrium between \( R \) and \( S \) field dependent because of a difference in permanent dipole moment: The orientation of the ionophore as a whole being rigid in the membrane, the reorientation of the polar groups when the field is changed induces the transition from one state to the other. The effect is such that a depolarization favors the permeable (\( R \)) state.

**D. Effect of the Electric Field on the Ionic Transport**

The number of charges that exert an electrostatic repulsion against the passage of the ions depends on the conformational state of the ionophores and their neighborhood. This number varies when the orientation of the polar groups attached to the ionophores is modified by a field variation.

**E. Environment of the Ionophores**

As a whole, the membrane is a coherent phase, which creates a permeability barrier between two volumic phases of different electrochemical potential; on both sides of the membrane, however, the ionophores are not in direct contact with the bulk solutions. There exists, in between a physical medium, or “equilibration layer,” where the activity and diffusion coefficients of the ligand may be different from both that of the bulk solutions and that of the ionophores.

**III. FORMULATION OF THE MODEL**

**A. Kinetics of the Transition Between the \( R \) and \( S \) Conformations**

We assume that the ionophores present in electrically excitable membranes have a fixed axis of orientation, which spans the lipid layer, and along which their spatial structure is a repetition of similar segments. Each segment may exist in two conformational states characterized by different ionic permeabilities and different net dipolar moments. Models for such ionophores are the polycyclic hexapeptides described recently by Urry\textsuperscript{12}.

The transition of an ionophore from the state in which all its segments are in the \( S \) conformation to the state in which they all are in the \( R \) one appears as a complex process involving a considerable number of intermediate states. One may, however, expect that there are, at a minimum, two types of elementary processes between which it is necessary to distinguish: (1) the nucleation of a \( R \) segment within a fully \( S \) ionophore, (2) the growth of the number of segments in one confor-
mation in comparison with the number of segments in the other conformation. The transition between the two extreme states of the ionophore can thus be written as

\[
\begin{align*}
    x_{0,n} & \xrightarrow{l_{RS}} x_{1,n-1} \xrightarrow{k_1}{k_2} x_{2,n-2} \xrightarrow{k_2} \cdots \xrightarrow{k_2} x_{n,0}
\end{align*}
\]

where \( n \) is the number of segments per ionophore; the \( x_{i,j} \) are the molar fractions of ionophores per unit area with \( i \) segments in the \( S \) conformation and \( j \) segments in the \( R \) conformation; \( l_{RS}, l_{SR} \) are the rate constants associated with the nucleation step; and \( k_1, k_2 \) are the rate constants for the "growth" process. These constants are field dependent because of the existence of the nonvanishing dipole moments \( \mu_R \) and \( \mu_S \) characteristic of the \( R \) and \( S \) conformations

\[
\begin{align*}
    k_1 &= k_{1}^e \mu_R \left( \frac{e}{RT} \right), \\
    k_2 &= k_{2}^e \mu_S \left( \frac{e}{RT} \right), \\
    \frac{k_1}{k_2} &= l_{R} \left( \frac{\mu_R - \mu_S}{e} \right) \left( \frac{e}{RT} \right) = l_{R} k
\end{align*}
\]

where \( E \) is the electric field across the membrane. We define the fraction \( \langle r \rangle \) of ionophores that have at least one segment in the \( R \) conformation:

\[
\langle r \rangle = \sum_{i=0}^{n-1} x_{i,n-i}
\]

The time change of the \( x_{i,j} \) fractions can then be described by the following set of kinetic equations:

\[
\begin{align*}
    \frac{d\langle r \rangle}{dt} &= l_{RS} x_{0,n} - l_{SR} x_{1,n-1} \\
    \frac{dx_{i,n-i}}{dt} &= k_1 x_{i-1,n+1-i} - (k_1 + k_2) x_{i,n-i} + k_2 x_{i+1,n-1-i} \quad (i \geq 3)
\end{align*}
\]

together with the conservation relation

\[
\sum_{i=0}^{n} x_{n-i,i} = 1
\]

We consider that the nucleation reaction is rate limiting compared to the "growth" process, and thus have the inequality

\[
k_1, k_2 \gg l_{RS}, l_{SR}
\]
which permits us to reduce (3.4) to a single kinetic equation:

$$\frac{d\langle r \rangle}{dt} = l_{RS} \left( 1 - \langle r \rangle - \frac{l_{SR}}{l_{RS}} \frac{(1 - l_R k^n)}{1 - l_R^n k^n} \langle r \rangle \right)$$  \hspace{1cm} (3.7)$$

and \(n-2\) algebraic relations for the intermediate \(x_{ij}\) states:

$$x_{i,n-i} = \frac{(l_R k)^{i-1}(1 - l_R k^n)}{1 - l_R^n k^n}$$  \hspace{1cm} (3.8)

It has often been suggested that cooperative structural interactions might exist between the ionophores and facilitate the transition from the nonpermeable to the permeable state.\(^{13,16}\) To take the possibility of such effects into account, we shall suppose here that the free energy of the nucleation step depends on the average conformational state of the membrane, and can be approximated in the following manner:

$$\Delta F = \epsilon - \eta U(x_{ij})$$  \hspace{1cm} (3.9)$$

where \(\eta\) is a constant and \(U(x_{ij})\) is given by

$$U(x_{ij}) = \sum_i i x_{i,n-i} = \frac{\langle r \rangle}{n(1 - l_R^n k^n)} \left( \frac{1 - l_R^n k^n}{1 - l_R k^n} - n l_R^n k^n \right)$$  \hspace{1cm} (3.10)$$

represents the average fraction of segments in the \(R\) state per ionophore. On the other hand, the analogy with artificial ionophores suggests that the difference in dipole moment between the two conformations could be related to a change in orientation of only a small number of peptide bonds; in that case the orders of magnitude to expect for \(\mu = \mu_R - \mu_S\) are probably only of a few tens of debyes. It is easy to see from Equation (3.7) that the conformational equilibrium will nevertheless exhibit a strong field dependence in such a system as long as the segments are sufficiently numerous. For example, when \(l_R > 1\) and \(n \gg 1\), Equation (3.7) can be rewritten as

$$\frac{d\langle r \rangle}{dt} = l_{RS} \left( 1 - \langle r \rangle - \frac{l_{SR}}{l_{RS}} \frac{k^{1-n}}{l_R^{n-1}} \langle r \rangle \right)$$  \hspace{1cm} (3.11)$$

and has the same form as if it corresponded to ionophores bearing a single large dipole of \((n-1)(\mu_R - \mu_S)\) D. An ionophore consisting of 20
segments in two conformations differing by a dipolar moment of 20 D may thus have a conformational equilibrium that depends on the field in the same way as if there were a single dipole of 400 D.

B. Derivation of the Current-Voltage Relation

Within the ionophore, the electrolyte is assumed to be completely dissociated; Na\(^+\) and K\(^+\) are transported as monovalent cations. The effect of the electrical field on the electrolytic dissociation (Wien effect) is neglected. Its influence on the ionic transport becomes significant when the rate-limiting step is the passage of the ions across the interface.\(^{21}\) It has been shown that then, at least in the case of lipid bilayer membranes, considerable nonlinearity in the \(I-V\) curves may arise from the Wien effect. Here, however, we expect that the rate-limiting step in the ionic transport is the conformational change of the ionophore, and that it is this step, rather than an electrolytic dissociation reaction that controls the ionic flows. Nonlinearity in the \(I-V\) curves appears also as a result of the image forces acting on the ion during its transport across the membrane.\(^{28}\) This additional complication is not taken into account here. For simplicity we also suppose that at the interfaces between ionophores, equilibration layers, and bulk solutions, the ionic concentrations vary in a discontinuous manner; the dielectric constant of the equilibration layers is taken as equal to that of the ionophore.

The transport of the ions is viewed according Eyring's rate theory as a series of discrete jumps through consecutive energy barriers. The electric field is constant through the membrane and influences the transport by altering the height of the energy barriers inside the membrane.

The permeability of a given ionophore will of course depend on the height of the energy barriers that it opposes to the translocation of the ions. These barriers in turn are related to the conformational state of the various segments forming the molecule; a detailed description of the translocation process would therefore require the consideration of all the intermediate states \(x_{i,j}\) together with their particular energetic configuration with respect to the passage of the ions. This would yield very difficult and bulky algebraic expressions, so instead of considering the transport across each particular type of ionophore separately, we choose a single scheme for the energy barriers met by the ion in the membrane phase, and define the kinetic constants across these barriers as functions of the average conformational state of the membrane. In Fig. 1 we have represented the arrangement of barriers chosen. For simplicity, only one barrier is considered in the equilibration layer. The ionophore itself has
Figure 1. Location of the energy barriers in the membrane. The position of the equilibrium layers depends on the value of the parameter $p$: When $p = 0$, both sites are at the interface with the bulk solutions; when $p = 1$, both sites are at $d/2$. The $\beta_j$ are the ionic densities at the various transition sites inside the membrane.

$m$ barriers whose heights vary linearly in such a way that

$$\frac{\Delta H_{i-1}}{\Delta H_i} = h$$

(3.12)

where $h$ is a constant. This organization has been suggested by Woodbury and is preferred because it leads straightforwardly to linear instantaneous $I-V$ curves when the condition $n \to \infty$ with $nh = zFV_Na / RT$ is satisfied. It is, however, only a mathematical convenience. In practice the $I-V$ curves are already linear within a few percent over an interval of potential $-150 \text{ mV} < V < 150 \text{ mV}$, when $n \geq 3$.

Let us now consider the transition rate across a particular energy barrier. The electrical field, which is constant through the membrane, simply adds a constant term to the activation energy of the transition probability $k_{i \to i+1}$ from site $i$ to site $i+1$. On the other hand, this activation energy is decreased when the average number of segments per ionophore in the $R$ conformation increases (Section II.D). This property is expressed by the addition of a term $qU(x_{ij})$, where $q$ is a
dimensionless parameter and $U(x_{i,j})$ is given by (3.10).* The total activation energy at a given barrier can thus be written as

$$
\Delta H_{i\rightarrow i + 1} = \Delta H_1 + (1-i)h - \frac{zF}{RT} (p-1) \frac{\Delta V}{n} - qU(x_{i,j})
$$

(3.13)

where $z$, $F$, $R$, $T$ have their usual meaning, $\Delta V = V_i - V_0$ is the potential difference between the inside and the outside. The transition probability $k_{i\rightarrow i + 1}$ is now given by

$$
k_{i\rightarrow i + 1} = \epsilon_i \exp\left( \frac{(p-1)\chi}{n} + qU(x_{i,j}) \right)
\frac{\Omega^U}{l}
$$

(3.14)

with $\epsilon = e^{-\Delta H_1}$, $\Omega = e^q$, $\chi = zF\Delta V / RT$, $l = e^{(p-1)\chi/n}$. In the equilibration layers we define the inward transition probability simply as

$$
c_p = \epsilon_p \exp\left( -\frac{p\chi}{4} \right) = \epsilon_p k_p
$$

so that we can finally write down the equations for the time change of the ionic concentrations $\alpha$, $\beta$ in the inner and outer equilibration layers and $\beta_j$ inside the membrane:

$$
\frac{d\beta}{dt} = k_{\alpha}^{\alpha}\left[ \gamma (k_\beta \beta_0 - k_\beta^{-1} \beta) - \langle r \rangle \Omega^U (l_\beta l^{-1} \beta_1) \right]
$$

$$
\frac{d\alpha}{dt} = k_{\alpha}^{\beta}\left[ \gamma (k_\alpha^{-1} \beta_1 - k_\alpha^{-1} \alpha) - \langle r \rangle \Omega^U (l^{-1} \alpha - l \beta_{m-1}) \right]
$$

$$
\frac{d\beta_j}{dt} = \epsilon_{i} \Omega^{(r)} \left[ \frac{\epsilon_j \beta_{j-1} - (l^{-1} \epsilon_j + l \epsilon_{j+1}) \beta_j}{\epsilon_{i}} + \frac{l^{-1} \epsilon_{j+1}}{\epsilon_{i}} \beta_{j+1} \right]
$$

(3.15)

* As recently reported by Haydon et al.,$^{18}$ alamethicin molecules incorporated in black lipid membranes reveal transport and structural properties of the type assumed here: (1) alamethicin exists in a conducting and a nonconducting conformation; the transition from one state to the other corresponds to a discrete jump in conductance whose magnitude is constant when the alamethicin molecule is isolated within the membrane; (2) under some conditions the molecules form small aggregates of 6 or 7 units; the permeability of an alamethicin molecule within the aggregate then depends on the conformational state of its neighbors; it is bigger when the latter already are in the conducting state. Furthermore, Haydon et al. also demonstrated that the transition probability from the nonconducting to the conducting state increases when the neighbors already are in the conducting conformation.
where $k'_d$, $k''_d$ are constants proportional to the number $N$ of ionophores per unit membrane area, and $\gamma = \epsilon_p / \epsilon_1 N$. In order to be in agreement with the fact that the equilibrium potential corresponding to zero flux through the membrane is the sodium potential given in first approximation by Nernst’s law, we have to require that the internal concentrations $\beta_j$ equilibrate very quickly compared to $\alpha$, $\beta$. Accordingly the inequality

$$k'_d, k''_d \ll \epsilon_1$$

needs to be satisfied, and the current through the membrane becomes

$$I_{Na} = \frac{\epsilon_n}{\epsilon_1} \frac{\langle r \rangle \Omega U (l^n \beta - l^{-n} \alpha)}{l^{1-n} \left( 1 + \epsilon \sum_{j=1}^{n-1} l^{2(n-j)} \right)}$$

(3.17a)

or equivalently

$$I_{Na} = \frac{\epsilon_n}{\epsilon_1} \langle r \rangle \Omega U l^{-1}(l^{2n} \beta - \alpha)(1 - e^{2e_h})$$

$$1 - e^{2e_n \epsilon h}$$

(3.17b)

The equilibrium potential is then given by

$$V_{Na} = -\frac{RT}{F(1-p)} \ln \frac{\beta}{\alpha}$$

(3.18)

and by analogy with HH we define the sodium conductance as

$$g_{Na} = \frac{I_{Na}}{V - V_{Na}}$$

(3.19)

Taking (3.16) and (3.17) into account, we can reduce the set of equations (3.15) to a system of two equations:

$$\frac{d\beta}{dt} = k'_d \left[ \gamma (k_p \beta_0 - k^{-1} \beta) - I_{Na} \right]$$

$$\frac{d\alpha}{dt} = k''_d \left[ \gamma (k_p^{-1} \beta - k_p \alpha) + I_{Na} \right]$$

(3.20)

which together with Equation (3.7) describe the ionic transports through the membrane.
IV. PROPERTIES OF THE MODEL AND EXPERIMENTAL PREDICTIONS UNDER VOLTAGE CLAMP

In this section, we shall compare the results obtained on the basis of Equations (3.7) to (3.20) for the time change of the sodium conductance with the experimental results obtained by HH in voltage-clamp experiments.

It is clear from Equations (3.17a,b) that in the variation of the Na current that follows a depolarization of the membrane, two distinct processes happen sequentially: first, corresponding to the opening of the channels, we observe an increase of the function $\langle r \rangle \Omega^U$ in time, and thereafter occurs a variation of the ionic concentrations $\alpha$, $\beta$ in the equilibration layers, resulting from the discharge of this intermediate electrochemical gradient across the open channels. These two processes can be seen in Fig. 2, where we have represented theoretical curves calculated from Equations (3.20) and (3.7) for the time course of $g_{Na}$ at various clamping potentials. The similarity between these curves and the experimental properties summarized in the introduction for the time course of the Na$^+$ conductance in the giant axon is striking. We now

![Figure 2. Time course of the sodium conductance following depolarizations of 10, 20, 30, 40, 50, and 60 mV. The resting state corresponds to $-70$ mV, and the following numerical values have been given to the parameters: $k'_2 = k''_2 = 1$, $q = 3.711$, $K = 0.089$, $\mu_3 = 0$, $\mu_R = -600$, $p = 0.5$, $\gamma = 0.418$, $m = 1$, $I' = 200$, $\eta = 2.196$, $\beta_0 = 10$, $\beta_1 = 1$.](image)

consider the activation and inactivation processes of Na\(^+\) in more detail.

A. Kinetics of the Sodium Activation

We call the function

\[ g = \langle r \rangle \Omega^U \]  \hspace{1cm} (4.1)

the permeability function of the membrane. It has a role comparable to \( g^3m^3 \) in the HH model. However, after a depolarization when \( n \), the number of segments, is greater than one, \( g \) exhibits here a behavior that is twofold: An instantaneous variation of \( U(x_i) \) results from the jump of the value of \( I_Rk^* \); a slower response is associated with the time change of \( \langle r \rangle \) and constitutes the activation process itself. In Fig. 3, as might be expected from the presence of the exponential factor in (4.1), it is clearly seen that the rise in conductance during the activation follows a high-order kinetics. It should be noticed, however, that the range of potentials over which the curves display a sigmoid shape depends simply on the value of \( q \). By increasing \( q \) it becomes possible to obtain sigmoid curves even for small depolarizations and with conditions under which the resting state remains stable infinitesimally. If, on the contrary, \( q \) were chosen equal to zero, the only way to recover a sigmoid shape within the framework of our hypotheses would be to relate the rise of \( \langle r \rangle \) to a phase transition or instability of the membrane such that \( d\langle r \rangle/dt, \ d^2\langle r \rangle/dt^2 > 0 \). In that case, however, there would exist a threshold in the course of the sodium activation, which has not yet been detected up to now.

Concerning the maximal conductances, we observe, in agreement with experiment, that for increasing depolarizations the position in time of the maxima first moves to the right and thereafter presents a reversed displacement to the left. On the other hand HH and also Dodge and Frankenhauser\(^{20} \) have shown that the height of the peak as a function of \( V \) might vary very steeply, an \( e \)-fold increase of \( g_{Na}^{max} \) resulting from a change in membrane potential of 5 mV. Several authors have suggested\(^{13,16,23} \) that this steep variation found its origin in the existence of structural cooperative interactions between channels. The model proposed here permits us to obtain some indications of this possibility. Without inactivation, the maximum conductance is simply proportional

* The instantaneous response of \( U \) may be neglected as long as \( \langle r \rangle \ll 1 \), that is, with the resting state as initial condition; on the contrary when \( \langle r \rangle \approx 1 \), a repolarization of the membrane leads to an instantaneous decrease of \( U \), which produces an instantaneous significant fall of the permeability function.
Figure 3. Fitting of the experimental values of Hodgkin and Huxley for $g_{Na}$ (squares) on the basis of Equations (3.5) to (3.15). The numerical values are $K = 0.39$, $\eta = +2.945$, $q = 3.737$, $\mu_R - \mu_S = -407$ D. In this fitting, we have tried to minimize the absolute value of $\mu_R - \mu_S$; it may therefore be considered as an estimation of the minimal dipole moment necessary in the case of a conformational equilibrium depending on the orientation of a single dipole. Much lower values become sufficient when the number of dipoles per ionophore increases.

to the equilibrium values of (4.1) as a function of $V$. This equilibrium is an intricate function of the parameters entering Equations (3.7) to (4.1). However, as a crude test we may consider the simplified case corresponding to Equation (3.11). Then $U(x_{ij}) \rightarrow \langle r \rangle$, and we are left with the problem of finding values of the effective dipole moment $(n-1) (\mu_R - \mu_S)$ and of the cooperativity parameter $\eta$ that permit us to fit the experimental points satisfactorily. The result is reported in Fig. 4; it yields a value of about 400 D for the dipole moment, and $-2.95$ for $\eta$. This is only a rough approximation, and we shall not try to make it more precise. Let us simply say that in order to diminish the importance
of cooperativity one necessarily would have to look for bigger dipole moments. A more detailed discussion of these orders of magnitude would require a rigorous knowledge of the kinetic parameters involved, and in particular of the ratio $k_d'/I_{RS}$, which, as we shall see in the discussion of inactivation, largely determines the maximum height measured for the peaks.

**B. The Spontaneous Inactivation of $g_{Na}$**

The spontaneous inactivation of $g_{Na}$ is, in general, considered as a process distinct from the reversal of the activation process. Among the mechanisms invoked one generally mentions "the movement of a block-
ing particle to a certain region of the membrane" (HH), the blocking of Na$^+$ transport by calcium ions,$^{24}$ and the presence of a third conformation of the Na$^+$ ionophore in addition to the resting state and active state.$^{25}$ The explanation adopted here, related to the presence of equilibriation layers, was first indicated by Tasaki$^{19}$ in his book, who wrote, “The fall of the membrane potential from the peak of the action potential may be considered as a result of accumulation of the interdiffusing cations in and near the axon membrane.” Let us now look at the implications of this mechanism in more detail.

Figure 2 clearly shows that the time course of Na$^+$ inactivation at various clamping potentials can be reproduced satisfactorily: The inactivation follows a simple exponential law as a function of time, in contrast with the high-order kinetics for activation, and vanishes for small variations of the potential, as in the experimental case. In Fig. 4 are compared the time dependences of the three fundamental functions of the theory: $g_{Na}$, the permeability function $g$, and the environmental functions $\alpha, \beta, \nabla C = \beta t^{2n} - \alpha$. The plots correspond to a small supra-threshold depolarization (40 mV). It becomes evident that changes of the Na$^+$ gradient within the equilibriation layers suffice to bring about an almost complete inactivation of $g_{Na}$. Interestingly, and in contrast with the generally accepted concepts, the permeability of the membrane remains high and constant while the Na$^+$ current vanishes; in other words, the sodium “channel” remains open during the inactivation.

A striking advantage of this interpretation of inactivation is that it furnishes a simple explanation of HH’s two-pulse experiments (effect of a small conditioning pulse on the changes of Na$^+$ conductance following a supra-threshold consecutive pulse). They showed, for instance, that a conditioning pulse of small amplitude (8 mV) but lasting more than 20 msec might cause up to 40% reduction of the maximal change of sodium. The permeability $g$ and $\langle r \rangle$ return rapidly to their resting values, while $g_{Na}$ remains very small, but $\alpha$ and $\beta$ take a long time to recover their initial values; in the equilibriation layers, Na$^+$ ions take a long time to reequilibrate with the bulk solutions. According to the model, the time necessary for the reequilibration would correspond to the refractory period. Most of the experimental findings relative to the process of Na$^+$ inactivation can thus be understood on the basis of limited diffusion conductance caused by a subsequent 40-mV pulse. For shorter conditioning pulses the decrease of $g_{Na}^{\text{max}}$ follows a smooth exponential curve. This is indeed the behavior obtained here. In the upper part of Fig. 5 we have plotted $\langle r \rangle$, the permeability function $g$, as a function of $V$. In the lower part we have plotted the maximum conductance that would be
Figure 5. In the upper part of the figure, we have drawn the steady-state solutions for \( \langle r \rangle \) and \( g \) as a function of \( V \), as well as the corresponding normalized values of \( g_{Na} \) found by Hodgkin and Huxley (squares). In the lower part of the figure we have reported, for values of \( p \) equal to 0, 0.01, 0.05, 0.5, the effect of a conditioning pulse, of amplitude given by the abscissa, on the maximum conductance of the membrane in a depolarization of 70 mV. The curves are normalized by dividing by the maximum conductance that would be attained in the absence of conditioning pulse. All parameters are as in Figure 2.

recorded in a depolarization of 70 mV preceded by a conditioning pulse of amplitude given by the abscissa. The curves are drawn for different values of the parameter \( p \), which fixes the location of the equilibration layer. It is seen that the effect increases rapidly as \( p \) varies from 0 to 1. In particular, for \( p = 0.5 \), a depolarization of 8 mV produces a decrease in the peak conductance of more than 50\%, although the permeability function \( g \) remains practically unchanged. Furthermore it is also predicted that if the conditioning pulse is of larger amplitude (15 mV < \( V_c \),
<70 mV), the current recorded in the test pulse could be reversed and be outward.

Finally, if the equilibration layers cause inactivation by creating a diffusion barrier to the permeant ion, the recovery from inactivation should be much slower than the inactivation itself. This is indeed what is seen with excitable membranes, where the refractory period lasts much longer than the action potential. The right-hand parts of the curves in Fig. 4 illustrate the variation in time of the fundamental functions of the theory following an abrupt termination of the clamp. between ionophores and bulk solutions. In this respect it is interesting here to underline the recent results of Dubois and Bergman,26 who have demonstrated the existence on both sides of the membrane of an adsorption site on which the fixation appears as a preliminary rate-limiting step to the transport. On the other hand, it is known that calcium ions enhance inactivation. One simple interpretation of this effect would be that calcium alters the properties of the equilibration layers, or of the fixation sites studied by Dubois and Bergman, in such a manner that the diffusion of Na⁺ becomes more difficult at their level. In other words, Ca⁺ binding would decrease. A similar interpretation could also account for the prolonged action potentials observed by Rojas27 after the treatment of the membrane with pronase.

In the coupling between activation and inactivation, the parameter whose value is critical is the ratio $k'_d/l_{RS}$. In Fig. 6, we have plotted as a function of potential, for a set of parameters, the theoretical maximum conductance as it is given by the equilibrium solutions of (4.7), and the actually observed maximum conductances for several values of $k'_d/l_{RS}$. It is manifest that when this ratio increases, the deviations between the theoretical curve and the values that would be observed increases drastically. Eventually, if $k'_d/l_{RS} \to \infty$, there would be no rise in conductance at all.

A test for the validity of our interpretation of sodium inactivation would be offered by the direct demonstration that during the inactivation phase the permeability of the membrane remains constant while the ionic concentrations in the equilibration layers vary. Such an experiment appears feasible as long as one possesses a physical parameter characteristic of the state and environment functions. Much more information certainly could be obtained concerning the latter quantities by the detailed study of the behavior of $V_{Na}$ under various sets of conditions. On the other hand, adequate spectroscopic probes might be found that selectively bind to the ionophore or to the equilibration layer and represent the change of ionophore conformation with Na⁺ concentration.
Figure 6. Maximum conductance as a function of the ratio $k'_d/l'$. All parameters are as in Figure 2. $k'_d = 1$; $l' = 100, 200, 400, 800$.

C. The Instantaneous Linearity of the $I-V$ Curves.

When the number of energy barriers crossed by the ions inside the membrane increases, it has been shown\(^\text{17}\) that the instantaneous $I-V$ curves rapidly become linear. It can be seen in Fig. 7 that, in varying $p$, a similar result is easily obtained even if only one barrier in the membrane is considered. For $p = 0.5$ and with the resting state as initial condition, the $I-V$ curves are linear within a few percent over a considerable range. This property is moreover preserved in the course of the transition towards the active state, as long as this process is sufficiently rapid with respect to the relaxation of the gradient of concentration. It must, however, be recognized that this is not sufficient to fully account for the experimental observations: Instantaneous linearity is required not only for the resting state, but for any state of the membrane as initial condition; it is verified whether the channels are activated or inactivated, and can also be observed in the case of the potassium conductance changes. One is therefore tempted to suggest
that the instantaneous linearity of the $I-V$ curves is related to structural properties like those described by $U(x_{ij})$ and that are capable of causing an instantaneous and important variation. In any case, a more detailed analysis of the field effect on the conformation equilibrium and of the fast charge motion characteristic of the capacitive currents is necessary.

V. STABILITY OF SPACE-CLAMPED ACTION POTENTIALS IN THE ABSENCE OF SODIUM INACTIVATION

In this last section we would like to consider some stability properties associated with the action potential itself. We investigate a simplified situation corresponding to a space-clamped nerve in the absence of sodium inactivation. It is indeed well known that sodium inactivation can be attenuated in various ways, and in particular by perfusing the nerves with pronase$^{27}$; such a situation is therefore not unrealistic.

We suppose that the opening of the potassium channels may appropriately be described by an equation analogous to (3.11).
Furthermore, for the sake of simplicity, both for sodium and for potassium we neglect the effect of structural cooperativity. Under those conditions, the time change of the active fractions \( \langle r \rangle \) and \( \langle t \rangle \) of the sodium and potassium channels is described by the simple set of two equations:

\[
\frac{d\langle r \rangle}{dt} = l_{RS}[1 - \langle r \rangle - \alpha \langle r \rangle e^{\mu_{Na}E/RT}] = F_r
\]

\[
\frac{d\langle t \rangle}{dt} = l_{IS}[1 - \langle t \rangle - \alpha' \langle t \rangle e^{\mu_{K}E/RT}] = F_t
\]

where \( \mu_{Na} \) and \( \mu_{K} \) are the differences in dipole moment between the Na and K active and nonactive conformations; \( l_{RS}, l_{IS}, \alpha, \text{ and } \alpha' \) are constants. On the other hand, since the equilibration layers play no role here, it is sufficient in order to describe the time change of \( V \) to consider the equation:

\[
- C \frac{dV}{dt} = \langle r \rangle e^{q(r)}(V - V_{Na}) + \langle t \rangle e^{q(t)}(V - V_{K}) = F_v
\]

The steady states of the system can be obtained by solving the set of simultaneous equations

\[
\langle r \rangle_0 = \frac{1}{1 + \alpha \exp(\mu_{Na}E_0/RT)} ; \langle t \rangle_0 = \frac{1}{1 + \alpha' \exp(\mu_{K}E_0/RT)}
\]

\[
V_0 = \frac{\langle r \rangle_0 e^{q(r)_0}V_{Na} + \langle t \rangle_0 e^{q(t)_0}V_{K}}{\langle r \rangle_0 e^{q(r)_0} + \langle t \rangle_0 e^{q(t)_0}}
\]

Their stability properties then depend on the nature of the normal modes of the following secular determinant:

\[
\begin{vmatrix}
\omega - \frac{\partial F_r}{\partial r} & 0 & - \frac{\partial F_r}{\partial V} \\
0 & \omega - \frac{\partial F_t}{\partial t} & - \frac{\partial F_t}{\partial t} \\
- \frac{\partial F_v}{\partial r} & - \frac{\partial F_v}{\partial t} & - \omega - \frac{\partial F_v}{\partial V}
\end{vmatrix} = 0.
\]

On the basis of (5.4) and (5.5) a stability diagram of the type shown in
Fig. 8 can straightforwardly be established. All other parameters being fixed, it is seen that as a function of $\mu_{Na}$ and $\alpha$, the system may present at least four distinct domains. In domain I, (5.4) admits only one steady-state solution, corresponding to the resting state of the membrane, and which is a stable node. A similar stable node is also found in domains II, III, and IV, but furthermore we then have two other solutions, which in II correspond to saddle points, in III to a saddle point and an unstable focus, and in IV to a saddle point and a stable focus. Essentially, we thus see that in going from domain I to domain IV, we pass from membranes that present a unique steady and stable regime, corresponding to the resting state, to membranes that may exist either in a polarized stable state or in a depolarized stable state. The latter state is assimilable to a prolonged action potential.

![Stability diagram as a function of the difference in the dipole moment of sodium between the R and S states and of the conformational equilibrium constant $\alpha$. All other parameters are fixed: $\alpha' = 0.661, \mu_K = 250, I_{IS} = 1, I_{RS} = 30$.](image)

On the other hand, it is important to realize that the existence of multiple-steady-state regimes in a membrane is not necessarily directly related to the presence of a threshold or all-or-none effect during the action potential. In Fig. 9 we have plotted, for various depolarizations, the time course of $V$, obtained by solving equations (5.1) to (5.3) numerically in the case of a system that lies in domain II and presents...
Figure 9. Space-clamped action potentials obtained in domain II. $\mu_{Na} = 469; \alpha = 0.708$. All other parameters are as in Fig. 8.

typical action-potential behavior. In Fig. 10, the maximum values of $V$ are given as a function of the amplitude of the initial depolarizing stimulus. It is clear from this curve that the threshold for depolarization is reached and lies between 41 and 42 mV. On the contrary, the steady-state values of $V$ correspond to 0.0017 mV. The parameter that critically controls the position of the threshold here is the ratio $I_{RS}/I_{IS}$, which measures the delay in potassium activation with respect to sodium activation: When $I_{RS}/I_{IS}$ diminishes, the value of the threshold tends to increase. In Fig. 11 we have graphed the behavior in time of the fractions $\langle r \rangle$ and $\langle t \rangle$ following a depolarization of 75 mV; it can be seen that at the peak of sodium conductance, when 95% of the sodium channels are open, one has only 40% of the potassium channels open.
VI. CONCLUSIONS

We have presented an interpretation of the changes of conductance observed during electrical excitation, based on the properties of ionophores, which may exist in two conformational states characterized by different permeabilities and dipole moments. Primarily, we have considered the case of sodium transport and shown that rather low dipole-moment differences between the conformational states (let us say, less than 100 D) permit us to account for the steep variation of the maximum conductances as a function of the applied depolarization; however, several dipoles per ionophore would then necessarily be implicated in the conformational transition. The existence of cooperative interactions among the ionophores might, on the other hand, both
facilitate this conformational transition and increase the transport capacity of the ionophores; in this way, cooperativity permits us to account for the high-order kinetics characteristic of the activation process.

We also investigated in detail the behavior of our transporting systems when they are separated from the bulk solutions by the presence of equilibrium layers, which create a diffusion barrier hindering the ionic motion. On the basis of this simple hypothesis, the essential characteristics of sodium inactivation can easily be reproduced; several predictions have been made that should be experimentally testable.

Finally, we considered the type of stability problems that may arise in space-clamped nerves when the sodium inactivation process has been
abolished. Surprisingly, it was shown that the absence of inactivation does not necessarily imply the occurrence of prolonged action potentials. Depending on the values of the parameters, we have, even for this simplified situation, quite complex stability diagrams, in which certain domains may contain multiple-steady-state regimes.

References