A DIPOLE MODEL FOR NEGATIVE STEADY-STATE RESISTANCE IN EXCITABLE MEMBRANES

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ABSTRACT A dipole model is presented for ion flow in excitable membranes. This model considers the membrane to be composed of two distinct regions: a polar region and a nonpolar region. Further, the construction of an electrodiffusive formalism which takes explicit account of the energy of partition required by an ion for passage from external fluid to nonpolar region is presented. In the polar region a cooperative effect is considered which produces a configurational transition of the polar group dependent only on membrane voltage. A resulting change in voltage drop across the polar group is brought about by this configurational transition. This gives rise to a negative steady-state resistance for the equimolar case, in reasonable agreement with observation. The theory, in addition, is in reasonable accord with nonequimolar ion flow, and provides an explanation for such effects as the following: the intercept of the voltage-current characteristic, the ion membrane concentrations inferred from electrodiffusion theories, and the effects of polyvalent cations

I. INTRODUCTION

In any attempt to elucidate the fundamental molecular mechanisms involved in the behavior of excitable membranes, one must consider both the complex transient phenomena associated with excitation activity and the steady-state current-voltage characteristics. Since the steady-state measurements are by far the simpler observations, any theory must certainly first explain these observations. Cole (1968) points out that perhaps the most vexing of these steady-state observations is the appearance of a negative steady-state resistance in membranes that are immersed in equimolar solutions of potassium salt. He notes that the explanation of the negative steady-state resistance has been a stumbling block of many theories, most notably the classical electrodiffusion models, and suggests that the origin of excitation lies in the understanding of this steady-state phenomena. In this paper we attempt to explain quantitatively this negative steady-state resistance in terms of molecular configurational changes and show this explanation to be consistent with other steady-state phenomena.

As pointed out, the classical electrodiffusion models based on the Nernst-Planck equations do not give negative steady-state resistance. Earlier attempts to explain this phenomenon have involved modification of the classical electrodiffusion formalism, usually by the addition of fixed charge or pressure as variables. Agin (1969) has recently reviewed these and other attempts and pointed out their shortcomings.

In the present work we consider a modification of an electrodiffusion theory based on a traditional bimolecular leaflet type of membrane scheme (Fig. 1) which yields a steady-state negative resistance in reasonable agreement with observation. This theory modifies the usual classical formalism in two key respects. We first consider explicitly the water-lipid partition energy as a boundary condition for transport across the membrane interface and as the chief barrier to permeation (section II). Secondly, we consider in detail a configurational transition of the polar groups of the membrane molecules. This transition involves a turning of the polar portions of the molecules and change in the voltage across the polar portions which is an explicit function of membrane voltage. It is this change in voltage across the polar portions of the membrane which produces the negative resistance (section III).

The theories developed in sections II, III, and IV describe the steady-state membrane in terms of 11 basic molecular parameters. In section V experimental data on equi- and nonequimolar membrane flows is utilized to obtain quantitative estimates of these parameters. It is shown there that a wide range of steady-state membranes phenomena, including steady-state negative resistance, can be accounted for by the present theory and that the kind of membrane structures that may be inferred are in reasonable accord with present knowledge of membrane structure.

Perhaps the most recent theoretical explanations of negative steady-state resistance have been by Goldman (1969) and Agin (1969). Agin has developed a theory for negative resistance based on departures of the ions fluxes from the Nernst-Planck equations due to the presence of ion sites in the membrane and a single file migration mechanism. Then assuming that the steady-state currents produce only a minor perturbation in the equilibrium (resting) concentration profile, a current-voltage relation is derived. This current-voltage characteristic, with certain values of the parameters chosen, does yield a steady-state negative resistance. Perhaps the main shortcoming of the theory, which is recognized by Agin (1969), is the completely *ad hoc* assumption of current-independent concentration profiles in the membrane. Agin (1969) points out that it is this assumption which gives rise to negative resistance. Independence of concentration profile of current flow is not a notable characteristic of physical transport systems. Therefore one must consider the results of the theory fortuitous until a definite mechanism can be proposed to provide for such independence.

Goldman (1969) has proposed an explanation of the phenomena based on a polar mechanism similar to that put forth here. In fact Goldman (1964) was the first to expound a detailed mechanism of excitation which centered on configurational changes of the polar groups. Goldman suggested that radical changes in calcium adsorbability by the polar groups can change the energy requirement for ions to enter the lipid. The energy requirements of this hypothesis are as yet unclear. Goldman's (1969) explanation of the negative steady-state resistance makes use of this polar hypothesis, and in addition, considers a current of anions in order to complete the theory.

A recent polar hypothesis has also been put forth by Wei (1966). Wei assumes that changes in the dipole orientation and voltage can mediate the changes in Na^+ current flow during an action potential. In this paper we demonstrate that while changes in the voltage across the polar portions of the molecule are responsible for the negative steady-state resistance they cannot be responsible for changes of sodium flux by a factor of 250. Wei (1966) ignores the main energy barrier to Na^+ permeation: the partition energy necessary to go from water to lipid. Changes in polar voltage can at most account for changes of flux by factors of only 3–5. The polar group theory which most resembles the present work is given by Wobschall (1968). He considers the membrane bilayer to consist of layers of electrets which can respond in a cooperative fashion to changes in external electric field and postulates that this response in turn produces changes in ion permeability. The physics of the cooperative effect is modeled by means of an equivalent circuit. The present model differs from this in that we consider distinct polar and nonpolar membrane regions and analyze in detail the physics of the cooperative dipole transition. In addition we employ a detailed analysis of the electrodiffusive flow of ions rather than utilizing equivalent circuit models. We do point out that we have in common with Wobschall (1968) the idea of a cooperative dipole transition rather than dipoles individually lining up in the external field as proposed by Goldman (1964) and Wei (1966).

II. ELECTRODIFFUSION MODEL

The basic membrane model assumed is the bimolecular leaflet model of Davson and Danielli (1943). Fig. 1 shows a schematic view of this membrane which is considered to be composed of three major regions: region I, the extracellular fluid; region II, the nonpolar, hydrophobic region of the membrane (NPR); and region III, the intracellular fluid. The outer portion of the membrane, separating region I and II is assumed to be composed of the hydrophilic polar regions of the membrane molecules (PR) and an absorbed layer of ions, the counterion layer. This layer is considered to exist in the extracellular fluid immediately adjacent to the membrane molecules and to be held by the negatively charged part of the polar group, when oriented by the external field, and possibly by fixed negative charge. The inner portion, separating regions II and III, is considered to be composed of a hydrophilic polar region. The inner boundary is assumed not to have a counterion layer. The inner and outer PR are assumed to have different properties so that the voltage

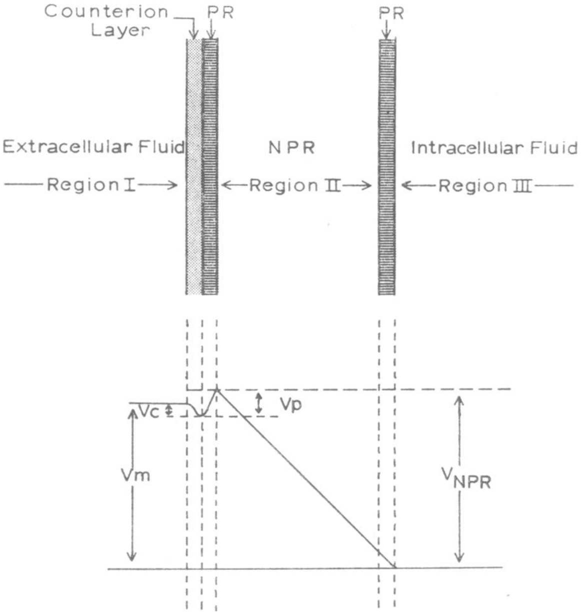

Figure 1 Membrane schematic. Top, division of membrane into regions. Bottom, voltage drops within membrane. PR, polar region; NPR, nonpolar region; V_m, membrane voltage; V_c, counterion voltage; V_p, polar voltage; V_{NPR}, nonpolar voltage.

across the inner polar region is minimal under normal circumstances and will be neglected. This assumption is suggested by some results discussed in section V. Its justification and implications will be discussed further there. Fig. 1 also shows a sketch of the assumed potential drops implicit in this choice of model. We note from Fig. 1 that when the inside of the cell is made negative with respect to the outside, our assumptions imply that the major drop in potential occurs across the hydrophobic regions of the membrane (Fig. 1, V_{NPR}). There will, however, also be significant potential gradients across the polar interfaces (Fig. 1, V_p), and a diffuse double layer potential (V_c) will develop across the counterion layer adjacent to the polar groups.

The basic view that we take of ion movements across the membrane is that the dominant over-all barrier to permeation is the energy required to carry an ion from the water phase into the NPR, i.e., the partition energy. The ions, once they enter the NPR, and finding few, if any, polar or charged groups with which to form association, scatter from the geometric barrier that these molecules present. This is to say, the NPR will appear to the ion as a polymeric network with thermal motion represented by folding and unfolding of the chain links. It is from these chain links that the ions scatter. This scattering represents the permeation barrier within the NPR, and has certain significant implications. If one assumes the ion-scattering model, one may also assume that the ion-scattering processes will keep the ions in the membrane close to a Maxwell-Boltzmann equilibrium distribution so that the use of the Nernst-Planck diffusion equations within the membrane will be justified.

This view of permeation is quite consistent with that of Stein (1967). Stein assumes that the permeability may be considered as a purely hydrocarbon term times an exponential term involving the energy of dehydration of the permeating species. He shows an impressive correlation between the number of hydrogen bonds broken in leaving the water and the permeability. From this correlation he obtains separate estimates of the contribution to permeability of the membrane interface and the hydrocarbon portions of the membrane and notes that the hydrocarbon contribution is similar to polymeric networks.

We also note that the view assumed here is in contradistinction to that of ions permeating through water-filled pores or channels. As Stein (1967) points out, the observed activation energies for ion permeation are much larger than those for ions diffusing through water so that such a water-filled pore is not likely. Our view is equally at odds with the idea of a charged membrane with sites for ions. We point out that the NPR region has few, if any, polar or charged groups with which ions might chemically associate. That is to say, given the general situation in which a structure is composed of molecules containing both charged and uncharged portions, and is in intimate contact with a polar environment, such as an aqueous solution, it is simplest and most reasonable to assume that the charged portions of the molecules will associate with the polar environment, while the uncharged portions will tend to associate one with the other. Since we have adopted the bimolecular leaflet model of the membrane, this clearly implies that the NPR portion of the membrane must be free of charge groups.

In considering the three membrane regions (Fig. 1) we note that in region II, the nonpolar region, we consider the ion density to be so small that the Debye length of the ions will be much greater than the NPR dimensions and so the electric field will be constant. We further assume the Nernst-Planck equations hold, defining a diffusion coefficient D and dielectric coefficient which are both constant. We neglect the diffusional resistances in regions I and III because the diffusion coefficient in water is much larger than in NPR.

We first write for the current J of a univalent cation:

$$J = D\left[-\frac{dn}{dx} + \frac{eEn}{kT}\right], \qquad \text{(II 1)}$$

the usual Nernst-Planck equation, where D is the diffusion coefficient in the NPR, x specifies the position within the membrane, E is the electric field, k is the Boltzmann constant, T the absolute temperature, and e the electronic charge. The equation of mass conservation is written as:

$$\frac{dJ}{dx} = 0. \qquad \text{(II 2)}$$

For the electric field we have the usual Poisson equation:

$$\frac{dE}{dx} = \frac{\rho}{\epsilon_0}, \tag{II 3}$$

where ρ is the net charge density at any point within the membrane.

Before proceeding to the analysis, we derive the form of the boundary condition for the interface resistances between regions I and II and regions II and III. We first attempt a rather precise definition of what these interfaces encompass. In Fig. 1 we see the membrane is considered to consist of a hydrophobic region (NPR) and a polar region (PR) bounded by an aqueous fluid. We consider the interface to be between the aqueous regions and the NPR. The polar groups are considered to be approximated by a sheet and actually imbedded within the interface. Therefore, the flux across this interface will be the usual thermal flux of ions from the aqueous region, but with considerable modification, due to the energy for partial dehydration as well as from the voltage across the interface.

If we refer to the flux into the NPR as J_{in}, we write:

$$J_{\text{in}} = J_{\text{thermal}} \left[\exp - \left(\frac{U_0}{kT} + \frac{eV_p}{kT} \right) \right], \tag{II 4}$$

where $J_{\text{thermal}} = n_w \bar{v}_w$ and n_w is the concentration of the ion in the water adjacent to interface, $\bar{v}_w$, its thermal velocity in the water, U_0 the partial dehydration energy and V_p the voltage drop across the polar group.

We assume that the flux of ions from NPR to water (which we call J_{out}) is given simply by a thermal flux:

$$J_{\text{out}} = n\bar{v}_{\text{NPR}}\,. \tag{II 5}$$

The rationale for this assumption is that when an ion in the vicinity of the aqueous region can free itself from the geometric barriers (folding and unfolding of chain links) that the NPR presents to it, the ion will go into the aqueous region with no further energy barrier to its passage. Because the energy gradient is so favorable for passage from NPR to water, V_p should have little effect on J_{out}.

Therefore, at the I, II interface:

$$D \left[-\frac{dn}{dx} + \frac{eEn}{kT} \right]_{\text{NPR}} = J_{\text{in}} - J_{\text{out}}$$

where

$$J_{\text{in}} = n_w \bar{v}_w \exp \left[- \left(\frac{U_0}{kT} + \frac{eV_p}{kT} \right) \right]$$

$$J_{\text{out}} = n\bar{v}_{\text{NPR}} \tag{II 6}$$

where n is the concentration of ions in the NPR (at the interface) and n_w is the concentration of ions in water immediately adjacent to the membrane. Due to the counterion layer voltage, V_c, $n_w = n_e \exp - (V_c e/kT)$ where n_e is the concentration of the ion in the external fluid. At the II, III interface:

$$-D\left[-\frac{dn}{dx}+\frac{eEn}{kT}\right]_{\mathrm{NPR}} = J_{\mathrm{in}} - J_{\mathrm{out}},$$

where

$$J_{\mathrm{in}} = n_w \bar{v}_w \exp\left(-\frac{U_0}{kT}\right)$$
$$J_{\mathrm{out}} = n\bar{v}_{\mathrm{NPR}} \qquad \text{(II 7)}$$

and $n_w = n_i$ the concentration of ions within the cell since no significant counterion layer is developed internally. We now integrate the flux equation

$$J = D\left(-\frac{dn}{dx}+\frac{eEn}{kT}\right),$$

and obtain for constant field:

$$n = n(0)\left[\exp\left(\phi_{\mathrm{NPR}}\frac{x}{L}\right)\right] + \frac{J}{D\phi_{\mathrm{NPR}}}\left(1 - \exp\left(-\phi_{\mathrm{NPR}}\frac{x}{L}\right)\right), \qquad \text{(II 8)}$$

where $\phi_{\mathrm{NPR}} = eV_{\mathrm{NPR}}/kT$, with V_{NPR} defined in Fig 1; where x is measured from the exterior surface of the membrane (i.e. $x = 0$ at the I, II interface), L is the thickness of region II, and $n(0)$ the ion concentration in region II at the I, II interface. We note that equation II 8 has two unknowns, $n(0)$ and J. These may be determined by an application of the two interfacial conditions of equations II 6 and II 7. Combining equations II 8, II 6, and II 7 we may now obtain an expression for J:

$$J = \frac{J_{\mathrm{sat}_e} e^{-(\phi_p-\phi_c)}[e^{-\phi_m} - e^{-\phi_r}]\phi_{\mathrm{NPR}}}{B(1 - e^{-\phi_{\mathrm{NPR}}}) + \phi_{\mathrm{NPR}}(1 + e^{-\phi_{\mathrm{NPR}}})},$$

where

$$J_{\mathrm{sat}_e} = n_e \bar{v}_w \exp\left(-\frac{U_0}{kT}\right); \qquad \phi_m = \frac{eV_m}{kT}$$

$$\phi_p = \frac{eV_p}{kT}, \qquad \phi_c = \frac{eV_c}{kT}, \qquad B = \frac{L\bar{v}_{\mathrm{NPR}}}{D}$$

and

$$\phi_r = \frac{eV_r}{kT} = \ln\frac{n_e}{n_i} \qquad \text{(II 9)}$$

the resting potential. We note that in equation II 9 all voltages are taken to be positive when membrane potential is inside negative. When the membrane potential is inside positive $\phi_r > 0$ and all other voltages are now negative.

It is important now to ask under what circumstances will equation II 9 become equivalent to the usual electrodiffusion results. If in equation II 9 we take the limit of $B \rightarrow \infty$, and V_p and V_c both zero we obtain:

$$J = \frac{\frac{J_{\mathrm{sat}_e}}{B}\phi_m[e^{-\phi_m} - e^{-\phi_r}]}{(1 - e^{-\phi_m})}, \tag{II 10}$$

which can be related to the usual electrodiffusion result, provided that J_{sat_e}/B is defined as the ion permeability. This is quite interesting, since now we have a more fundamental basis for defining permeability (P):

$$P = \frac{D}{L}\left(\frac{\bar{v}_w}{\bar{v}_{\mathrm{NPR}}}\right)e^{-U_0/kT} \tag{II 11}$$

so that the usual permeability is seen to be because of a term which involves the NPR diffusion barrier D/L, a term associated with the activation of NPR chain links $\bar{v}_w/\bar{v}_{\mathrm{NPR}}$, and a term which involves the partial dehydration energy e^{-U_0}/kT.

In practice, B will not be large but will typically be of order one. This will result in the present theory giving a qualitatively different description of steady-state phenomena than that given by the Goldman equation.

In summary we now list those physical parameters which the present section suggests are necessary for the description of steady-state ion permeation:

(*a*) U_0, the energy for partial dehydration of an ion,
(*b*) D, the diffusion coefficient of an ion in NPR,
(*c*) L, the thickness of the NPR,
(*d*) $\bar{v}_{\mathrm{NPR}}$, the exit velocity of an ion from the NPR, and
(*e*) $\bar{v}_w$, the thermal velocity of an ion in water,

While these are the fundamental molecular parameters, these parameters appear in equation II 9 in essentially two groupings: J_{sat_e} and B. In the usual electrodiffusion equation only the permeability appears.

The major assumptions of this section are:

(*a*) The membrane is composed of an NPR and PR.
(*b*) A counterion layer exists immediately adjacent to the membrane.
(*c*) For the NPR, the Nernst-Planck relation holds and the field is constant.
(*d*) The PR may be considered as an interface of negligible thickness.
(*e*) V_p and V_c are negligible on the inner surface at the membrane.

III. CONFIGURATIONAL TRANSITION OF THE POLAR GROUP

In this section we develop a theory for a configurational transition in the polar groups of the membrane molecules. The polar groups involved in this transition may be phospholipids or protein; the only requirement is that we associate a dipole moment to this region. The transition is found to be only voltage dependent, and when used in conjunction with the steady-state current theory, it produces a negative resistance.

First we consider the polar groups as being approximated by a row of dipoles of some known dipole moment. In general the position of dipoles relative to each other, which corresponds to a minimum energy configuration (assuming no thermal motion of the dipoles) is for the dipoles to lie in the same plane (Fig. 2). In this configuration, if one dipole were to be rotated out of the plane, a large restoring force due to dipole-dipole interaction would be felt. This would tend to restore the dipole to its original position. If however many dipoles were simultaneously rotated out of the plane, the net restoring torque would only be proportional to those that remained. In this way if all the dipoles were to rotate far out of the original plane, there would be no restoring torque and the dipoles would essentially be free. This in fact is the state of affairs which exists when the temperature (and subsequent thermal motion) increases. At low temperatures the dipoles will all be very close to their minimum potential energy configuration in the plane. As the temperature increases, more dipoles rotate out of the original plane, and this in turn weakens the dipole-dipole forces that created the original well-ordered configuration. Therefore, as the temperature increases, a cooperative order-disorder transition takes place and the dipoles all become free to rotate at high temperatures. To quantitatively describe this transition, various investigators have applied statistical physics to the

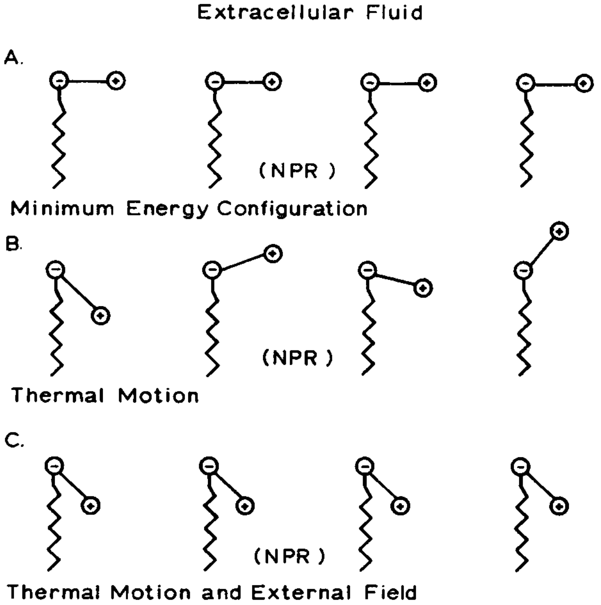

FIGURE 2 Configurations of polar group.

problem of order-disorder. One technique, which is simple and gives a reasonable result is due to Fowler (1935).

Let N_1 = bound dipoles (those not free to rotate) and N_2 = free dipoles (those that may rotate), where $N_1/N = S$ and $N = N_1 + N_2$. If we say that dipoles with a thermal energy $>(eE_0p)/3$ are free to rotate (where E_0 is the dipole-dipole field, and p the dipole moment) then from the Boltzmann distribution we know the fraction of dipoles with thermal energy $<(eE_0p)/3$ is:

$$1 - S = \exp\left[\frac{-pEe}{3kT}\right]. \qquad \text{(III 1)}$$

The physical basis of the cooperative effect is that as S decreases, the dipole-dipole field, E, which tends to order the dipoles also decreases. If we assume that $E = E_0S$, where E_0 is the original, fully ordered field, we can write:

$$1 - S = \exp\left[\frac{-epE_0S}{kT}\right]. \qquad \text{(III 2)}$$

This equation may now be solved for S. The solution indicates that at a high temperature, $S = 0$. As one lowers the temperature an abrupt transition occurs at a temperature $T = (epE_0)/3k$. This temperature is usually referred to as a Curie temperature. Below this temperature, the system becomes ordered and $S \rightarrow 1$. This can be seen by expanding the exponential in equation III 2:

$$\left(\frac{epE_0}{3kT} - 1\right)S - \frac{1}{2!}\left(\frac{epE_0S}{3kT}\right)^2 + \frac{1}{3!}\left(\frac{epE_0S}{kT}\right)^3 + --- + = 0, \qquad \text{(III 3)}$$

where we have an alternating series. It can be seen that for this equation we only have a nonzero solution for $S(S > 0)$ if $(epE_0)/3kT > 1$. So that $S = 0$ for $(epE_0)/3kT < 1$ and $S \rightarrow 1$, rapidly, below this temperature.

The difficulty with directly applying this cooperative effect to the polar groups of the membrane is that we generally consider biological systems to be isothermal. Let us now examine the possibility that the membrane field acts in a manner similar to that in which temperature acts. Consider that the membrane field, which acts perpendicularly to the original plane of the dipoles, will disrupt the ordered array of dipoles. That is to say its effect will be to rotate the dipoles away from their ordered configuration and cause the dipole-dipole interaction to be weakened. This weakened dipole-dipole interaction allows a free rotation of the polar portions of the molecule which will now align in the membrane field. To express this effect quantitatively, using statistical physics, we consider a new effective temperature: $T_e = T + (epE_m)/k$, where E_m is the membrane field, T the absolute temperature, and k the Boltzmann constant. We point out that the assumption implicit here, is that the orienting field, E_m, that disrupts the dipole-dipole interaction is the constant field which exists in the NPR. The effects of the dipole self field themselves are already accounted for, since it is the self field which acts to create the ordered dipole configuration of Fig. 2 A.

We may now proceed by utilizing equation III 2 in modified form:

$$1 - S = \exp\left[\frac{\dfrac{-pE_0\,e}{3kT}\,S}{1 + \dfrac{pE_m e}{kT}}\right], \tag{III 4}$$

where the effects of the membrane field now appear explicitly. We see that when

$$\frac{\dfrac{epE_0}{3kT}}{1 + \dfrac{epE_m}{kT}} < 1,$$

the dipoles are free to align in the membrane field (Fig. 2 C), while for

$$\frac{\dfrac{epE_0}{3kT}}{1 + \dfrac{epE_m}{kT}} > 1$$

The dipole-dipole interaction takes hold, producing an ordered configuration (Fig. 2 A). We now have introduced a configurational transition of the polar group which depends only on the membrane voltage and defines an order-disorder transition.

As the dipoles align in the membrane field, a voltage develops across the polar region (V_p) and is given by:

$$V_p = \frac{p\,\overline{\cos\theta}}{A\kappa\epsilon_0}(1 - S), \tag{III 5}$$

where A is the area per dipole in m^2, κ is the dielectric constant, $\epsilon_0 = [36\pi \times 10^9]^{-1}$ F/m, and $\overline{\cos\theta}$ is the average angle a dipole makes with the membrane field. We calculate $\overline{\cos\theta}$ by use of the Langevin equation:

$$\overline{\cos\theta} = \mathrm{ctnh}\left(\frac{peE_m}{kT}\right) - \left[\frac{epE_m}{kT}\right]^{-1}. \tag{III 6}$$

The assumption here is that those dipoles that are free to rotate are distributed in angles around the direction of membrane field, according to Boltzmann statistics. An integration over the Boltzmann distribution, for the average cosine of the angle with respect to the field, yields equation III 6.

If we now consider the polar group associated with the outer membrane surface, as the membrane potential is made increasingly inside negative, the number of free dipoles increase and V_p increases. This increase is most rapid over the range of voltage that produces the change of S from one to zero. V_p will have a polarity which opposes the membrane field. The inward current will therefore be decreased as the membrane field increases. At the voltage (V_m(max)), for which the following equation is satisfied,

$$1 = \frac{\dfrac{epE_0}{3kT}}{1 + \dfrac{epE_m}{kT}}. \qquad \text{(III 7)}$$

The configurational transition will be complete and all the dipoles will be free to rotate and align in the membrane field. As the membrane field increases beyond this point, the normal component of the force between adjacent positive and negative charges (Fig. 3) increases. This causes a vertical adjustment of the dipoles, which we call "easement." This will result in a decrease in polar voltage. This point is discussed further in section V.

In order to make a quantitative calculation of the V_p we require a number of molecular parameters, these are:

(*a*) A, area per dipole (number of dipoles/unit area)$^{-1}$,

(*b*) p, dipole moment,

(*c*) E_0, the dipole-dipole interaction force in the ordered configuration.

Unfortunately these are not readily available for the molecular species associated with membranes, and so in section V we utilize the data on steady-state negative resistance to give reasonable values for these parameters. We stress that these parameters are not *ad hoc* interaction variables but rather quantities which are reflective of the molecular state of the polar group.

The major assumptions of this section are:

(*a*) V_{NPR} acts in a manner analogous to temperature in relation to order-disorder transitions.

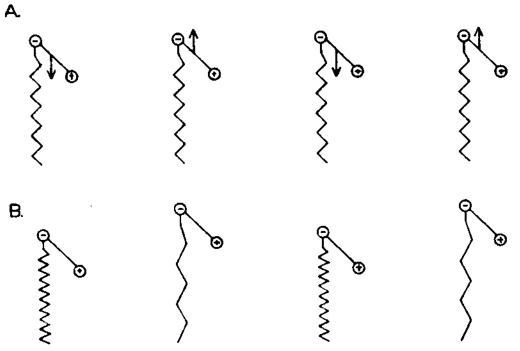

FIGURE 3 Dipole easement. (A) Forces acting on dipoles for voltages near V_m (max). (*B*) Easement configuration at voltages greater than V_m(max).

(*b*) The electric field acting on a single dipole is composed of contributions from V_{NPR} and dipole-dipole self field.

IV. EFFECTS OF POLYVALENT CATIONS

It has been observed in recent years that the concentration of polyvalent cations, especially calcium, can have a profound effect on steady-state currents and negative steady-state resistance, e.g., Blaustein and Goldman (1968) and Gilbert and Ehrenstein (1969). We postulate that the polyvalent cations have two basic actions on the membrane that are primarily responsible for the phenomena observed. The first of these is to change the configurational transition of the polar group and therefore effect the steady-state negative resistance associated with this transition. The second is the replacement in the counterion layer of the univalent cation with the polyvalent cation thus causing a reduction of steady currents (in both the equi- and nonequimolar case). In what follows we consider these two actions to develop a quantitative description of the effects of calcium (we use calcium interchangeably with the term polyvalent cation) on steady-state currents and negative steady-state resistance.

We first consider the effects of calcium on the polar transition. In Fig. 4 we see in the top line a row of dipoles, with ratio of spacing to chain length, a/d, arranged in an ordered, minimum energy configuration. We postulate that calcium ions, when added to the external fluid will be adsorbed by the polar groups. We now focus on dipoles M and N in Fig. 4, and assume a calcium ion is adsorbed by dipole N, so creating a group with a double positive change. We ask what effect does the N dipole now have on its neighbors and on the entire row of dipoles.

We recall that the ordered configuration in Fig. 2 is created by the dipole-dipole fields, which exert a restoring torque on any dipole which is perturbed from the ordered configuration. This dipole-dipole interaction, is however, disrupted by the

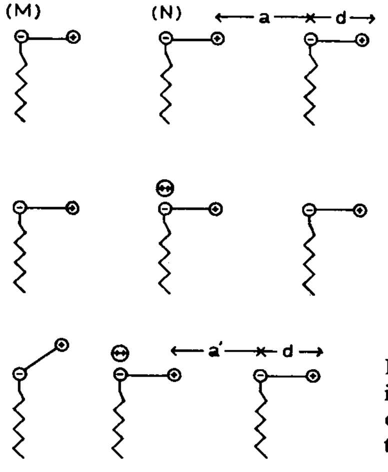

FIGURE 4 Effects of [Ca^{++}]. Top, molecular arrangement in the absence of Ca^{++}; a, distance between dipoles; d, dipole length. Middle, Ca^{++} adsorbed to dipole N. Bottom, molecular changes due to adsorbed Ca^{++}.

presence of the double positive-charged group N. The neighbor dipole M, will now experience a torque which in fact rotates it out of its originally ordered configuration. We now postulate that the rotation of dipole M will disturb the force equilibrium of the dipole chain. In order to reestablish this equilibrium there will be a movement of the negatively charged part of the dipole M. This movement can be estimated to have a value of $d/2$, and will be shared by all the dipoles of the chain.

If β is the fraction of dipoles in the chain that have adsorbed calcium, then the dipole spacing will be increased by an amount $(d/2)\beta$. This increase in spacing (which in practice will be 1–2 A) will decrease the dipole-dipole interaction and thus profoundly affect the configurational phase transition. To make an estimate of this effect, we note that the dipole-dipole force E_0 can be estimated by considering the effect of only the nearest neighbor. This will be:

$$E_0 \doteq \frac{1}{(a/d)^2} + \frac{1}{(a/d + 2)^2} - \frac{2}{(a/d + 1)^2}. \qquad \text{(IV 1)}$$

Therefore as a/d is increased by the presence of calcium, E_0 will be decreased. We consider that with calcium the spacing between dipoles will be given by:

$$\frac{a'}{d} = \frac{a}{d} + \beta/2 \qquad \text{(IV 2)}$$

then the modified electric field E_0' can be written:

$$\frac{E_0}{E_0'} = \frac{\dfrac{1}{\left(\dfrac{a}{d}\right)^2} + \dfrac{1}{\left(\dfrac{a}{d} + 2\right)^2} - \dfrac{2}{\left(\dfrac{a}{d} + 1\right)^2}}{\dfrac{1}{\left(\dfrac{a}{d} + \dfrac{\beta}{2}\right)^2} + \dfrac{1}{\left(\dfrac{a}{d} + \dfrac{\beta}{2} + 2\right)^2} - \dfrac{2}{\left(\dfrac{a}{d} + \dfrac{\beta}{2} + 1\right)^2}}. \qquad \text{(IV 3)}$$

In order to see the effect on the cooperative transition discussed in section III, we only need note that the weakened dipole-dipole field E_0' should replace E_0. The use of E_0' will have a significant effect on the voltage at which the transition begins as well as on the steepness of the transition. In calculating the dipole voltage, we must now also take account of the fact that those dipoles with adsorbed calcium are no longer dipoles and do not contribute to the polar voltage. We therefore write V_p:

$$V_p = \frac{(1 - \beta)\, \overline{\cos\theta}\, p}{A\kappa\epsilon_0}(1 - S).$$

Another important effect of calcium will be to decrease the steady-state currents. This effect will occur because of the replacement of the univalent ions in the vicinity of the polar group by calcium. We first assume the univalent ion currents originate from a region of fluid of thickness (5–10 A) near the polar group. These ions are assumed only to make hydrogen bonds with the water—they are not adsorbed by the polar group. With the presence of adsorbed hydrated calcium on the polar group, there will be a volume V_e, in the vicinity of an adsorbed calcium ion, from which univalent ions are excluded. This exclusion is assumed to occur from steric and charge considerations. That is the adsorbed polyvalent ion will have a 2–4 A dimension and in addition have a positive charge. These considerations are assumed to effectively exclude univalent ions from the immediate vicinity of the calcium ion. This exclusion will decrease the thermal flux from the water to the hydrocarbon.

To make this discussion quantitative we first need to determine a relation between the concentration of external polyvalent cation and the fraction of dipoles, β, which have adsorbed a polyvalent cation. To do this we choose the simplest adsorption isotherm, the Langmuir isotherm (Davies and Rideal [1963]):

$$\beta = \frac{N_{\mathrm{Ca}^{++}}/\gamma}{1 + \dfrac{N_{\mathrm{Ca}^{++}}}{\gamma}}, \qquad \text{(IV 4)}$$

where $N_{\mathrm{Ca}^{++}}$ is the concentration in the external fluid of calcium in moles per liter. The constant γ contains the adsorption energy W:

$$\gamma = N_D A_1 \exp\left(-\frac{W}{kT}\right), \qquad \text{(IV 5)}$$

where N_D is the number of dipoles in the polar group per cm^2 and A_1, is 1.2×10^{-14}. We certainly do not insist that this precludes other more complex isotherms, only that it represents the simplest one.

If we then assume that for each adsorbed polyvalent cation there will be an effective exclusion volume V_e, then the thermal flux of univalent cations will be reduced and we write a modified expression for J_{sat}:

$$J_{\mathrm{sat}} = J_{\mathrm{sat}_0}\,[1 - \beta[N_D V_e/l]]. \qquad \text{(IV 6)}$$

In general V_e will be a function of percentage of surface covered by calcium and can only be determined from complex theoretical considerations or by fitting the data, and $l \sim 5$–10 A.

We may now summarize the number of additional parameters introduced by the presence of polyvalent cations:

(*a*) a/d, the ratio of dipole spacing to chain length,

(*b*) V_e/V_t, the fraction of volume in the adjoining fluid, from which univalent cations are excluded by the presence of adsorbed cations, and

(*c*) W, the adsorption energy of the cation.

V. RESULTS

In the previous sections we developed a quantitative theory for the steady-state behavior of excitable membranes. This theory requires the specification of 11 parameters. These parameters for the most part describe the membrane in terms of quantities at the molecular level, which are as yet inaccessible experimentally, and unavailable from basic theory. In this section we utilize experimental data on steady-state ion transport to find quantitative value for these parameters.

The experimental data on steady-state ion transport is voluminous and we could not hope to cover even a fraction of this body of data in this report. What we have done here is to choose data which represents equi- and nonequimolar potassium ion flow. The data utilized for the equimolar case is shown in Cole (1968) and is due to Gilbert and Ehrenstein (1969). For nonequimolar transport we have chosen the data of Blaustein and Goldman (1968).

The analysis of experimental results and determination of parameters will be discussed in three basic groupings: (A) the general permeation characteristics, (B) steady-state negative resistance, and (C) the effects of polyvalent cations.

A. General Permeation Characteristics

Here we consider those molecular parameters which are necessary to describe steady-state phenomena in the absence of negative resistance. This will include: U_0, D, and $\bar{v}_w/\bar{v}_{\mathrm{NPR}}$. The concept of ion permeability in relation to this model, and significant features of the current-voltage characteristic (i.e. rectification, intercepts, and saturation) will also be discussed.

To estimate U_0, the energy of dehydration of an ion, we note that the current J_{sat} in equation II 9 is expressed as $(J_{\mathrm{thermal}}) \exp(-U_0/kT)$, where $J_{\mathrm{thermal}} = n_w\bar{v}_w$. An inspection of equation II 9 reveals that when $V_m/kT \gg 1$, the theory predicts that the current will saturate. This behavior is displayed in Fig. 5 for the equimolar case. We note that for this data $J_{\mathrm{sat}} = 1.2$ ma. To calculate J_{thermal} we use: $n_w = 0.550$ moles/liter and $\bar{v}_w$, the thermal velocity of an ion in water; $\bar{v}_w = 10^5$ cm/sec. We thus find U_0 to be 13.5 kcal/mole, a value which is consistent with those listed in Stein (1967).

To estimate the diffusion coefficient, D, within the NPR we have the results of the Appendix for the potassium voltage-clamp transient: $D = 10^{-9}$ cm^2/sec. We note that a similar estimate by Cole (1968) for this quantity gives $D = 10^{-8}$–10^{-9} cm^2/sec.

We are able to obtain estimates of the parameter B. $B = (L\bar{v}_{\mathrm{NPR}})/D$ by fitting equation II 9 to the data for squid in Fig. 5 and lobster in Fig. 6. We find $B = \frac{1}{4}$ for Gilbert and Ehrenstein (1969) while $B = 1$ for Blaustein and Goldman (1968).

Knowing B and D and assuming an L (NPR width) of 50 A, we can now estimate $\bar{v}_{\mathrm{NPR}}$ the exit velocity of an ion from the NPR. We find $\bar{v}_{\mathrm{NPR}} = \frac{1}{2} \times 10^{-3}$ cm/sec

To see the significance of this estimate we now make use of it to establish a value for n/n_w .

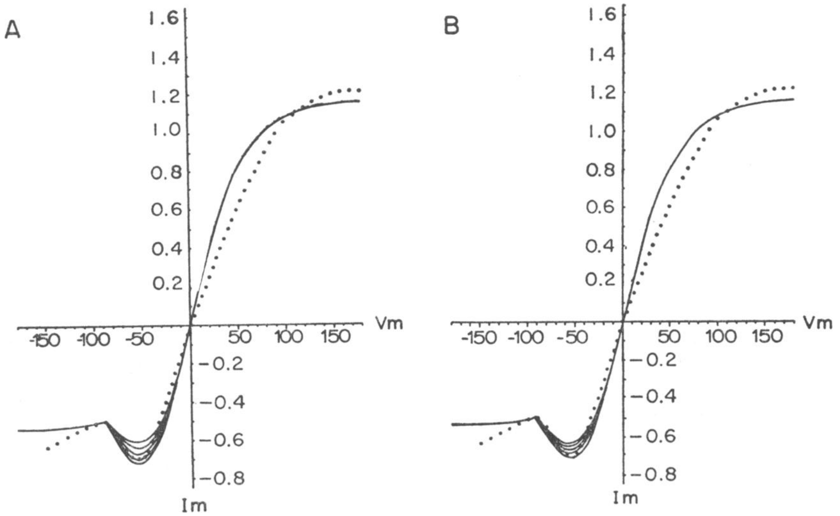

FIGURE 5 Equimolar current-voltage characteristics. Dotted curves, data points after Gilbert and Ehrenstein (Cole, 1968), by permission of the Regents of the University of California. Solid curves, theoretical calculations. (A) calculations parametrically on dipole moment, p = 6.25 Debye units (top curve), 12.5, 25.0, 50.0, 800.0 (bottom curve). NPR width = 30 A. (B) calculations parametrically on NPR width, L = 20 A (bottom curve), 40.0, 60.0, 80.0, 100.0 (top curve). Dipole moment = 50 Debye units.

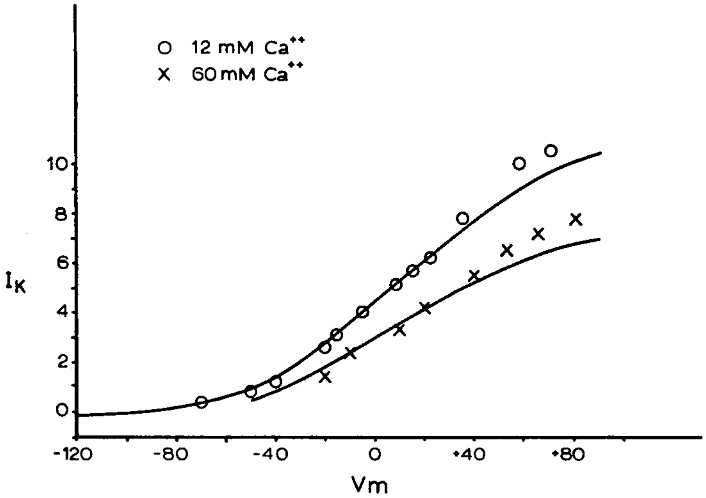

FIGURE 6 Nonequimolar voltage-current characteristics. Circles and crosses, data from Blaustein and Goldman (1968), by permission of The Rockefeller University Press. Solid lines: theoretical curves.

If no current flows through the membrane, then the current flow from water to lipid must of course equal the flow from membrane to water. We can write

$$n_w \bar{v}_w e^{-U_0/kT} = n\bar{v}_{\mathrm{NPR}}$$

so that

$$\frac{n}{n_w} = e^{-U_0/kT}\left(\frac{\bar{v}_w}{\bar{v}_{\mathrm{NPR}}}\right). \tag{V 1}$$

Utilizing previously determined values of U_0 and $\bar{v}_w/\bar{v}_{\mathrm{NPR}}$, we find that $n/n_w = \frac{1}{2} \times 10^{-2}$ or $n = 2.5$ mM of potassium. This value is quite high but consistent with the values obtained by Cole (1968) ($n = 1$–100 mM of potassium) from a rather different approach. We offer here a possible explanation of this large value of n which bears very significantly on membrane structure. We note in equation V 1 that if $\bar{v}_w/\bar{v}_{\mathrm{NPR}}$ were to be of order unity, then n/n_w would be described purely in terms of the partition energy, U_0, and so we would find $n/n_w = 10^{-10}$. However, because $\bar{v}_w/\bar{v}_{\mathrm{NPR}} \gg 1$, n/n_w achieves its large value. We therefore need to inquire into why $\bar{v}_w/\bar{v}_{\mathrm{NPR}} = \frac{1}{2} \times 10^8$.

If an ion were to achieve a condition close to thermal equilibrium in the polymeric membrane network, $\bar{v}_{\mathrm{NPR}}$ would more closely correspond to $\bar{v}_w$ or to 10^5 cm/sec. The fact that $\bar{v}_{\mathrm{NPR}} \doteq \frac{1}{2} \times 10^{-3}$ cm/sec suggests that there exists a barrier in the membrane to the exiting of ions into water. Because there are few if any charged or polar groups in the lipid, we can most probably discount chemical or van der Waal forces. However, as noted in section II, we do consider the ions to be scattered from the polymeric like network, which we assume composes the interior of the membrane. The thermal motion of this network, if it is characteristic of polymer chains, has an activation energy which inhibits the bending of the chains. We assume that it is the thermal motion of these chains which provide openings for the ions to exit to the water. Then, although each ion will be thermalized by scattering from the chain, and their mean speed will be close to 10^5 cm/sec, they will be trapped within the network by the geometric barrier this network presents. It will be the bending of chains by thermal motion which will provide pathways for the ions to leave the lipid and enter the water. If the temperature increases and the chain links become freer to bend and rotate, the exit flux may increase and so $\bar{v}_{\mathrm{NPR}}$ will also increase. We may therefore crudely estimate $\bar{v}_{\mathrm{NPR}}/\bar{v}_w$ as the Boltzmann factor for the network activation. If we do this, we find an activation energy of 11 kcal/mole for the polymeric network. This is quite close to the figure of 10 kcal/mole which Stein (1967) computes from the temperature dependence of D for permeation in ox erythrocyte. The high values of n, results therefore from the geometric trapping of ions in the lipid and the rather small exit fluxes. We stress that this process need not rely on relatively high energy chemical forces between ion and lipid but rather can result from simple geometric obstruction to ion flow, which the polymer-like chain presents to an ion.

As Cole (1968) points out, one of the difficulties introduced by large ion concentrations in the lipid is breakdown of the constant-field assumptions. It becomes necessary then to consider the fields produced by the ions in the lipid. This produces considerable complication in the analysis and it is difficult to anticipate what changes in the theory may result, except to hope that the very reasonable agreements of the results of constant-field approximation with experiment portends that the present approximation will at least prove to be qualitatively correct.

Another aspect of general permeation is the estimate of ion permeability. As pointed out in section II, the present theory does not directly produce a parameter which has the usual meaning attributed to permeability. It is shown in section II, however, that the usual P_k is related to the present theory by $P_k = J_{\text{sat}}/B$. For the equimolar squid data we find $P_k = 10 \times 10^{-5}$ cm/sec, while for the lobster data, $P_k = 14 \times 10^{-5}$ cm/sec; these may be compared to the usual experimental values of 17×10^{-5} cm/sec. We point out that if one were to determine the permeability from $dJ/d\phi_m$ near $\phi_m = 0$ for the equimolar case, the usual electrodiffusion result (i.e. the Goldman equation) would yield $dJ/d\phi_m = eP_kn$ while the present theory gives $dJ/d\phi_m = (eP_kn)/(1 + 2/B)$, so if $B = \frac{1}{4}$, the present theory will give a value of P_k which is a factor of 9 larger than the classical electrodiffusion result and one which is closer to experimental values. In Fig. 6 we see the general voltage current characteristic predicted by this theory for nonequimolar flow. The curve has several interesting features which correct, in part, certain defects of the classical electrodiffusion theories. We first note that for $|\phi_m| \gg 1$, the current will saturate. This predicted saturation has not been experimentally observed thus far, for the nonequimolar case. For the equimolar case, as seen in Fig. 4, the current does exhibit a definite saturation which is in accord with the theory. An important test of the theory will certainly be the question of whether current saturation does occur when $|V_m/kT| \gg 1$ in the nonequimolar case. Another feature of the curve is the asymptote of the linear region near $V_m = 0$. The present theory predicts that, V_s, the intercept voltage, is simply given as:

$$V_s = \left(\frac{2kT}{e}\right)\left[\frac{1 - \exp\left(-\frac{V_r}{kT}\right)}{1 + \exp\left(-\frac{V_r}{kT}\right)}\right], \qquad (\text{V } 2)$$

where for $T = 300°\text{K}$, $(2\,kT)/e = 52$ mv. This corrects a major defect of single ion classical theories which predict the asymptote to occur at the origin. Another aspect of classical theories which has been vexing has been the explanation of the rather large rectification that is observed experimentally in the nonequimolar case. The classical theories predict the rectification to be equal to n_i/n_e. In the present theory, an inspection of Fig. 6 indicates that there is no linear region on the hyperpolarized side of the curve. Since the current saturates, it is possible to find rather large rectification because the conductance here eventually approaches zero.

B. Negative Steady-State Resistance

Here we apply the results of section II and III to the quantitative determination of negative steady-state resistance. We consider the equimolar data of Gilbert and Ehrenstein (1969) to determine the molecular parameters which underlie this phenomena: p, dipole moment of the polar group; A, the area per dipole in the polar group; and E_0, the interdipole electric field.

The physical mechanism we propose involves the voltage drop across the outer polar region during polarization of the membrane (inside negative). This V_p will always oppose the direction of the membrane field, and so for the inside negative situation, it will tend to impede the flow of positive ions from the external fluid into the membrane. From the results of section III, we see that V_p will increase as the membrane voltage increases and so, given appropriate values of the molecular parameters, a decrease in current can result. This can be seen quite clearly when we specialize equation II 9 to the equimolar case:

$$J = \frac{J_{\text{sat}}\, e^{-V_p e/kT}[1 - e^{-V_m/kT}]\, \dfrac{e\, V_{\text{NPR}}}{kT}}{B(1 - e^{-V_{\text{NPR}}/kT}) + (1 + e^{-V_{\text{NPR}}/kT})\, \dfrac{e\, V_{\text{NPR}}}{kT}}, \qquad (\text{V } 3)$$

where all quantities are previously defined and all $V > 0$ for the membrane-polarized inside negative. We see that the factor $e^{-V_p e/kT}$ will decrease the current when V_p increases with increasing polarization. We point out that a significant V_p is considered to exist only on the exterior surface of membrane. The V_p of the inside polar region is not considered to have a significant effect. This asymmetry is suggested by the data since no pronounced region of steady-state negative resistance is observed for the inside positive polarization equimolar case. If in fact the internal V_p were significant, one should observe the negative resistance phenomena in the inside positive case. Perhaps the asymmetry of the membrane and its apparent lack of an internal V_p is due to the gel-like nature of the axoplasm which might be pictured as forming an adherent lattice which inhibits the rotation of the internal polar groups. Moreover, we have established that the addition of a symmetrical interior V_p to the model has little effect on the negative resistance seen for inside negative polarization. In connection with these comments, it is interesting to point out that the steady-state negative resistance observed in artificial membranes occurs for inside negative and inside positive cases. This suggests a symmetry in polar groups for the artificial membranes which does not seem to occur for the natural membrane.

In producing a current voltage curve the present theory requires the following four values be determined from the experimental data to be modeled: first, the maximum value of V_p; second, the membrane voltage at which V_p maximum occurs, we call this $V_m(\text{max})$; third, the thickness (L) of the NPR; and fourth, the dipole moment (p) to be associated with PR. Utilizing equation V 3 and the equimolar potassium data (dotted curve Fig. 5) to supply V_m(max) and a reasonable value of V_p(max), we infer a current-voltage relation as seen in Fig. 5 A (parametrically on p) and Fig. 5 B (parametrically on L). These curves show a negative resistance characteristic in quite good agreement with the experimental data. The negative resistance is due to the increasing V_p (Figs. 7 A and B) caused by the increasing number of dipoles in the PR which are free to align in the membrane field, i.e., $V_p = ([1 - S]p \cos \theta)/\kappa\epsilon_0$. (The number of dipoles free to rotate as a function of membrane voltage is pictured in Figs. 8 A and B. The mean angle the rotating dipoles make with the membrane surface as a function of V_m is given in Figs. 9 A and B). The main difficulty seen (Fig. 5) is the increased I_k at high values of V_m, (i.e. $V_m > V_m$(max)) exhibited by the experimental data as compared to the smaller increase in I_k in this region for the theoretical curves. The explanation we propose to account for this discrepancy is that in the membrane as the dipoles rotate, the normal forces between adjacent negative and positive charges (see Fig. 3) force the dipoles to move against their bonds and decrease the net distance of separation between positive and negative charge. This "easement" of charge will decrease the polar voltage and produce an increase in the inward I_k as seen in the experimental data. We have not quantitatively assessed this effect here. The theoretical curves have assumed that V_m(max) represents that voltage at which the configurational transition is complete. Therefore for voltages larger than V_m(max) the theoretical V_p produced is constant rather than decreasing and the theoretical I_k is therefore smaller than the experimental I_k. This procedure is not entirely satisfactory but it

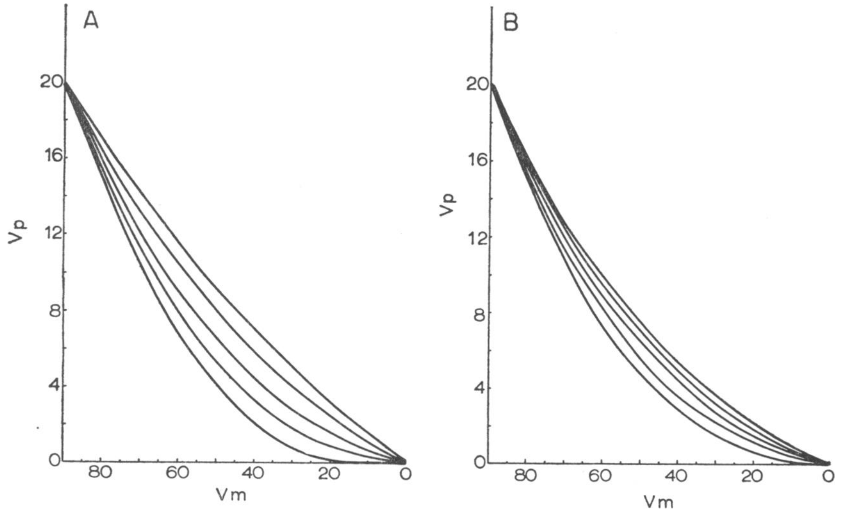

FIGURE 7 Polar voltage (V_p) vs. membrane voltage (V_m). (A) calculations parametrically on dipole moment, p = 6.25 Debye units (top curve), 12.5, 25.0, 50.0, 800.0 (bottom curve). NPR width = 30 A. (B) calculations parametrically on NPR width, L = 20 A (bottom curve), 40.0, 60.0, 80.0, 100.0 (top curve). Dipole moment = 50 Debye units.

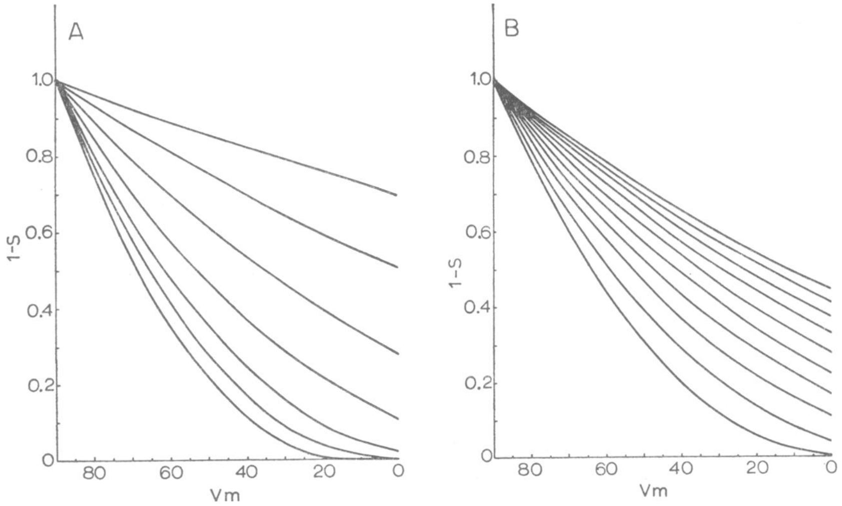

FIGURE 8 Order-disorder variable $(1 - S)$ vs. membrane voltage (V_m). (A) calculation parametrically on dipole moment, $p = 6.25$ Debye units (top curve), 12.5, 25.0, 50.0, 100.0, 200.0, 800.0 (bottom curve). NPR width = 30 A. (B) calculations parametrically on NPR width, $L = 10$ A (bottom curve), 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0, 100.0 (top curve). Dipole moment = 50 Debye units.

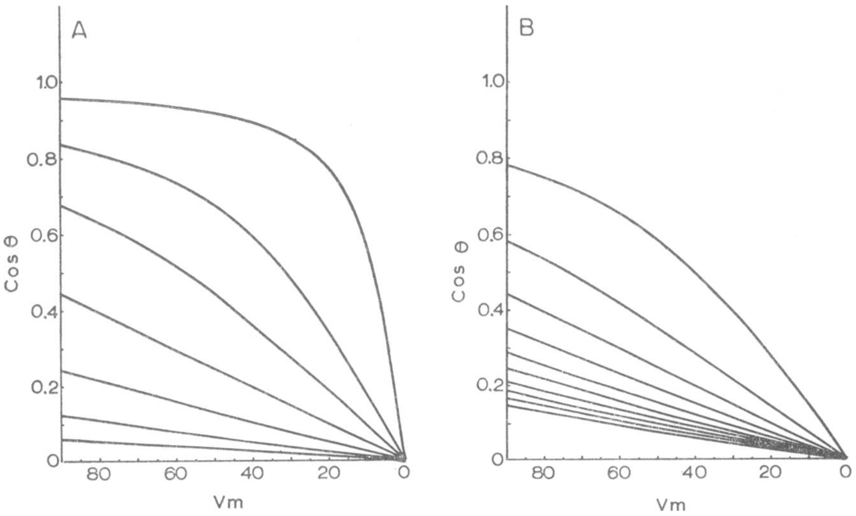

FIGURE 9 Angular orientation of dipole $(\cos \theta)$ vs. membrane voltage (V_m). (*A*) calculations parametrically on dipole moment, $p = 6.25$ Debye units (bottom curve), 12.5, 25.0, 50.0, 100.0, 200.0, 800.0 (top curve). NPR width = 30 A. (B) calculations parametrically on NPR width, $L = 10$ A (top curve), 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0, 100.0 (bottom curve). Dipole moment = 50 Debye units.

must suffice because of the large uncertainties in molecular bonds which would be required to correctly assess this "easement" phenomena.

Taking this approach we can now say that the configurational transition begins at V_m(max) and so we determine epE_0/kT from the equation:

$$3 = \frac{epE_0/kT}{1 + \frac{ep(E_m)_{\max}}{kT}} \tag{V 4}$$

where $(E_m)\max = V_m(\max)/L$. We further have at V_m(max)

$$\frac{p}{A} = \frac{(V_p)_{\max}}{\overline{\cos\theta}_{\max}} (\kappa\epsilon_0), \tag{V 5}$$

where $\overline{\cos\theta}_{\max}$ is the value of $\overline{\cos\theta}$ at V_m(max). Using equations V 4 and V 5 we can reduce the problem to selection of the parameters L and p. We now ask over what range of NPR width, L, and dipole moment, p, will the negative resistance phenomena be possible. In Figs. 5 A and B we see that the phenomena does not seem to be extremely sensitive to the choice of L or p, and that a value of p of about 50 Debye units with an L of about 30 A adequately represents the experimental facts. With this choice of NPR width and dipole moment we find: $(epE_0)/kT = 5.25$ and the area per dipole $A = 315$ A^2/dipole.

The data also shows that for increasingly nonequimolar solutions the negative steady-state resistance clearly disappears. To show that the theory predicts this effect, in Fig. 10 we show the current-voltage characteristic for an internal solution of 500 mM potassium chloride and external solution whose concentration is varied from 500 to 50 mM. We see clearly the disappearance of negative resistance. This occurs because the current into the membrane at the exterior surface decreases linearly with concentration and so the effects of polar voltage on this flow become decreased.

Perhaps the main shortcoming of this analysis of the data is that we have assumed V_p is entirely a voltage across the PR. We neglect the counterion voltage in this paper. It is of course necessary in the analysis of transient phenomena and in a future paper, we will address ourselves to this question. Its inclusion in the present paper would only change the values of the molecular parameters somewhat—it would have no effect on the theoretical explanation of the negative steady-state resistance phenomena.

C. The Effects of Polyvalent Cations

In the previous Results section we have considered the general permeation parameters as well as those which affect the negative steady-state resistance. Here we consider the effects of polyvalent cations, principally calcium.

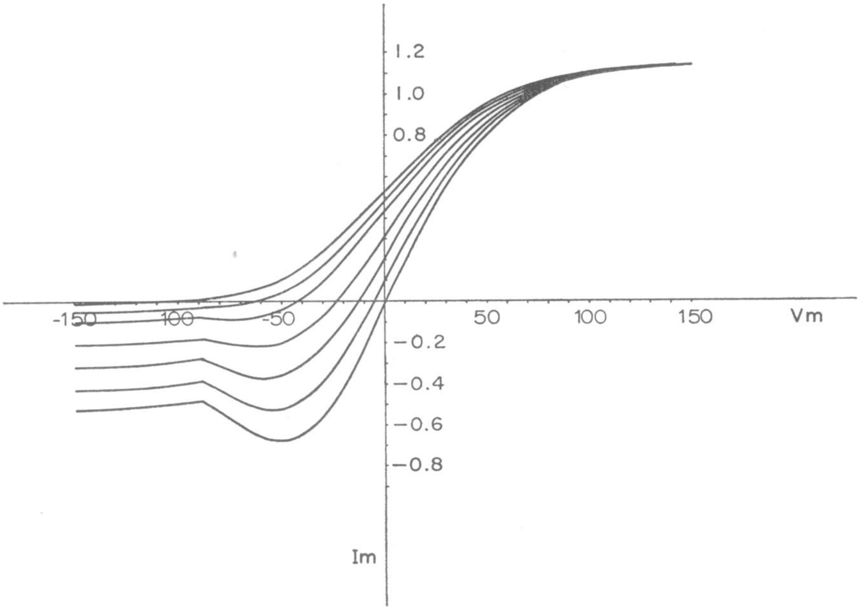

Figure 10 Effects of external potassium concentration on current-voltage characteristic $[K]_i = 500$ mM, $[K]_0 = 500$ mM (bottom curve), 400, 300, 200, 100, 50, 10 (top curve).

In discussing the effects of calcium in section IV, the hypothesis we make is that calcium's main effect is on the polar transition and the steady-state currents. Its principal effect on the polar transition is to decrease the $V_m(\max)$ at which the transition begins. We see this quite graphically in Fig. 11. If we were to combine equations III 7 and IV 3 we would see that $V_m(\max) = f([\mathrm{Ca}^{++}], a/d, W/kT)$. Since two of these parameters are unknown, it is necessary that one be chosen and the other determined from the data. The value of a/d is perhaps most reasonable to choose since we have already estimated the area per dipole to be 315 A². If d is 6–8 A then a will be 15–18 A and a reasonable estimate for a/d is 2. We now find W/kT by utilizing the data on the change of $V_m(\max)$, i.e. the peak of the negative resistance curve, which accompanies the additional Ca^{++} to the bathing media as found in the work of Gilbert and Ehrenstein (1969) and the curves in Fig. 11. The data shows a decrease in $V_m(\max)$ to be associated with each of several values of external calcium concentration. Fig. 11 allows each of these values of external calcium to associate with a particular β for $a/d = 2$. These points are shown as "x's" in Fig. 12 on which are also shown a family of Langmuir adsorption isotherms. By matching the points derived from the data to a reasonable isotherm we are able to find the constant γ in the Langmuir expression and therefore also to estimate W, the adsorption energy of a calcium ion. This procedure yields an estimate of $W = 800$ cal. Interestingly, the choices we made for these parameters predict a shift in the plot of sodium conductance vs. membrane potential of 11.6 mv for a 5-fold increase in

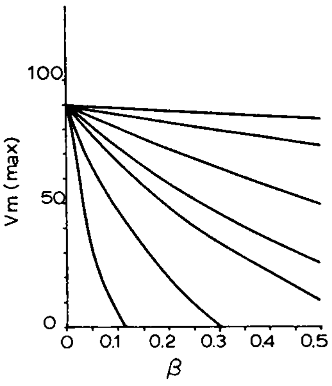

FIGURE 11 V_m(max) vs. β, calculations parametrically on a/d; $a/d = 0.1$ (lower curve), 0.3, 0.7, 1.0, 2.0, 5.0 (upper curve).

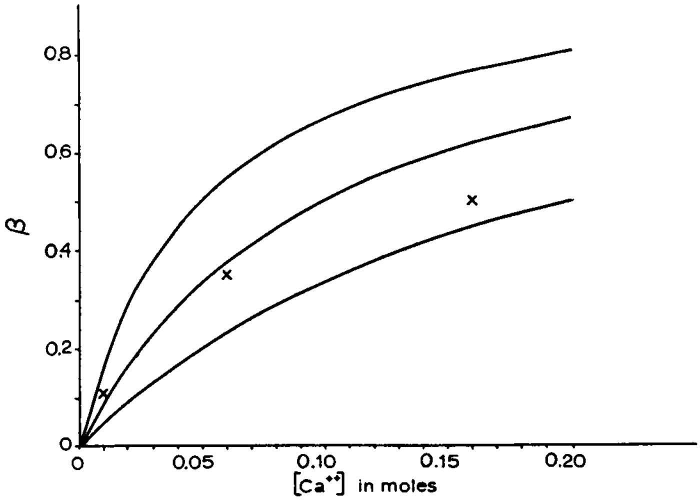

FIGURE 12 Fraction of dipoles with adsorbed Ca^{++} (β) as a function of the concentration of Ca^{++} in the bathing medium. ×: points derived from data. Solid curves: calculated values, $\gamma = 0.05$ (top curve), 0.01, 0.02 (bottom curve).

Ca^{++} from 12 mM to 60 mM. This compares well to the 12 mv experimental shift reported by Blaustein and Goldman (1968).

The effects of calcium on the level of steady-state currents are difficult to quantify from a first principles consideration and must be obtained by a fit to the data. Using the curves of Fig. 13 which are based on the equimolar data of Gilbert and Ehrenstein (1969) we obtain an estimate of V_e, the exclusion volume referred to in equation IV 6 of 1.5–6 × 10^4 A^3. This estimate, while only approximate, is seen to be reasonable when it is used to fit the nonequimolar steady-state current measurements of Blaustein and Goldman (1968) in Fig. 6. The outward currents in this curve are calculated by assuming that a portion of the externally applied Ca^{++} will leak to the inside membrane surface, i.e. for 60 mM external Ca^{++}, 3 mM is assumed

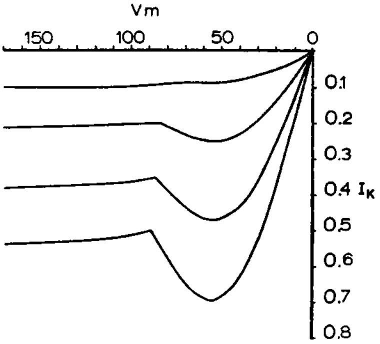

FIGURE 13 Effects of [Ca^{++}] on negative resistance. V_m, membrane voltage (inside negative). I_k, membrane current. $\beta = 0$ (bottom curve), 0.025, 0.050, 0.35 (top curve).

to leak to the inside. This internal concentration of Ca^{++} is based on the assumption that the ratio of external to internal concentration is the same for high external concentrations as it is for normal external concentrations (i.e. about 4–5%) (Potts and Parry, 1964). The absolute amount of the internal concentration dictated by this assumption (3 mM for 60 mM external Ca^{++}) is in the light of the well-known deleterious effects of internal Ca^{++}, abnormally high, and may eventually require an alternative explanation of the effects of Ca^{++} on steady-state outward currents.

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APPENDIX

Estimate of Diffusion Coefficient

In order to estimate the diffusion coefficient of the membrane, we consider that the potassium current transient that results when a voltage clamp is applied to the membrane is controlled by electrodiffusion. If n is the potassium number density we may write for n the usual diffusion equation:

$$\frac{\partial n}{\partial t} = \frac{\partial^2 n}{\partial x^2} + \phi \frac{\partial n}{\partial x}$$

where

$$x = x'/L, \quad \phi = \frac{eE_0L}{kT}$$

$$t = \frac{t'}{L^2/D}, \quad n = n'/n_i$$

and x' is the distance measured from the centerline of the membrane, ϕ the membrane voltage, L the membrane NPR thickness, D the NPR diffusion coefficient, and t' the time. If we consider a voltage clamp to zero voltage the mathematics is considerably simplified and an exact solution is possible. For this case the equation becomes:

$$\frac{\partial n}{\partial t} = \frac{\partial^2 n}{\partial x^2}.$$

To facilitate calculation we further assume that, at rest we have zero potassium in the external fluid and a specified concentration, n_i, internally, and that at time equal to zero, $n = 0$ within the membrane. We may then utilize the boundary conditions developed in section II:

$$x = -\frac{1}{2}, \quad -Bn = \frac{\partial n}{\partial x}$$

$$x = +\frac{1}{2}, \quad B[A_2 - n] = -\frac{\partial n}{\partial x},$$

Where $B = (\bar{\nu}_{\text{NPR}} L)/2D$ and is approximately one-eighth (from section V), $A_2 = \bar{\nu}_w/\bar{\nu}_{\text{NPR}}$. The exact solution of this transient problem is given by Carslaw and Jaeger (1959). For $t \gg 1$ we may write the approximation for the current J, as:

$$J/J_{ss} \doteq 1 - 1.3 \exp{(0.23t)},$$

where J_{ss} is the new steady-state current. If we now match this solution to the data of Hodgkin and Huxley (1952) for the potassium transient, this gives the time constant for the potassium transient to be:

$$\tau_c = \frac{7\left(\frac{L}{2}\right)^2}{D}.$$

If $L = 50$ A, then $D = \frac{1}{2} \times 10^{-9}$ cm²/sec. We point out that this estimate differs from that given by Cole (1968) mainly in the fact that we do not neglect the surface barriers to flow. Cole (1968) obtains $D = 10^{-8}$ cm/sec.

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