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A Theory for the Effects of Neutral Carriers Such as the Macrotetralide Actin Antibiotics on the Electric Properties of Bilayer Membranes

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Summary. To develop a quantitiative theoretical treatment for the effects of neutral macrocyclic antibiotics on the electrical properties of phospholipid bilayer membranes, this paper proceeds from the known ability of such molecules to form stoichiometric lipid-soluble complexes with cations and deduces the electrical properties that a simple organic solvent phase would have if it were made into a membrane of the thinness of the phospholipid bilayer. In effect, we postulate that the essential barrier to ion movement across a bilayer membrane is its liquid-like hydrocarbon interior and that the neutral macrocyclic antibiotics bind monovalent cations and solubilize them in the membrane as mobile positively charged complexes. Using the Poisson-Boltzmann equation to describe the equilibrium profile of the electrical potential, it is shown that an excess of the positive complexes over all the other ions is expected in the membrane as a net space charge for appropriate conditions of membrane thickness and values of the partition coefficients of the various ionic species and without requiring the presence of fixed charges. Describing the fluxes of these complexes by the Nernst-Planck equation and neglecting the contribution to the electric current of uncomplexed ions, theoretical expressions are derived for the membrane potential in ionic mixtures, as well as for the limiting value of the membrane conductance at zero current when the membrane is interposed between identical solutions. The expressions are given in terms of the ionic activities and antibiotic concentrations in the aqueous solutions so as to be accessible to direct experimental test. Under suitable experimental conditions, the membrane potential is described by an equation recognizible as the Goldman-Hodgkin-Katz equation, in which the permeability ratios are combinations of parameters predicted from the present theory to be independently determinable from the ratio of membrane conductances in single salt solutions. Since this identity between permeability and conductance ratios is expected also for systems obeying the "Independence Principle" of Hodgkin and Huxley, the applicability of this principle to membranes exposed to antibiotics is discussed, and it is shown that this principle is compatible with the permeation mechanism proposed here.

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Neutral macrocyclic molecules, such as the Macrotetralide Antibiotics Nonactin, Monactin, Dinactin, and Trinactin (Gerlach & Prelog, 1963; Graven, Lardy, Johnson, & Rutter, 1966) are known to increase markedly the cation permeability of phospholipid bilayer membranes, whether natural (Pressman, 1965) or artificial (Mueller & Rudin, 1967; Lev & Buzhinsky, 1967; Andreoli, Tieffenberg, & Tosteson, 1967). The mechanism by which these permeability-inducing antibiotics produce their effects is not presently known. They have been variously conjectured to create ion-specific tunnels by Mueller and Rudin (1967), or alternatively to act as molecular carriers by Lardy, Graven, and Estrade-O (1967), Pressman (1968*a*), and Tosteson (1968). The question of whether their action is on the bulk properties of the membrane or on its surface properties has also been raised (Tosteson, 1968). These conceivable mechanisms have been discussed briefly elsewhere by Eisenman (1968).

On the other hand, the equilibrium chemistry of such molecules is well understood. Simon and his colleagues have demonstrated that Nonactin and Monactin form stoichiometric one-to-one complexes with K^+ and Na^+ in methanol (Pioda, Wachter, Dohner, & Simon, 1967); Pressman (1968*a*, *b*) has presented data for such stoichiometry in the salt extraction into toluence-butanol produced by valinomycin and a variety of other cyclic antibiotics. We have shown how the effects of such molecules on the ionic distribution equilibria between aqueous solutions and an appropriate solvent (e.g., n-hexane) chosen to represent the interior of the membrane can be compared unambiguously with their effects on the membrane potential and electric resistance properties of artificial phospholipid bilayers (Eisenman, Ciani, & Szabo, 1968). The configuration of the Nonactin-K^+ complex has been identified in crystals (Kilbourn, Dunitz, Pioda, & Simon, 1967), and studies on the rate of formation and dissociation of such complexes is under active study by Eigen and his colleagues (Eigen & DeMaeyer, 1969). It is therefore possible to attack the question of the mechanism of action of such molecules by proceeding from the known equilibrium chemistry of these molecules in bulk solvents to their expected effects on membranes.

Given the knowledge of the chemistry of these molecules in bulk solvent phases [which will be characterized for the macrotetralide antibiotics in the second paper of this series (Eisenman, Ciani, & Szabo, 1969), referred to hereafter as II], the present paper will deduce theoretically what electrical properties such a solvent phase would have if it were made into a membrane of the thinness of a phospholipid bilayer. The third paper (Szabo, Eisenman, & Ciani, 1969*b*; referred to hereafter as III) will

test to what extent the properties of phospholipid bilayers are observed to be similar to or different from this. Such an approach, which may seem oversimplified, is not unreasonable in view of the following facts. (1) The hydrocarbon tails in the interior of the membrane are liquid-like (Schmitt, 1939; Luzatti & Husson, 1962; Van Deenen, 1965; Chapman, 1966; Cass & Finkelstein, 1967), and the interior of artificial bilayers contains significant amounts of solvents such as decane (Henn & Thompson, 1968). (2) The presence of the charged polar-head groups of the lipid can be shown theoretically to be unimportant over a significant range of experimental conditions (Ciani, Szabo, & Eisenman, 1969*b*). (3) The rate processes of forming and dissociating the complexes in aqueous solutions are so rapid that these are unlikely to be rate limiting in the aqueous phase (Eigen & DeMaeyer, 1969); and the possibility that such complexes, when once formed in the membrane interior, may not dissociate within the lifetime of diffusion across the membrane will be shown not to alter the expectations deduced in the present paper.

We begin this series of papers by carrying out a theoretical analysis, using no arbitrary assumptions as to electroneutrality or as to profiles of concentration or electric potential within the membrane, of the effects of neutral macrocyclic molecules on the electrical properties of a simple model in which the phospholipid bilayer membrane is represented as a thin liquid hydrocarbon phase some 60 A thick interposed between two aqueous solutions, thereby explicitly neglecting the effects of the polar-head groups of the lipid which are analyzed elsewhere (Ciani et al., 1969*b*). For such a membrane it is possible to deduce expressions for the membrane potential and membrane resistance at zero current as a function of the concentrations of antibiotic and ions in the aqueous solutions. In addition, quantitative interrelationships between such properties as membrane potential and electric resistance are predicted unambiguously.

The following paper, II, examines the equilibrium chemistry of such molecules and shows how the salt extraction properties conferred by these molecules on organic solvents are expected to be related to the electrical properties measureable for phospholipid bilayer membranes. The effects of such molecules on the ionic distribution equilibria between aqueous solutions and organic solvents are deduced theoretically and measured experimentally, and an appropriate set of equilibrium constants is characterized for these antibiotics from which a variety of their effects on bilayer membranes can be "predicted".

The third paper of this series, III, characterizes the experimentally observed effects of the Macrolide Actin antibiotics on the electrical prop-

erties of phospholipid bilayer membranes and compares these effects with the quantitative expectations of the theory of the present paper. Remarkably good agreement is found not only between theory and experiment for bilayers, but also between the observed bilayer electrical properties and those "predicted" from the equilibrium measurements of the second paper. These results strongly support the validity of the initial postulate that neutral antibiotics such as the Macrotetralide Actins produce their effects on lipid bilayer membranes by acting as molecular carriers of cations.

In two further articles, the effects of the charged polar head groups of the lipid are examined (Ciani et al., 1969*b*) and the rate limiting step for ion permeation of bilayer membranes is elucidated (Ciani et al., 1969*a*). Some salient conclusions from these five papers have been presented to several recent Symposia (Eisenman et al., 1968; Szabo et al., 1969*a*).

Description of the System

The simplest model for the effects of neutral macrocyclic molecules (such as the Macrotetralide Actins illustrated in Fig. 1) on lipid bilayer membranes consists of a thin (e.g., 60 A) membrane phase composed of a low dielectric constant liquid interposed between two aqueous solutions of univalent electrolytes containing a single species of a neutral ion-binding molecule, which will be referred to as a "neutral carrier" and will be denoted by S (or s when used as a subscript). The macrocyclic molecules are assumed to be preferentially partitioned in the organic phase (Szabo, 1969) and to form stoichiometric complexes with cations (Pioda et al., 1967), thereby solubilizing them in the membrane. Such a membrane is schematized in Fig. 2, omitting for simplicity of presentation the effects of charged polar-head groups of the lipid. The effects of this surface charge are considered elsewhere and shown to be important only in the limits of very low antibiotic and ionic concentrations (Ciani et al., 1969*b*).

The organic phase need not be thin (although this is the only membrane situation we will examine here) nor need it be studied as a membrane. Indeed, for purposes of comparison with measurements of equilibrium salt extraction, the membrane will be considered in paper II to be expanded into a bulk liquid phase, and the effects of neutral macrocyclic molecules on salt extraction equilibria will be deduced for confrontation with the appropriate membrane measurements given in paper III.

Denoting the generic cation and anion by I^+ and X^- (i and x when used as subscripts), respectively, the following reactions are assumed to

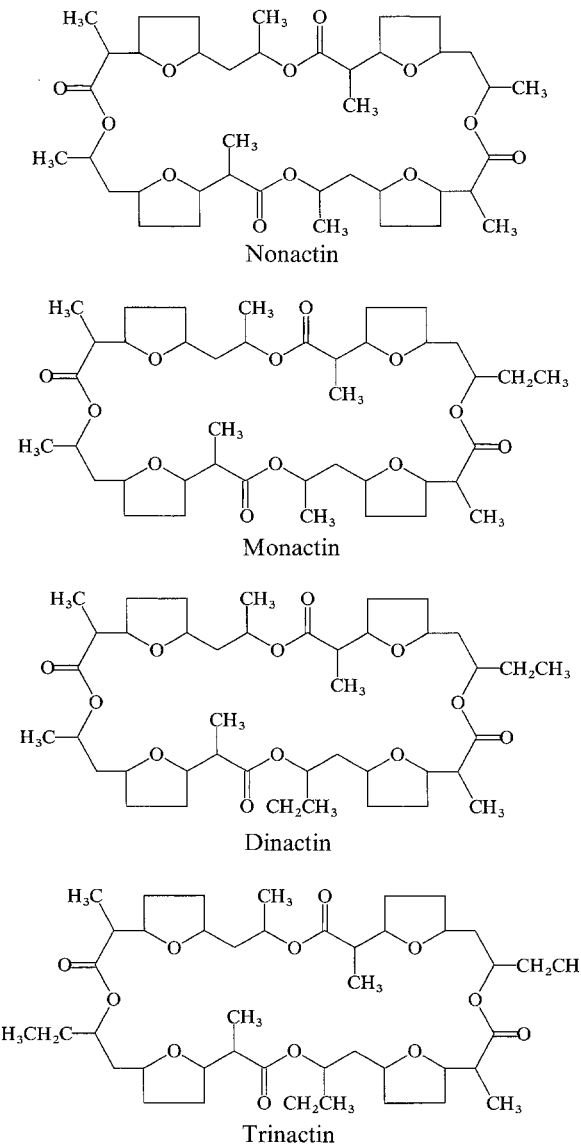

Fig. 1a. Chemical formulas of the macrotetralide actin antibiotics

occur in the aqueous, as well as in the membrane phases:

$$I^+ + S \underset{K_{is}^+}{\rightleftharpoons} IS^+; \qquad K_{is}^+ = \frac{a_{is}}{a_i a_s} \tag{1}$$

and

$$IS^+ + X^- \underset{K_{isx}}{\rightleftharpoons} ISX; \qquad K_{isx} = \frac{a_{isx}}{a_{is} a_x} \tag{2}$$

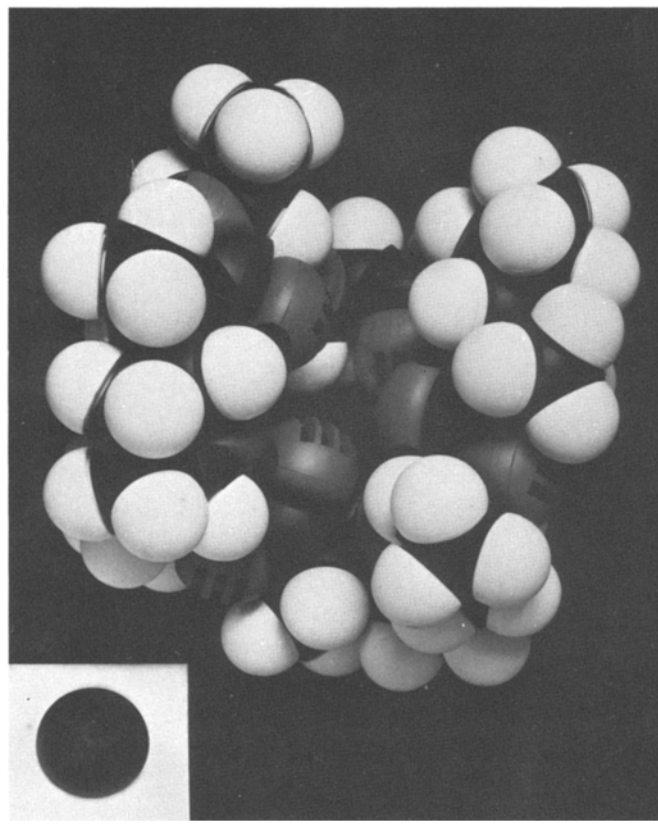

Fig. 1b. Space-filling model for Nonactin. A Corey-Pauling-Koltun model of the Nonactin molecule is shown in the configuration which we believe to be likely to exist in a low dielectric constant solvent or in the interior of the membrane, where the molecule is folded around the cation, sequestering it in relation to the four carbonyl oxygens within its interior (in the configuration similar to that of the Nonactin-K^+ complex in crystals; Kilbourn et al., 1967). The size of the cavity is seen to be appropriate to accomodate the potassium ion of ionic radius 1.33 A illustrated below. It can be seen that the overall configuration and external size of the molecule is not expected to vary greatly for different alkali cations within the interior. Note also that the addition of methyl groups to form the more highly methylated members of the series would not alter greatly the external size of the molecule

where a denotes the activity of the species in moles per liter. Reaction (1) describes the formation of a charged complex (IS^+) between the cation and the neutral carrier; reaction (2) takes into account the possibility of neutralization of this charged complex (or "complexed-cation") by association with the anion X^-. If n is the number of species of cations and m that of the anions, n species of charged complexes, IS^+, and $n \cdot m$ neutral complexes, ISX, will be present in the system as a result of the occurrence of reactions (1) and (2).

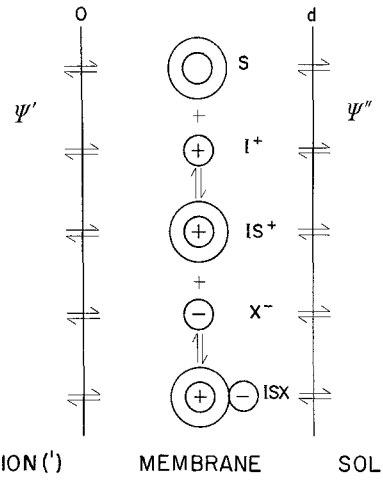

Fig. 2. Diagram of the membrane. A diagram of the membrane is indicated in which it is seen to be interposed between two aqueous solutions whose electric potentials are designated by ′ and ″. The species I^+, S, IS^+, X^-, and ISX refer to the free ion, the neutral molecular carrier, the complexed-cation, the free anion, and the neutralized complex, respectively. Although these species are illustrated within the membrane phase, the arrows at the membrane-solution interfaces indicate that equilibria exist between these species and their counterparts in the aqueous solutions

Moreover, the condition of heterogeneous equilibrium at the membrane-solution boundary can be symbolically described by partition equilibria of the type

$$S \underset{k_s}{\rightleftharpoons} S^*; \qquad k_s = \frac{a_s^*}{a_s} \tag{3}$$

for the neutral species (e.g., S and ISX), and of the type

$$R^{\pm} \rightleftharpoons R^{\pm *}; \qquad k_r = \frac{a_r^* \exp\left[\dfrac{z_r F \psi^*}{RT}\right]}{a_r \exp\left[\dfrac{z_r F \psi}{RT}\right]} \tag{4}$$

for the charged species (e.g., $R^{\pm} = I^+$, IS^+, X^-), where ψ is the electrostatic potential, z_r is the valence of R^+, and the asterisk ($*$) will be used to designate any quantity characteristic of the membrane phase.

Assumption (i). Assuming ideal behavior for all species in the membrane phase as well as for the species, S, IS^+, and ISX in the aqueous phase (activities will be used for the ionic species I^+ and X^- in the aqueous phase since activity coefficient corrections can be made for these), the

equilibria of reactions (1) and (2) in the aqueous phase are given by

$$K_{is}^{+}=\frac{C_{is}}{a_i C_s}; \qquad i=1, 2, \ldots, n \tag{5}$$

and

$$K_{isx}=\frac{C_{isx}}{C_{is} a_x}; \qquad i=1, 2, \ldots, n;\ x=1, 2, \ldots, m \tag{6}$$

where C_s, C_{is}, C_{isx} are concentrations in moles per liter, a_i and a_x the ionic activities in the same units, and K_{is}^{+}, K_{isx} the equilibrium constants in liters per mole of reactions (1) and (2), respectively. Experimental evidence in support of such ideal behavior will be presented in the following paper (Eisenman et al., 1969).

Condition of Thermodynamic Equilibrium

When the membrane is interposed between solutions of identical composition at the same temperature and pressure (as is done in paper III when measuring the limiting value of the resistance at zero current of the membrane exposed to the same antibiotic and salt concentrations on both sides), the system is in thermodynamic equilibrium; consequently, the electrochemical potentials of all the permeant components are constant throughout the whole system. Considering for simplicity the aqueous solutions to be infinitely extended in the x-direction normal to the membrane boundaries, we can therefore write

$$\bar{\mu}_\alpha(x)=\text{const}_\alpha; \qquad -\infty<x<+\infty \tag{7}$$

where α designates any of the permeant components and $\bar{\mu}$ is the electrochemical potential in units of energy per mole.

Following the general convention of thermodynamics applied to electrochemical systems, $\bar{\mu}$ is separable into two parts

$$\bar{\mu}_\alpha=\mu_\alpha+z_\alpha F\psi \tag{8}$$

ψ being the electric potential and μ_α the chemical potential. (For neutral components, for which $z_\alpha=0$, there is no electrical contribution to $\bar{\mu}_\alpha$.) The chemical potential can also be written as a function of molar concentration as

$$\mu_\alpha=\mu_\alpha^{(c)0}(T, P)+RT\ln y_\alpha C_\alpha \tag{9}$$

where y_α is the molar activity coefficient, and the standard chemical potential $\mu_\alpha^{(c)0}$ is a constant for a given temperature and pressure.

Since the electric potential terms, $z_\alpha F\psi$, in Eq. (8) cannot be assumed a priori to be constant near the membrane-solution interfaces, the number of variable parameters in the set of Eq. (7) (e.g., the concentrations C_α and the electric potential ψ) exceeds by one the number of equations. Therefore, an additional relation, namely the Poisson equation, is required to define the system and, in particular, to evaluate the concentration profiles of the individual ionic species inside the membrane. The determination of these concentration profiles will be shown later to be essential to the calculation of the membrane conductance; but it will be found unnecessary to evaluate these explicitly when calculating the membrane potential, as will now be demonstrated.

Membrane Potential at Zero Current

The electric current density, I, is defined as the sum of the fluxes of all charged species:

$$\frac{I}{F}=\sum_{i=1}^{n} z_{is}J_{is}+\sum_{i=1}^{n} z_i J_i+\sum_{x=1}^{m} z_x J_x. \tag{10}$$

For the present system $z_{is}=z_i=-z_x=1$.

Assumption (ii). Following the proof to be given in the Appendix that at equilibrium the concentrations in the membrane of the bare ions I^+ and X^- are negligible compared to the concentration of the complexed-cations IS^+, it is reasonable to assume, at least for small deviations from equilibrium, that the fluxes J_i and J_x can be neglected compared to J_{is}, provided the mobility of the IS^+ complex is not unduly low. Therefore,

$$I\simeq F\sum_{i=1}^{n} J_{is}. \tag{11}$$

The membrane potential data to be presented in paper III support the validity of this approximation.

Expressing J_{is} in terms of the Nernst-Planck flux equations, Eq. (11) becomes for zero current

$$0=\sum_{i=1}^{n} u_{is}^*\left[\frac{dC_{is}^*}{dx}+C_{is}^*\frac{F}{RT}\frac{d\psi^*}{dx}\right] \tag{12}$$

where u_{is}^* is the mobility of the IS^+ complex in the membrane.

Assumption (iii). Assuming the mobilities of IS^+ to be constant and dividing by $\sum_{i=1}^{n} u_{is}^* C_{is}^*$, we obtain

$$-\frac{d\psi^*}{dx}=\frac{RT}{F}\frac{d}{dx}\ln\left[\sum_{i=1}^{n} u_{is}^* C_{is}^*(x)\right] \tag{13}$$

which can be integrated directly across the membrane thickness from 0 to d to give

$$\psi^*(d)-\psi^*(0)=-\frac{RT}{F}\ln\frac{\sum_{i=1}^{n} u_{is}^* C_{is}^*(d)}{\sum_{i=1}^{n} u_{is}^* C_{is}^*(0)}. \tag{14}$$

This is the expression for the diffusion potential within the membrane in terms of $C_{is}^*(d)$ and $C_{is}^*(0)$, the membrane concentrations of IS^+ at the interfaces with solutions ($''$) and ($'$), respectively. Note that the result expressed by Eq. (14) requires only the validity of the Nernst-Planck differential equation within the membrane and is completely independent of the particular profiles of the concentrations and of the electric potential; consequently, it implies neither an assumption as to steady state nor as to the existence of equilibrium for reactions (1) within the interior of the membrane.

Assumption (iv). Provided the flux of IS^+ does not perturb the equilibrium existing at the membrane-solution interfaces as well as in the aqueous solutions, the membrane concentrations at 0 and d can be expressed in terms of the bulk solution concentrations through Eq. (4) as

$$C_{is}^*(0)=C'_{is}\,k_{is}\exp\left[-\frac{F}{RT}\left(\psi^*(0)-\psi'\right)\right], \tag{15}$$

$$C_{is}^*(d)=C''_{is}\,k_{is}\exp\left[-\frac{F}{RT}\left(\psi^*(d)-\psi''\right)\right]. \tag{16}$$

When Eqs. (15) and (16) are inserted into Eq. (14), a fortunate cancellation of the diffusion potential, underlined below, is seen to occur

$$\underline{\psi^*(d)-\psi^*(0)}=-\frac{RT}{F}\ln\frac{\sum_{i=1}^{n} u_{is}^* k_{is} C''_{is}}{\sum_{i=1}^{n} u_{is}^* k_{is} C'_{is}}+\psi'-\psi''+\underline{\psi^*(d)-\psi^*(0)}$$

so that the total membrane potential V_0 (i.e., the potential difference between solutions ($''$) and ($'$)) is given simply by

$$V_0 = \psi'' - \psi' = \frac{RT}{F} \ln \frac{\sum_{i=1}^{n} u_{is}^* k_{is} C'_{is}}{\sum_{i=1}^{n} u_{is}^* k_{is} C''_{is}} \tag{17}$$

which expresses the dependence of the membrane potential on the concentrations of the complexed-cations in the aqueous phases.

Since the aqueous concentrations of C_{is} (as well as of C_s and C_{isx}) are generally unknown, it is desirable to express these in terms of the known ionic activities and of the total concentration of neutral carriers, C_s^{Tot}, present in the aqueous phase. This can be done (*see* Appendix A) by solving the system of Eqs. (5) and (6) with respect to C_{is} and C_{isx} and combining with the equation of conservation of mass for the species S:

$$C_s^{\mathrm{Tot}} = C_s + \sum_{i=1}^{n} C_{is} + \sum_{i=1}^{n} \sum_{x=1}^{m} C_{isx}. \tag{18}$$

Using the equilibria (1) and (2) and Eqs. (5A) and (6A) deduced in the Appendix, Eq. (17) can be written

$$\begin{aligned} V_0 = {} & \frac{RT}{F} \ln \frac{\sum_{i=1}^{n} \left[\frac{u_{is}^* k_{is} K_{is}^+}{u_{js}^* k_{js} K_{js}^+} \right] a'_i}{\sum_{i=1}^{n} \left[\frac{u_{is}^* k_{is} K_{is}^+}{u_{js}^* k_{js} K_{js}^+} \right] a''_i} + \frac{RT}{F} \ln \frac{C_s^{\mathrm{Tot}\,\prime}}{C_s^{\mathrm{Tot}\,\prime\prime}} \\ & + \frac{RT}{F} \ln \frac{1 + \sum_{i=1}^{n} K_{is}^+ a''_i + \sum_{i=1}^{n} \sum_{x=1}^{m} K_{is}^+ K_{isx} a''_i a''_x}{1 + \sum_{i=1}^{n} K_{is}^+ a'_i + \sum_{i=1}^{n} \sum_{\lambda=1}^{m} K_{is}^+ K_{isx} a'_i a'_x} \end{aligned} \tag{19}$$

in terms of the known composition variables in the aqueous solutions and the indicated parameters ($u_{is}^*, k_{is}, K_{is}^+, K_{isx}$) of the system.

No restrictions as to the constancy of the individual fluxes have been made in the above derivation; and it therefore applies not only in the steady state, but also transiently (i.e., as soon as equilibrium conditions are established throughout the aqueous phases subsequent to a change in aqueous concentrations). It is also important to emphasize that the derivation of Eq. (19) requires no assumption of electroneutrality nor the explicit separate evaluations of the boundary and diffusion potentials,

and that it follows directly from the condition that the predominant permeant charged species are the cation complexes.

The first term of Eq. (19) is recognizible as equivalent to the classical Goldman-Hodgkin-Katz equation (Goldman, 1943; Hodgkin & Katz, 1949), which is also characteristic of a variety of ion exchange membranes (Sandblom & Eisenman, 1967). It is convenient to define the bracketed combination of parameters in this term as the permeability ratio, P_i/P_j.

$$\frac{P_i}{P_j}=\left[\frac{u_{is}^* k_{is} K_{is}^+}{u_{js}^* k_{js} K_{js}^+}\right]. \tag{20}$$

This combination of parameters, which is a constant for a given membrane and antibiotic, determines the relative effects of the ionic species on the membrane potential. It is seen to consist of the ratio of mobilities of the complexed cations IS^+ and JS^+ within the membrane multiplied by a particular combination of equilibrium parameters: namely, the partition coefficients and the association constants defined in Eqs. (4) and (1), respectively. This particular product of equilibrium parameters is shown in paper II to be measurable experimentally by characterizing the equilibrium extraction of salt by antibiotics into appropriate bulk solvent phases.

The second term of Eq. (19) indicates that the membrane potential is expected to depend linearly on the logarithm of the ratio of the total antibiotic concentrations in the two aqueous solutions. This term becomes zero when the antibiotic concentration is kept the same on both sides of the membrane, as is the case in paper III. We will therefore restrict the present considerations to this case, anticipating however that the effects of varying the ratio of antibiotic concentrations on the two sides of the membrane has been the subject of a separate study (Ciani et al., 1969*a*), where it is shown that the analysis of the potential and conductance behavior under these conditions permits the identification of the rate limiting step for carrier permeation (*see* Szabo et al., 1969*a*).

The third term of Eq. (19) results from the possibility of significant formation of IS^+ and ISX complexes in the aqueous solution. For sufficiently dilute solutions, this term reduces to zero. It will also be zero even at high salt concentrations provided the constants K_{is}^+ and K_{ISX} are sufficiently small in the aqueous solution, as is expected to be the case from the values of K_{is}^+ characteristic of Nonactin and Monactin in methanol (Pioda et al., 1967), as well as from the lack of any sign of significant formation of aqueous complexes of these species observed in the distribution equilibria between H_2O and CH_2Cl_2 phases presented in paper II.

Therefore, for the same concentration of antibiotic on both sides of the membrane and over a wide range of salt concentrations, Eq. (19) is expected to reduce to the simple form:

$$V_0 = \frac{RT}{F} \ln \frac{\sum_{i=1}^{n} \frac{P_i}{P_j} a_i'}{\sum_{i=1}^{n} \frac{P_i}{P_j} a_i''} \tag{21}$$

which for the usual experimental situation of a mixture of only two species, I^+ and J^+, can be written:

$$V_0 = \frac{RT}{F} \ln \frac{a_j' + \frac{P_i}{P_j} a_i'}{a_j'' + \frac{P_i}{P_j} a_i''}. \tag{22}$$

Paper III will demonstrate the completely satisfactory manner in which Eq. (21) describes the membrane potentials characteristic of phospholipid bilayers exposed to Nonactin, Monactin, Dinactin, and Trinactin.

When the third term of Eq. (19) is not negligible, its effect is to flatten, and even to reverse [when the neutralization reaction (2) predominates over the complexing reaction (1)], the slope expected from the first term. This can be seen most easily by considering Eq. (19) for the case in which a single salt, IX, is present in both solutions at different concentrations. For simplicity, we will assume ideal behavior of the ions in the aqueous phases

$$a_i = C_i; \qquad a_x = C_x \tag{23}$$

and note that the concentration of the antibiotic, C_s^{Tot}, is in practice so low with respect to the salt concentration as to allow the electroneutrality condition

$$C_i + C_{is} = C_x \tag{24}$$

to be approximated by

$$C_i = C_x. \tag{25}$$

Under these conditions, Eq. (19) can be differentiated with respect to $\ln C_i''$ to give

$$\frac{F}{RT} \frac{\partial V_0}{\partial \ln C_i''} = \frac{-1 + K_{is}^+ K_{isx} C_i''^2}{1 + K_{is}^+ C_i'' + K_{is}^+ K_{isx} C_i''^2}. \tag{26}$$

Eq. (26) shows that the Nernst slope for cations (-1) expected at high dilution can be reversed to that characteristic of anions $(+1)$ when

$K_{is}^{+} K_{isx} C_i''^{2} \gg 1 + K_{is}^{+} C_i''$. This comes about because, when reaction (2) predominates over reaction (1), an increase of the ionic concentration C_i'' actually decreases the concentration of the permeable charged species, IS^{+}.

Membrane Conductance at Zero Current

To calculate the electric conductance of the membrane when interposed between identical solutions of varying salt and antibiotic concentrations, the use of the Poisson equation is required for the evaluation of the concentration profiles of the individual ionic species within the membrane. The treatment given below follows the line indicated by Verwey (1940) and Verwey and Overbeek (1948) for the theory of double-layer interaction ocurring when two liquid phases are separated by a thin layer of a different liquid and, in particular, uses the same assumption that the dielectric constants are uniform throughout the individual phases while presenting a sharp discontinuity at the interfaces. The simple form of the Poisson-Boltzman equation is used, regarding all ions as point charges and neglecting discrete ion and image effects, as in these treatments, as well as in those given by Mauro for fixed charge membranes (1962) and by Laüger, Lesslauer, Marti, and Richter, (1967) and Everitt and Haydon (1968) for bilayer membranes.

The Poisson Equation in the Aqueous Phase

Denoting by D the permittivity of water and by $\rho(x)$ the charge density at x, the Poisson equation in the aqueous phase is:

$$\frac{d^2\psi}{dx^2} = -\frac{4\pi\rho(x)}{D}; \quad \begin{cases} -\infty < x \leqq 0 \\ d \leqq x < +\infty \end{cases} \tag{27}$$

where 0 and d designate the x coordinates of the left and right boundaries of the membrane, respectively. Recalling that in our model both species I^{+} and IS^{+} bear positive charges, the net charge density, resulting from the excess of ions of one sign, (e.g., cations less anions) at a given position x, is given by

$$\rho(x) = F\left[\sum_{i=1}^{n} C_i(x) + \sum_{i=1}^{n} C_{is}(x) - \sum_{x=1}^{m} C_x(x)\right]; \quad \begin{cases} -\infty < x \leqq 0 \\ d \leqq x < +\infty . \end{cases} \tag{28}$$

We can now express $\rho(x)$ in terms of the potential, $\psi(x)$, as well as of the concentrations in the bulk of the solutions: $C_i(\pm\infty)$, $C_{is}(\pm\infty)$ and $C_x(\pm\infty)$. Defining the value of the electric potential at $x = -\infty$ as zero,

and omitting, for brevity, the symbol $(\pm\infty)$ at these extremities (e.g., $\bar{\mu}_\alpha(\pm\infty)=\bar{\mu}_\alpha$; $\psi(\pm\infty)=\psi$, etc.), the condition for equilibrium, Eq. (7), can be rewritten as:

$$\bar{\mu}_\alpha(x)=\bar{\mu}_\alpha; \qquad \begin{cases} -\infty < x \leqq 0 \\ d \leqq x < +\infty \end{cases} \tag{29}$$

or, recalling Eqs. (8) and (9):

$$C_\alpha(x)=\frac{y_\alpha}{y_\alpha(x)}\, C_\alpha e^{-z_\alpha \phi(x)}; \qquad \begin{cases} -\infty < x \leqq 0 \\ d \leqq x < +\infty \end{cases} \tag{30}$$

where we express the potential in units of RT/F as:

$$\phi = F\psi/RT. \tag{31}$$

Substituting Eq. (30) in Eq. (28) yields:

$$\rho(x)=F\left[\sum_{i=1}^{n}\left(\frac{y_i}{y_i(x)}\, C_i+\frac{y_{is}}{y_{is}(x)}\, C_{is}\right)e^{-\phi(x)} - \sum_{x=1}^{m}\frac{y_x}{y_x(x)}\, C_x e^{\phi(x)}\right]; \qquad \begin{cases} -\infty < x \leqq 0 \\ d \leqq x < +\infty . \end{cases} \tag{32}$$

Assumption (v). Provided the values of the activity coefficients $y(x)$ near the membrane solution interfaces do not vary appreciably from those of the bulk solutions, so that they can be approximated by constants in the entire aqueous phases, and recalling that electroneutrality

$$\sum_{i=1}^{n} C_i+\sum_{i=1}^{n} C_{is}-\sum_{x=1}^{m} C_x=0 \tag{33}$$

must be satisfied in the bulk of the solution (i.e., at $x=\pm\infty$), Eq. (32) can be reduced to

$$\rho(x)=-2F\left(\sum_{x=1}^{m} C_x\right)\sinh\phi(x); \qquad \begin{cases} -\infty < x \leqq 0 \\ d \leqq x < +\infty \end{cases} \tag{34}$$

which gives the explicit dependence of the charge density at a given position on the bulk concentrations and the difference of electric potential of that point from that of the bulk solution.

Introducing the Debye length in the aqueous phase,

$$L=\left(\frac{RTD}{8\pi F^2 \cdot \sum_{x=1}^{m} C_x}\right)^{\frac{1}{2}}. \tag{35}$$

Eq. (27) becomes

$$\frac{d^2\phi(x)}{dx^2}=\frac{1}{L^2}\sinh\phi(x); \qquad \begin{cases}-\infty<x\leqq 0\\ d\leqq x<+\infty\end{cases} \tag{36}$$

which is the well known Poisson-Boltzman equation (Verwey & Overbeek, 1948).

The Poisson Equation in the Membrane Phase

For the present equilibrium case, Eq. (7) implies

$$\bar{\mu}_\alpha^*(x)=\bar{\mu}_\alpha; \qquad 0\leqq x\leqq d \tag{37}$$

where, as usual, we denote by an asterisk the quantities in the membrane. By use of the explicit form given by Eqs. (8) and (9) for the electrochemical potentials in both aqueous and membrane phases, and recalling that the electric potential has been defined as zero at $x=\pm\infty$ for the present situation where the solutions are identical on both sides of the membrane, Eq. (37) may be written:

$$C_\alpha^*(x)=k_\alpha a_\alpha e^{-z_\alpha\phi^*(x)}; \qquad 0\leqq x\leqq d. \tag{38}$$

The activity of α in the aqueous phase, a_α, is related to the molar concentration and the molar activity coefficient by

$$a_\alpha=y_\alpha C_\alpha \tag{39}$$

and the partition coefficient of the species α, k_α, is defined as

$$k_\alpha=\exp\left[\frac{\mu_\alpha^{(c)0}-\mu_\alpha^{(c)0*}}{RT}\right] \tag{40}$$

in terms of the standard chemical potentials.

Using relation (38) to express the concentrations of the ionic species I^+, IS^+, and X^- inside the membrane, the net charge density in the membrane is

$$\rho^*(x)=F\left[\left(\sum_{i=1}^{n}k_i a_i+\sum_{i=1}^{n}k_{is}C_{is}\right)e^{-\phi^*(x)}-\sum_{x=1}^{m}k_x a_x e^{\phi^*(x)}\right]; \qquad 0\leqq x\leqq d \tag{41}$$

where the aqueous concentrations C_{is} have been used instead of the aqueous activities in accord with assumption (i).

To write the Poisson equation in the same compact form as Eq. (36), we introduce the following definitions:

$$A=\sum_{i=1}^{n} k_i a_i+\sum_{i=1}^{n} k_{is} C_{is}; \qquad B=\sum_{x=1}^{m} k_x a_x, \tag{42}$$

$$L^*=\left(\frac{RTD^*}{8\pi F^2 (AB)^{\frac{1}{2}}}\right)^{\frac{1}{2}}, \tag{43}$$

$$Y=\frac{1}{2}\ln\frac{A}{B}. \tag{44}$$

By straightforward manipulations of relations (41)–(44), the Poisson equation in the membrane may be then written as:

$$\frac{d^2\phi^*(x)}{dx^2}=\frac{1}{L^{*2}}\sinh[\phi^*(x)-Y]. \tag{45}$$

Eq. (45) can be integrated under the following two assumptions:

Assumption (vi). In accord with the postulate that the macrocyclic antibiotics solubilize cations in the membrane in the form of charged complexes IS^+, it is assumed that the partition coefficients of such complexes are so much higher than those of the anions, as well as of these of the uncomplexed cations, that the relations

$$k_{is} C_{is} \gg k_x a_x, \qquad k_{is} C_{is} \gg k_i a_i \tag{46}$$

are satisfied for the normally explored range of concentration, despite the fact that the concentrations C_{is} of the complexed cations in the aqueous solutions are generally lower than activities of the free cations and anions, a_i and a_x.

Assumption (vii). It is also assumed that in the normal range of the concentrations the following relation is satisfied between the thickness of the membrane and the parameters of the system

$$d<\frac{4D^*}{D}L\left\{\left[1+Ye^{-Y}\left(\frac{DL^*}{D^*L}\right)^2\right]^{\frac{1}{2}}-1\right\}. \tag{47}$$

We show in Appendix B that, as a consequence of assumptions (vi) and (vii) Eq. (45) can be approximated by

$$\frac{d^2\phi^*(x)}{dx^2}=-\frac{8\pi F^2 A}{RTD^*}e^{-\phi^*(x)}; \qquad 0\leqq x\leqq d. \tag{48}$$

For the zero electric field, at the center of the membrane ($x=d/2$), which can be deduced from the symmetry of the system, Eq. (48) can be integrated to yield

$$\phi^*(x)=\ln\left[\frac{2\pi d^2F^2A}{RTD^*}\cdot\frac{\cos^2\frac{\lambda}{d}\left(\frac{d}{2}-x\right)}{\lambda^2}\right];\qquad 0\leqq x\leqq d \tag{49}$$

where the constant of integration λ is calculated in terms of solution concentrations and activities in Appendix C.

The Limiting Value of the Membrane Conductance at Zero Electric Current

One consequence of assumptions (vi) and (vii) is shown in the Appendix to be that the complexes IS^+ are the predominant charged species present in the membrane, justifying assumption (ii) that the contribution of the anions and of the uncomplexed cations can be neglected compared to the contributions due to the complexed cations IS^+ in any flow of electric current across the membrane. We may therefore describe the current density in terms of the Nernst-Planck equation for the fluxes of the IS^+ species as:

$$I=-RTF\sum_{i=1}^{n}u_{is}^*\left[\frac{dC_{is}^*}{dx}+C_{is}^*\frac{F}{RT}\frac{d\psi^*}{dx}\right]. \tag{50}$$

After dividing by $\sum_{i=i}^{n}u_{is}^*C_{is}^*$, Eq. (50) can be integrated formally with respect to x from the left to the right boundary, yielding in the steady state, for which I is independent of x

$$\frac{I}{F^2}\int_0^d\frac{dx}{\sum_{i=1}^{n}u_{is}^*C_{is}^*}=-\frac{RT}{F}\ln\frac{\sum_{i=1}^{n}u_{is}^*C_{is}^*(d)}{\sum_{i=1}^{n}u_{is}^*C_{is}^*(0)}+\psi^*(0)-\psi^*(d) \tag{51}$$

where $\psi^*(0)-\psi^*(d)$ will be recalled to be the internal potential difference between the membrane boundaries and $C_{is}^*(0)$ and $C_{is}^*(d)$ are the membrane concentrations of the species IS^+ at the membrane-solution interfaces. Recalling assumption (iv) and combining Eq. (51) with Eqs. (15) and (16) for $C_{is}'=C_{is}''$, since the bulk concentrations of the two solutions

are identical, we obtain simply

$$\frac{I}{V} = -\frac{F^2}{\int_0^d \frac{dx}{\sum_{i=1}^{n} u_{is}^* C_{is}^*}} \tag{52}$$

where $V = \psi'' - \psi'$ is the difference of electric potential between the two solutions.

The limiting value of the conductance at zero current, G_0, is

$$-\lim_{I \to 0} \frac{I}{V} = G_0 = \frac{F^2}{\int_0^d \frac{dx}{\lim_{I \to 0} \sum_{i=1}^{n} u_{is}^* C_{is}^*}} \tag{53}$$

assuming that the limit extraction and integration operation can be mutually interchanged.

Since at zero current the system is at equilibrium, the limiting value of the concentrations $C_{is}^*(x)$ in the integral of the conductance are those that one obtains by combination of Eq. (49) with Eq. (38):

$$C_{is}^*(x) = k_{is} C_{is} \frac{RTD^*}{2\pi d^2 F^2 A} \cdot \frac{\lambda^2}{\cos^2 \frac{\lambda}{d}\left(\frac{d}{2} - x\right)}. \tag{54}$$

Inserting Eq. (54) in the denominator of Eq. (53) and carrying out the integration we obtain

$$G_0 = \frac{\lambda^2}{\left(1 + \frac{\sin \lambda}{\lambda}\right)} \cdot \frac{RTD^*}{\pi d^3 A} \cdot \sum_{i=1}^{n} u_{is}^* k_{is} C_{is} \tag{55}$$

where λ is given by Eq. (5C). It is shown in the Appendix that when Eq. (6C) is valid, Eq. (53) becomes, approximately

$$G_0 = \frac{F^2}{d} \cdot \sum_{i=1}^{n} u_{is}^* k_{is} C_{is} \tag{56}$$

which expresses the dependence of the membrane conductance on the aqueous concentrations of the complexed cations.

Expressing the aqueous concentrations C_{is} in terms of the aqueous activities of the free ions and the total concentration of the carriers, as in

Eq. (5A), we obtain

$$G_0 = \frac{F^2}{d} \cdot \frac{C_s^{\mathrm{Tot}} \cdot \sum_{i=1}^{n} u_{is}^* k_{is} K_{is}^+ a_i}{1 + \sum_{i=1}^{n} K_{is}^+ a_i + \sum_{i=1}^{n} \sum_{x=1}^{m} K_{is}^+ K_{isx} a_i a_x} \tag{57}$$

which is the general expression for the membrane conductance as a function of the (known) aqueous concentrations and the parameters of the system.

From Eq. (57) it is immediately apparent that the membrane conductance is expected to be directly proportional to the total concentrations of antibiotic in the aqueous phase, C_s^{Tot}, regardless of the ionic concentrations. This expectation is borne out by the experimental results of paper III. Moreover, when association in the aqueous solution is negligible (as is usually expected to be the case as previously mentioned), the denominator of Eq. (57) can be approximated by unity and the expression of the conductance can be reduced to the simpler one

$$G_0 \simeq \frac{F^2}{d} C_s^{\mathrm{Tot}} \cdot \sum_{i=1}^{n} u_{is}^* k_{is} K_{is}^+ a_i \tag{58}$$

which becomes

$$G_0(J) \simeq \frac{F^2}{d} C_s^{\mathrm{Tot}} u_{js}^* k_{js} K_{js}^+ a_j \tag{59}$$

when only a single cationic species J^+ is present in the solution. The simple linear dependence on the ionic activity predicted by Eq. (59), has also been verified in the experiments to be reported in paper III.

It is worth noting that in the situation where the neutralization reaction (2) can be neglected in the aqueous phase, Eq. (57) for a single cation species J^+ simplifies to

$$G_0(J) = \frac{F^2}{d} \cdot C_s^{\mathrm{Tot}} \frac{u_{js}^* k_{js} K_{js}^+ a_j}{1 + K_{js}^+ a_j} \tag{60}$$

from which it can be seen that the conductance tends to be the limiting value

$$\lim_{a_i \to \infty} G_0(J) = \frac{F^2}{d} C_s^{\mathrm{Tot}} u_{js}^* k_{js} \tag{61}$$

when $K_{js}^+ a_j$ is sufficiently large that $K_{js}^+ a_j > 1$*.

* From the perfect proportionality between KCl concentration and bilayer membrane conductance which has been observed to hold in paper III at concentrations at least as high as 0.1 M, it would appear that $K_{js}^+ a_i$ is smaller than unity even at 0.1 M. Therefore, K_{js}^+ for potassium-monactin complexation in the aqueous phase can be inferred to be of the order or less than 1 liter/mole.

Discussion

The Postulate That Neutral Macrocyclic Antibiotics Act as Cation Carriers

The results deduced here are based on the postulate that molecules such as neutral macrocyclic antibiotics of the Nonactin and Valinomycin type solubilize cations as mobile charged complexes in the liquid-like interior of a phospholipid bilayer membrane. Under appropriate physical conditions, defined in the Appendix, the concentrations of such species are expected to exceed those of all the other ions inside the membrane, so as to determine completely its electrical properties. This postulate is, in itself, sufficient to account for the characteristic increase of conductance as well as the cationic permselectivity caused by such antibiotics. Its more detailed consequences from the present model lead to the experimentally testable expectation of a direct proportionality between membrane conductance and concentration of antibiotic in the aqueous solutions [*see* Eq. (63)], which is indeed observed (Eisenman et al., 1968; Szabo et al., 1969*a*, *b*). In addition, a proportionality to ionic concentration is also expected [*see* Eq. (59)] which is observed when ionic strength effects are properly controlled (Szabo et al., 1969*a*, *b*).

A further unambiguous expectation of the present treatment is a directly testable relationship between membrane potential and conductance ratio valid in the limit in which the conductance can be expressed by Eq. (58). Comparing the general expression of the potential, Eq. (17), with the two values of the zero current conductance, Eq. (56), measured successively for a membrane equilibrated with solution (′) and (″), respectively, we find

$$V_0 = \frac{RT}{F} \ln \frac{G_0'}{G_0''}. \tag{62}$$

In particular, when (′) and (″) refer to single cation solutions of I^+ and J^+ at the same ionic activity and in the presence of the same total concentration of the antibiotic, Eq. (62) reduces to:

$$\frac{G_0(J)}{G_0(I)} = \left[\frac{u_{js}^* k_{js} K_{js}^+}{u_{is}^* k_{is} K_{is}^+}\right] = \frac{P_j}{P_i} \tag{63}$$

when association is negligible in the aqueous solution. $G_0(I)$ and $G_0(J)$ denote the zero current value of the conductance in such experimental conditions.

Such a relationship is totally different from that expected (or observed) for ion exchange membranes of sufficient thickness that the excess charge density is negligible compared to the concentration of the fixed (charged)

sites (Conti & Eisenman, 1965; Eisenman, 1967)*; and it suggests the importance of comparing the results of independent measurements of membrane potential and membrane conductance. Such measurements have been reported for Li, Na, K, Rb, and Cs in the presence of Monactin (Eisenman et al., 1968) and are extended to Nonactin, Dinactin, and Trinactin in paper III of this series with remarkably precise verification of this expectation.

It should be noted that the postulate that the antibiotic molecules are free to move as cation carriers, rather than providing "tunnels" for cation movement as suggested by Mueller and Rudin (1967), is not strictly necessary for the validity of the above discussed identity between permeability and conductance ratios. Such an identity is a consequence of the assumption that the excess of the permeant cation species results from the presence of the antibiotic and can also be shown to exist for a mechanism in which the cations move through neutral antibiotic pores. It is more difficult for such a mechanism to deduce what sort of dependence on aqueous antibiotic and salt concentration is to be expected for the membrane conductance, but it is not impossible to conceive of the same expectations as for the present carrier model. Therefore, potential and conductance measurements cannot, by themselves, provide a unique test for the carrier hypothesis.

More pertinent to this question is the confrontation between the present expectations for the effects of the carrier molecules on the electrical properties of bilayer membranes and the equilibrium properties expected for their effects on the extraction of salts from aqueous solutions into bulk liquid phases or organic solvents, to be considered both theoretically and experimentally in paper II. It will be shown there that the same combination of equilibrium parameters $k_{js} K_{js}^{+}/k_{is} K_{is}^{+}$, appearing in the permeability and conductance ratios, can be measured from the ratio of the equilibrium constants of heterogeneous reactions of the type:

$$\underset{\text{(aqueous)}}{I^{+}} + \underset{\text{(aqueous)}}{X^{-}} + \underset{\text{(organic)}}{S^{*}} \overset{K_I}{\rightleftharpoons} \underset{\text{(organic)}}{IS^{+*}} + \underset{\text{(organic}}{X^{-*}} \tag{64}$$

where the asterisk (*) is now used to denote a bulk organic liquid phase in contact with an aqueous solution. Decomposing reaction (64) into

* In such ion exchangers, the ratio of conductance of the membrane when exposed to single salt solutions of different cations depends only on the mobility ratio of these ions and is independent of the partition coefficients. This is because the uptake of counterions by an ion exchanger from a single salt solution is (by electroneutrality) a function solely of the ion exchange capacity of a membrane, any effect on the uptake due to the different partition coefficients being counteracted by the occurrence of appropriately compensating phase-boundary potentials at the membrane solution interfaces.

appropriate elementary reactions, it is easily verified that

$$\frac{K_J}{K_I}=\frac{k'_{js}K^+_{js}}{k'_{is}K^+_{is}} \tag{65}$$

where k'_{js} and k'_{is} now represent the partition coefficients between the aqueous and bulk solvent (instead of membrane) phases. Despite the fact that the mobility ratio does not appear in relation (65) and that the partition coefficients k'_{is}, k'_{js} for the model solvent cannot be expected to be the same as those for the membrane, the experimental values of the ratio of equilibrium constants in Eq. (65) have been found experimentally to be identical to the corresponding permeability or conductance ratios measured electrically in bilayer membranes, independent of the lipid composition of the bilayer membrane as well as of the particular solvent chosen to represent the interior of the membrane in the equilibrium salt extraction experiments.

This result, at first sight surprising, becomes less so when one compares the relative dimensions of the alkali cations with those of the macrotetralide molecules, as can be done in Fig. 2, where a space-filling model of a Nonactin molecule is presented in the known configuration, characteristic of its K^+ complex in the crystalline state (Kilbourn et al., 1967), which is almost certainly its configuration in a medium of low dielectric constant. It can be inferred that the overall size of the molecule should be quite insensitive as to whether the sequestered ion is of the size of the K^+ illustrated in the figure or of the size of Na^+, Rb^+, Cs^+, NH_4^+, or H_3O^+ (or even the much smaller Li^+ or its monohydrate).

For these particular molecules, one is naturally led to postulate that the association reactions with the various cations will occur without altering the overall dimensions and shape of the carrier, leading to the formation of charged complexes which are indistinguishable in most of their properties. In such a case the following approximations are valid

$$\frac{u^*_{js}}{u^*_{is}}\simeq 1, \tag{66}$$

$$\frac{k_{js}}{k_{is}}\simeq\frac{k'_{js}}{k'_{is}}\simeq 1 \tag{67}$$

so that the ratio of permeabilities, conductances, salt extraction constants, and aqueous phase association constants are expected to be interrelated by

$$\frac{P_j}{P_i}=\frac{G_0(J)}{G_0(I)}\simeq\frac{K_J}{K_I}\simeq\frac{K^+_{js}}{K_{is}}. \tag{68}$$

It should be emphasized that the validity of the approximation of equal mobilities, Eq. (66), which is essential in deriving relation (68) and therefore in interpreting the experimentally found identity between bulk equilibrium and bilayer electrical properties, is justifiable by the above arguments only if the charged complexes themselves are the mobile entities within the membrane. No simple explanation for the validity of relation (66) would be found from the "tunnel model" in which the ions are supposed to move through channels opened across the membrane by aligned rings of stacked antibiotic molecules.

The remarkably simple relation (68) shows that the permeability and conductance ratios are expected to be independent of the lipid composition of the membrane [since K_{js}^{+} and K_{is}^{+} depend only on aqueous solution properties as can be seen from their definition Eq. (1)]. In fact, these ratios merely express the ratio of the equilibrium constants of the association reactions between the carrier molecule and the cations J^{+} and I^{+} in the aqueous solution phases. This can be most clearly seen by introducing relations (66) and (67) into Eq. (17), to yield the simple expression for the membrane potential

$$V_0 = \frac{RT}{F} \ln \frac{\sum_{i=1}^{n} C'_{is}}{\sum_{i=1}^{n} C''_{is}} \tag{69}$$

which indicates that the electrical potential in effect depends merely on the ratio of the total concentrations of the complexed-cations in the two aqueous solutions. The membrane thus behaves merely as a sensor of the total concentration of complexed cations in the aqueous phases. Since the concentrations of the complexes in a given solution in turn depend solely on the equilibrium constants K_{js}^{+} and K_{is}^{+}, it should be clear why the ion-binding constant of the carrier in an aqueous solution is expected to be the principle parameter determining the relative effects of ions on the membrane potential.

The Properties of the Present Model and the "Independence Principle" of Hodgkin and Huxley

Since the identity between permeability and conductance ratios found for the present model is expected also for a system obeying the "Independence Principle" (A. Hodgkin, *personal communication*) postulated by Hodgkin and Huxley (1952) to govern the ionic fluxes in the squid axon

membrane, it is worth examining the present system to see if the "Independence Principle" is characteristic of it.

The "Independence Principle" requires that the partial ionic current of I^+, I_i, can be written in the form

$$I_i = P_i f(V) \{C_i'' e^{\frac{FV}{RT}} - C_i'\} \tag{70}$$

where $f(V)$ is a function of voltage which is the same function for all ionic species. Following the line of the proof given by Hodgkin *(personal communication)*, let us consider the case in which a single permeant cation I^+ is present at the same concentrations in both solutions, $C_i' = C_i'' = C$. The conductance $G_0(I)$ at zero current (and therefore zero voltage since $C_i' = C_i''$) is then given by

$$G_0(I) = \lim_{V \to \infty} \left(\frac{\partial I_i}{\partial V} \right) = P_i f(0)\, C \tag{71}$$

so that, comparing the two values of the single ion conductances in the presence of I^+ and J^+, respectively, we have

$$\frac{G_0(J)}{G_0(I)} = \frac{P_j}{P_i}. \tag{72}$$

On the other hand, the voltage at zero current in the presence of a mixture of two cations, J^+ and I^+, is easily found from Eq. (70) to be given in the form

$$V_0 = \frac{RT}{F} \ln \frac{C_i' + \frac{P_j}{P_i} C_j'}{C_i'' + \frac{P_j}{P_i} C_j''} \tag{73}$$

so that, comparing Eqs. (72) and (73), it is apparent that the identity between permeability and conductance ratios of Eq. (63) also holds for a system of ionic currents obeying the "Independence Principle".

To express the partial current of the species IS^+ for our system in a form equivalent to Eq. (70) we consider the Nernst-Planck equation, written in the form:

$$\frac{I_{is}}{F u_{is}^*} e^{\phi^*(x)} = \frac{d}{dx} [C_{is}^* e^{\phi^*(x)}] \tag{74}$$

where $I_{is}=FJ_{is}$ and $\phi^*=\dfrac{F\psi^*}{RT}$. Integrating formally across the membrane, we get:

$$\frac{I_{is}}{Fu_{is}^*}=\frac{e^{\phi^*(0)}}{\int_0^d e^{\phi^*}\,dx}\left[C_{is}^*(d)\,e^{\phi^*(d)-\phi^*(0)}-C_{is}^*(0)\right]. \tag{75}$$

Inserting the boundary condition (15) and (16) and recalling that $\phi'=0$, we have:

$$I_{is}=\frac{Fu_{is}^*k_{is}}{\int_0^d e^{\phi^*}\,dx}\left[C_{is}''\,e^{\phi''}-C_{is}'\right]. \tag{76}$$

Expressing the aqueous concentrations C_{is}' and C_{is}'' in terms of the aqueous ionic activities and assuming low association in the aqueous phases ($C_s=C_s^{\mathrm{Tot}}$) we find

$$I_{is}=\frac{FC_s^{\mathrm{Tot}}u_{is}^*k_{is}K_{is}^+}{\int_0^d e^{\phi^*(x)}\,dx}\left[a_i''\,e^{\phi''}-a_i'\right]. \tag{77}$$

Eq. (77) has the same form of Eq. (70) where

$$P_i=FC_s^{\mathrm{Tot}}u_{is}^*k_{is}K_{is}^+ \tag{78}$$

and

$$f(V)=\frac{1}{\int_0^d e^{\phi^*(x)}\,dx}. \tag{79}$$

In particular, when $a_i'=a_i''=a$, Eq. (79) becomes

$$I_{is}=\frac{FC_s^{\mathrm{Tot}}u_{is}^*k_{is}K_{is}^+a}{\int_0^d e^{\phi^*(x)}\,dx}\left[e^{\phi''}-1\right] \tag{80}$$

where, in the limit of zero current, the integral can be calculated using the equilibrium profile of the potential given by Eq. (49). Considering such expression for the potential as well as the equation for the parameter λ, Eq. (5C), it is apparent that in the same limit, expressed by relation (6C), in which the identity between permeability and conductance ratios holds true, the potential $\phi^*(x)$ is approximately constant and equal to 0, so that Eq. (79) becomes simply

$$f(0)=\frac{1}{d}. \tag{81}$$

Therefore, for vanishingly small currents, at least one aspect of the "Independence Principle" is certainly compatible with an ion permeation mechanism utilizing a neutral carrier, namely the equality of the functions $f(V)$ at zero voltage for all the ionic species when present alone in the system at the same concentration in the solutions.

In the presence of a mixture of two or more ions, or even of a single ion at different concentrations in the solutions, Eq. (77) is still valid. However, the integral in the denominator, although being the same for all the ionic species, is in general a complicated function of all the parameters of the system. This problem will be dealt with explicitly in a subsequent paper (Ciani et al., 1969*c*), where the expectations for the conductance of a membrane exposed asymmetrically to ionic mixtures will be derived.

Conclusions

Starting from the knowledge that neutral macrocyclic antibiotics solubilize monovalent cations in hydrocarbon solvents as mobile positively charged complexes, a model for the effects of these molecules on phospholipid bilayer membranes is proposed in which the electrical properties are deduced for a simple solvent membrane of the thinness of the phospholipid bilayer. The following conclusions have been reached:

(1) Under appropriate conditions, an excess of positive complexes over all other charged species is expected in the membrane interior. This makes certain integrations of the flux equations relatively easy.

(2) An expression for the membrane potential in ionic mixtures is deduced in terms of the aqueous concentrations of ions and of antibiotic. Under usual conditions, this equation is identical in form to the Goldman-Hodgkin-Katz equation – with the permeability ratio representing combinations of such membrane parameters as the mobilities of the complexed-cations, the partition coefficients of the complexed-cations, and the formation constants of the complexes in aqueous solution.

(3) An equation for the membrane conductance in the limit of zero current is also derived for a membrane exposed on both sides to the same solution. This depends on the same parameters as did the membrane potential.

(4) Indeed, it is shown that the ratio of conductance measured in single salt solutions for two different cations should be identical to their permeability ratio, thereby providing an immediately testable expectation of the theoretical treatment.

(5) The membrane conductance is also expected to be proportional to the concentration of salt for dilute solutions but may become independent of concentrations at high concentrations.

(6) The membrane conductance is expected to be directly proportional to the total concentration of antibiotic in the aqueous solution.

(7) Although conclusions (2) through (5) are properties of a neutral carrier mechanism, they are also conceivable for membranes in which the neutral antibiotics might form "tunnels" for cation permeation.

(8) However, if the overall size of the complex is approximately the same regardless of the particular cation bound, as is likely for the usual macrocyclic antibiotics, the mobilities of the complexes will be the same for all cations. In this event, the permeability and conductance ratios are expected to depend only on equilibrium selectivity parameters, which will be shown in the following paper to be measurable independently by the bulk extraction of salt into an appropriate organic solvent phase. The comparison of membrane electrical properties with equilibrium extraction properties provides a means for distinguishing neutral carriers from neutral "tunnels".

(9) Lastly, the properties of the neutral carrier mechanism proposed here are shown to be compatible with the "Independence Principle" of Hodgkin and Huxley.

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Appendix A

Chemical Composition of an Ionic Solution in the Presence of Neutral Ion-Binding Carriers

In this appendix we express the aqueous concentration of the carrier, C_s and its complexes, C_{is} and C_{isx}, in terms of the ionic activities a_i, a_x, and of the total concentration of carriers, C_s^{Tot}.

If n is the number of species of cations and m that of the anions, Eqs. (1) and (2) and assumption (i) give

$$C_s = \frac{C_{is}}{K_{is}^+ a_i} = \cdots = \frac{C_{ns}}{K_{ns}^+ a_n} = \cdots = \frac{C_{isx}}{K_{is}^+ K_{isx} a_i a_x} = \cdots = \frac{C_{nsm}}{K_{ns}^+ K_{nsm} a_n a_m} \tag{1A}$$

or, adding the numerators and the denominators

$$C_s = \frac{\sum_{i=1}^{n} C_{is} + \sum_{i=1}^{n} \sum_{x=1}^{m} C_{isx}}{\sum_{i=1}^{n} K_{is}^+ a_i + \sum_{i=1}^{n} \sum_{x=1}^{m} K_{is}^+ K_{isx} a_i a_x}. \tag{2A}$$

From the conservation of mass of the carrier species, we have

$$C_s^{\mathrm{Tot}} - C_s = \sum_{i=1}^{n} C_{is} + \sum_{i=1}^{n} \sum_{x=1}^{m} C_{isx}. \tag{3A}$$

Substituting the left hand side of Eq. (3A) in the numerator of Eq. (2A) and solving with respect to C_s, we obtain

$$C_s = \frac{C_s^{\mathrm{Tot}}}{1 + \sum_{i=1}^{n} K_{is}^{+} a_i + \sum_{i=1}^{n} \sum_{x=1}^{m} K_{is}^{+} K_{isx} a_i a_x}. \tag{4A}$$

From Eqs. (1A) and (4A) the following relations are now immediately obtained

$$C_{is} = \frac{K_{is}^{+} a_i C_s^{\mathrm{Tot}}}{1 + \sum_{i=1}^{n} K_{is}^{+} a_i + \sum_{i=1}^{n} \sum_{x=1}^{m} K_{is}^{+} K_{isx} a_i a_x}; \quad i = 1, 2, \ldots, n, \tag{5A}$$

$$C_{isx} = \frac{K_{is}^{+} K_{isx} a_i a_x C_s^{\mathrm{Tot}}}{1 + \sum_{i=1}^{n} K_{is}^{+} a_i + \sum_{i=1}^{n} \sum_{x=1}^{m} K_{is}^{+} K_{isx} a_i a_x}; \quad i = 1, 2, \ldots, n; \; x = 1, 2, \ldots, m. \tag{6A}$$

Appendix B

Electric Potential and Concentration Profiles in the System at Equilibrium

In this appendix we examine, for a membrane interposed between identical aqueous solutions, the profiles of concentration and electric potential, which must be evaluated in order to assess the limiting value of the membrane conductance at zero current. The determination of the profiles of the electric potential and of the ionic concentrations throughout the system in the equilibrium situation where the compositions of the two aqueous phases are identical requires the integration of the Poisson-Boltzman equation as well as the use of appropriate boundary conditions at the membrane-solution interfaces. Schematizing such interfaces as ideal surfaces of discontinuity of the dielectric constant and of the standard chemical potential, we shall assume, consistently with electrostatics: 1) continuity of the electric potential, and 2) continuity of the electric displacement vector, defined as the product of the electric field by the permittivity of the dielectric medium.

Since, for identical compositions of solutions (′) and (″) of concern here, the electric potential as well as the concentration profiles are symmetrical at equilibrium with respect to the central region of the membrane ($x = d/2$), we need, when convenient, consider explicitly only the results referring to the left of such region; so, in particular, the integration of the Poisson-Boltzman equation in the aqueous solution, Eq. (36), for vanishing electric field and zero charge density at $-\infty$ gives

$$\phi(x) = \ln\left(\frac{1 + K e^{x/L}}{1 - K e^{x/L}}\right)^2; \quad -\infty < x \leqq 0 \tag{1B}$$

where K is an integration constant and the coordinate 0 refers to the left membrane-solution interface.

Defining

$$\tilde{\phi}^*(x)=\phi^*(x)-Y; \quad 0\leqq x\leqq d \tag{2B}$$

where Y is given by relation (44), the Poisson-Boltzman equation within the membrane phase, Eq. (45), becomes simply

$$\frac{d^2\tilde{\phi}^*(x)}{dx^2}=\frac{1}{L^{*2}}\sinh\tilde{\phi}^*(x); \quad 0\leqq x\leqq d. \tag{3B}$$

It is appropriate to observe that now, as a consequence of assumption (vi), Y is a positive quantity, representing the value of the potential difference expected theoretically (although not experimentally accessible) between the interior of the aqueous solution and that of a bulk membrane phase much thicker than its Debye length. It is therefore clear intituitively, and can be rigorously proven by a tedious sequence of mathematical steps, that the potential values throughout the whole system are positive and less than Y. This implies that $\tilde{\phi}^*(x)$, defined in Eq. (2B) satisfies the relation

$$\tilde{\phi}^*(x)<0; \quad 0\leqq x\leqq d \tag{4B}$$

so that, from Eq. (3B) and the elementary properties of the hyperbolic sine, we deduce

$$\frac{d^2\tilde{\phi}^*(x)}{dx^2}=\frac{d^2\phi^*(x)}{dx^2}<0; \quad 0\leqq x\leqq d. \tag{5B}$$

The only profile compatible with relation (5B) and with the symmetry of the system is a curve monotonously increasing from the left boundary up to the center, $x=d/2$, presenting a maximum there, and decreasing symmetrically in the remaining portion of the membrane. Defining for simplicity of notation

$$\frac{d\phi^*}{dx}=\phi^{*\prime} \tag{6B}$$

we have then

$$\phi^{*\prime}>0,\ 0\leqq x<\frac{d}{2}; \quad \phi^{*\prime}\left(\frac{d}{2}\right)=0; \quad \phi^{*\prime}(x)<0,\ \frac{d}{2}<x\leqq d. \tag{7B}$$

A first integration of Eq. (3B) gives

$$[\phi^{*\prime}(x)]^2-[\phi^{*\prime}(0)]^2=\frac{2}{L^{*2}}[\cosh\tilde{\phi}^*(x)-\cosh\tilde{\phi}^*(0)]. \tag{8B}$$

Recalling that the electric field and therefore $\phi^{*\prime}$ must vanish at $x=d/2$, Eq. (8B) gives, for $x=d/2$,

$$[\phi^{*\prime}(0)]^2=\frac{2}{L^{*2}}\left[\cosh\tilde{\phi}^*(0)-\cosh\tilde{\phi}^*\left(\frac{d}{2}\right)\right]. \tag{9B}$$

Substituting the right hand side of Eq. (9B) in Eq. (8B), we find

$$[\phi^{*\prime}(x)]^2=\frac{2}{L^{*2}}\left[\cosh\tilde{\phi}^*(0)-\cosh\tilde{\phi}^*\left(\frac{d}{2}\right)\right]. \tag{10B}$$

Taking the square root of both sides of Eq. (10B) and recalling that $\phi^{*\prime}(x)>0$ in the left half of the membrane [see relation (7B)], we get

$$\phi^{*\prime}(x)=\frac{\sqrt{2}}{L^*}\left[\cosh\tilde{\phi}^*(x)-\cosh\tilde{\phi}^*\left(\frac{d}{2}\right)\right]^{\frac{1}{2}}; \quad 0\leqq x\leqq\frac{d}{2}. \tag{11B}$$

Eq. (11 B) cannot be integrated analytically, unless an approximation is made whose justification and precise statement requires, however, some consideration of the properties of the exact Eq. (11 B), as well as some estimates on the dependence of its integral on the membrane thickness and the solution composition. Let us start by integrating Eq. (11 B) formally across the half of the membrane between 0 and $d/2$:

$$\int_{\tilde{\phi}^*(0)}^{\tilde{\phi}^*(d/2)} \left[\cosh\tilde{\phi}^* - \cosh\tilde{\phi}^*\left(\frac{d}{2}\right)\right]^{-\frac{1}{2}} d\tilde{\phi}^* = \frac{d}{\sqrt{2}\,L^*}. \tag{12B}$$

Eq. (12 B) shows that the integral on the left side vanishes when the ratio d/L^* is made to tend to zero, which can be done either by decreasing the thickness of the membrane or by diluting the outside solution with a consequent increase of L^* [see definition of L^* given by Eq. (41)]. We show now that the vanishing of the integral in (12 B) for $d/L^* < 0$ implies that the two limits of integration $\phi^*(0)$ and $\phi^*(d/2)$ approach indefinitely closely to each other. This can be seen by noting that, since $\tilde{\phi}^*(0) < \tilde{\phi}^*(x) < \tilde{\phi}^*(d/2) < 0$, the following inequalities hold

$$\cosh\tilde{\phi}^*(x) - \cosh\tilde{\phi}^*\left(\frac{d}{2}\right) < \sinh\tilde{\phi}^*(0)\left[\tilde{\phi}^*(x) - \tilde{\phi}^*\left(\frac{d}{2}\right)\right] < \frac{e^y}{2}\left[\tilde{\phi}^*\left(\frac{d}{2}\right) - \tilde{\phi}^*(x)\right]; \tag{13B}$$

$$0 \leqq x \leqq \frac{d}{2}$$

so that

$$\sqrt{2}\, e^{-\frac{y}{2}} \int_{\tilde{\phi}^*(0)}^{\phi^*(d/2)} \left[\tilde{\phi}^*\left(\frac{d}{2}\right) - \tilde{\phi}^*\right]^{-\frac{1}{2}} d\tilde{\phi}^* < \int_{\tilde{\phi}^*(0)}^{\tilde{\phi}^*(d/2)} \left[\cosh\tilde{\phi}^* - \cosh\tilde{\phi}^*\left(\frac{d}{2}\right)\right]^{-\frac{1}{2}} d\tilde{\phi}^* = \frac{d}{\sqrt{2}\,L^*}. \tag{14B}$$

Performing the integration in the first integral we find

$$\left[\tilde{\phi}^*\left(\frac{d}{2}\right) - \tilde{\phi}^*(0)\right]^{\frac{1}{2}} = \left[\phi^*\left(\frac{d}{2}\right) - \phi^*(0)\right]^{\frac{1}{2}} < \frac{e^{y/2}}{4}\,\frac{d}{L^*} \tag{15B}$$

which clearly shows that

$$\lim_{\frac{d}{L^*}\to 0}\left[\phi^*\left(\frac{d}{2}\right) - \phi^*(0)\right] = 0. \tag{16B}$$

It is now necessary to show that not only the difference $\phi^*(d/2) - \phi^*(0)$ but also the individual values of the potential $\phi^*(0)$ and $\phi^*(d/2)$, approach to zero when d/L^* vanishes. Recalling the boundary condition of continuity of the electric displacement vector, from Eq. (11 B), and the derivative of Eq. (1 B) we get at the left boundary ($x = 0$)

$$\frac{K}{1-K^2} = \frac{\sqrt{2}}{4}\cdot\frac{D^*L}{DL^*}\left[\cosh\tilde{\phi}^*(0) - \cosh\tilde{\phi}^*\left(\frac{d}{2}\right)\right]^{\frac{1}{2}}. \tag{17B}$$

Expressing K in terms of $\phi(0)$ by means of Eq. (1 B) and substituting in (17 B) we find

$$\frac{e^{\phi(0)} - 1}{e^{\phi(0)/2}} = \sqrt{2}\,\frac{D^*L}{DL^*}\left[\cosh\tilde{\phi}^*(0) - \cosh\tilde{\phi}^*\left(\frac{d}{2}\right)\right]^{\frac{1}{2}}. \tag{18B}$$

Since the ratio L/L^* is always finite, whereas the square root in the right hand side vanishes according to Eq. (16B), it is apparent that

$$\lim_{\frac{d}{L^*}\to\infty} \phi(0)=0. \tag{19B}$$

From the boundary condition of continuity of the electric potential, expressed at the left boundary as

$$\phi(0)=\phi^*(0) \tag{20B}$$

and from Eqs. (19B) and (16B) we get

$$\lim_{\frac{d}{L^*}\to 0} \phi^*(0)=\lim_{\frac{d}{L^*}\to 0} \phi^*\left(\frac{d}{2}\right)=0. \tag{21B}$$

The result expressed by relation (21B) proves that, either by reducing the thickness of the membrane or by diluting the outside solutions (which can be done without altering the value of Y), we can, in principle, get the potential as close to zero as we want. Therefore, since the relation

$$e^Y \gg 1 \tag{22B}$$

must hold as a consequence of assumption (vi), there is certainly a range for the values of the parameter d/L^* in which the following approximation can be used to integrate Eq. (11B)

$$\cosh \tilde{\phi}^*(x)=\frac{e^{\phi^*(x)-Y}+e^{-\phi^*(x)+Y}}{2}\simeq\frac{e^{-\phi^*(x)+Y}}{2} \tag{23B}$$

and analogously for $\cosh \tilde{\phi}^*(d/2)$.

Using Eq. (23B), Eq. (11B) becomes

$$[e^{-\phi^*}-e^{-\phi^*(d/2)}]^{-\frac{1}{2}}\, d\phi^*=\frac{e^Y}{L^*}\, dx \tag{24B}$$

or, recalling the definitions of Y and L^*, Eqs. (42) and (41),

$$[e^{-\phi^*}-e^{-\phi^*(d/2)}]^{-\frac{1}{2}}\, d\phi^*=\left(\frac{8\pi F^2 A}{RTD^*}\right)^{\frac{1}{2}} dx. \tag{25B}$$

Under the conditions that ϕ^* attains its maximum value at $x=d/2$, the integration of Eq. (25B) gives

$$\phi^*(x)=\ln\left\{\frac{2\pi F^2 d^2 A}{RTD^*\lambda^2}\cos^2\left[\frac{\lambda}{d}\left(\frac{d}{2}-x\right)\right]\right\};\qquad 0\leqq x\leqq d. \tag{26B}$$

Eq. (26B), which contains the as yet undetermined parameter λ, has been used to evaluate the concentration profiles as well as the integral conductance, given in Eqs. (56) and (57).

We can now show also that, in the same range of the values of d/L^* in which $\phi^*(x)\ll Y$, so that the approximate Eq. (25B) can be used instead of (11B), the concentrations in the membrane of the anions X^- are negligible with respect to those of the complexed cations IS^+:

Using Eq. (36), the ratio of the total concentrations of the IS^+ species to that of the anions is given by

$$\frac{\sum_{i=1}^{n} C_{is}^*(x)}{\sum_{x=1}^{m} C_x^*(x)}=\frac{\sum_{i=1}^{n} k_{is}\, C_{is}}{\sum_{x=1}^{m} k_x\, C_x}\, e^{-2\phi^*(x)}. \tag{27B}$$

By use of the definition of Y, Eq. (44), and recalling that $k_i a_i \ll k_{is} C_{is}$ (assumption (vi)) Eq. (27 B) gives directly the desired proof,

$$\frac{\sum_{i=1}^{n} C_{is}^*(x)}{\sum_{i=1}^{n} C_x^*(x)} \simeq e^{2[Y-\phi^*(x)]} \simeq e^{2Y} \gg 1. \tag{28 B}$$

So far we have shown that, given that $e^y \gg 1$, there is certainly a range of the aqueous ionic concentrations in which the condition

$$\phi^*(x) \leqq \phi^*\left(\frac{d}{2}\right) \ll Y \tag{29 B}$$

is satisfied, so that the approximation (23 B) used in the derivation of Eqs. (24 B) through (28 B) is valid. To define now this range in terms of a relationship between the thickness of the membrane, the aqueous composition, and the other parameters of the system, we proceed as follows. Recalling that $\phi(0) > 0$, and replacing 1 with $e^{\phi(0)/2}$ in the left hand side of Eq. (18 B), we find after straightforward manipulations

$$\phi(0) \leqq 2 \ln \left\{1 + \sqrt{2}\, \frac{D^* L}{D L^*} \left[\cosh \tilde{\phi}^*(0) - \cosh \tilde{\phi}^*\left(\frac{d}{2}\right)\right]^{\frac{1}{2}}\right\}. \tag{30 B}$$

Inserting Eq. (15 B) in Eq. (13 B) we get

$$\left[\cosh \tilde{\phi}^*(0) - \cosh \tilde{\phi}^*\left(\frac{d}{2}\right)\right]^{\frac{1}{2}} < \frac{1}{4\sqrt{2}}\, e^Y \frac{d}{L^*} \tag{31 B}$$

so that (30 B), combined with (31 B) leads to

$$\phi(0) \leqq 2 \ln \left[1 + \frac{1}{4} \frac{D^* L d}{D L^{*2}}\, e^Y\right]. \tag{32 B}$$

From (32 B) and (15 B) we finally get

$$\phi^*\left(\frac{d}{2}\right) \leqq 2 \ln \left[1 + \frac{1}{4} \frac{D^* L d}{D L^{*2}}\, e^Y\right] + \frac{1}{16}\, e^Y \frac{d^2}{L^{*2}} \tag{33 B}$$

or, recalling that $\ln(1+x) < x$

$$\phi^*\left(\frac{d}{2}\right) \leqq \frac{1}{2} \frac{D^* L d}{D L^{*2}}\, e^Y + \frac{1}{16} \frac{d^2}{L^{*2}}\, e^Y. \tag{34 B}$$

Comparing Eqs. (19 B) and (34 B), it can be easily seen that condition (29 B), which is of central importance for the validity of all other results, is certainly satisfied if the relation

$$d^2 + 8 \frac{D^*}{D} L d < 16\, Y e^{-Y} L^{*2} \tag{35 B}$$

or, equivalently,

$$d < \frac{4 D^*}{D} L \left\{\left[1 + Y e^{-Y} \left(\frac{L^* D}{L D^*}\right)^2\right]^{\frac{1}{2}} - 1\right\} \tag{36 B}$$

is satisfied. Note that when the bracketed term is bigger than one, relation (36B) reduces approximately to

$$d < \left(\frac{2RTD^* Y}{\pi F^2 A}\right)^{\frac{1}{2}} \tag{37B}$$

where A and Y have been defined in Eqs. (42) and (44), respectively.

Appendix C

Determination of the Parameter λ from the Boundary Condition

From the continuity of the electric potential and the electric displacement vector at the left interface we have

$$\phi(0) = \phi^*(0) \tag{1C}$$

and

$$D \frac{d\phi(0)}{dx} = D^* \frac{d\phi^*(0)}{dx}. \tag{2C}$$

Expressing $\phi(x)$ and $\phi^*(x)$ by means of Eqs. (1B) and (26B), relations (1C) and (2C) become

$$\frac{1+K}{1-K} = \left(\frac{2\pi F^2 d^2 A}{RTD^*}\right)^{\frac{1}{2}} \frac{\cos\frac{\lambda}{2}}{\lambda} \tag{3C}$$

and

$$\frac{K}{1-K^2} = \frac{D^* L}{2Dd} \operatorname{tg}\frac{\lambda}{2} \tag{4C}$$

respectively. Eliminating K between (3C) and (4C), and recalling the definitions of L, Eq. (35), we find

$$\frac{2\pi F^2 d^2 A}{RTD^*} \cdot \frac{\cos^2\frac{\lambda}{2}}{\lambda^2} = 1 + \left(\frac{D^* A}{D \cdot \sum_{x=1}^{m} C_x}\right)^{\frac{1}{2}} \sin\frac{\lambda}{2}. \tag{5C}$$

Although this equation cannot be explicitly solved with respect to λ, an approximate value of λ can be found when the aqueous solutions are sufficiently dilute that the condition

$$\frac{2\pi F^2 d^2 A}{RTD^*} \ll 1 \tag{6C}$$

is satisfied. Since the right hand side of Eq. (5C) is bigger than unity, $\left(0 \leqq \frac{\lambda}{2} < \frac{\pi}{2}\right)$ condition (6C) implies

$$1 \ll \frac{\cos^2\frac{\lambda}{2}}{\lambda^2} < \frac{1}{\lambda^2} \tag{7C}$$

so that

$$\lambda \ll 1 \tag{8C}$$

and Eq. (5C) reduces approximately to

$$\lambda^2 \simeq \frac{2\pi F^2 d^2 A}{RTD^*}. \tag{9C}$$

Substituting Eq. (9C) in Eq. (55) and recalling that because of (8C), $\sin\lambda/\lambda \simeq 1$, we obtain directly the approximate expression of the conductance given by Eq. (56).

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