J. Membrane Biol. 24, 1–34 (1975)

Effects of Unstirred Layers on the Steady-State Zero-Current Conductance of Bilayer Membranes Mediated by Neutral Carriers of Ions

S. Ciani, F. Gambale, A. Gliozzi and R. Rolandi

C.N.R. Laboratory of Cybernetics and Biophysics, Camogli, Institute of Physics of the University of Genova, Italy and Dept. of Physiology and Brain Research Institute, UCLA, Center for the Health Sciences, Los Angeles, California 90024

Received 10 March 1975; revised 19 May 1975

Summary. Some effects of diffusion polarization and chemical reactions on the steady-state zero-current conductance of lipid bilayers mediated by neutral carriers of ions have been studied theoretically and experimentally. Assuming that ion permeation across the interfaces occurs via a heterogeneous reaction between ions in the solution and carriers in the membrane, the relationship between the conductance and the aqueous concentration of carriers is shown to be linear only in a limited range of sufficiently low concentrations. At higher carrier concentrations, which for the most strongly bound cations are within the range of the experimentally accessible values, the conductance is expected to become limited by diffusion of the carried ion in the unstirred layers and therefore reach an upper limiting value independent of the membrane properties. This expectation has been successfully verified for glyceryl-monooleate membranes in the presence of the ions K^+, Rb^+ and NH_4^+ and carriers such as valinomycin and trinactin. The experimental results support, at least for the present system, the generally accepted view that complexation between ions and the macrocyclic antibiotics occurs at the membrane surface; it is shown, in fact, that for a different mechanism, such as that by which the complexes would form in the aqueous solutions and cross the interfaces as lipid-soluble ions, the same type of saturation would be expected to be observable only for unrealistically high values of the rate constants of the ion-carrier association. A previously proposed criterion to distinguish between these two mechanisms, based on the dependence of the conductance on the ion concentration, is discussed from the viewpoint of this more comprehensive model.

In this paper we discuss some effects of the aqueous unstirred layers on the steady-state zero-current conductance of thin lipid membranes induced by neutral carriers of ions, such as the macrocyclic antibiotics valinomycin and trinactin. Theoretical and experimental studies on carrier-mediated membrane properties, encompassing the effects of aqueous diffusion polarization, have been presented previously by LeBlanc (1971), Neumcke (1971*b*) and Hladky (1972, 1973). The first two authors concentrated on charged ion-complexing molecules, such as some uncouplers of

oxidative phosphorylation, while Hladky (1972) presented a theory in a generalized formalism, applicable to both charged and neutral carriers. The analysis given here, although restricted to the zero-current conductance for membranes between identical solutions, is more general than the previous ones in that it relaxes the assumption of local equilibrium of the ion-carrier complexation reaction in the solutions and considers explicitly the diffusion of the ions in the unstirred layers, thus allowing for an effect which is essential for a satisfactory understanding of the behavior of the steady-state conductance. We will show theoretically that, if the diffusion of the permeant ion in the unstirred layers is considered and if the mechanism of ion permeation across the interfaces is a heterogeneous reaction between ions from the solutions and antibiotics from the membrane [(R_{is}) mechanism], the membrane conductance as a function of carrier concentration is expected first to increase linearly, then bend, and finally reach a saturation value dependent solely on the properties of the aqueous unstirred layers. These expectations have been tested and verified successfully for glyceryl-monooleate membranes in the presence of the ions potassium, rubidium and ammonium, and carriers such as valinomycin and trinactin. Not only is the experimental behavior of the conductance qualitatively similar to that predicted theoretically, but also the thickness of the unstirred layers, as deduced from the analysis of the conductance, is consistent with that measured by an independent and more direct method.

The fact that the conductance reaches an upper value at high concentrations of carriers is not surprising and is intuitively expected if one considers that increasing the concentration of carriers decreases the membrane resistance, so that the current becomes eventually limited by diffusion of the ions in the unstirred layers. Nevertheless, it is interesting to ask whether the possibility of observing experimentally this limiting conductance depends on the particular nature of the mechanism of ion permeation across the interfaces; more precisely, is it expected to be observable only when the mechanism of permeation is the heterogeneous reaction [(R_{is}) mechanism], or would it be so also if the complexes formed in the solution and crossed the interfaces as lipophylic ions [(P_{is}) mechanism]? It will be shown in the Discussion section that, if (P_{is}) were the actual mechanism in our system, such a saturation would be expected to be detectable only for chemical rate constants higher than those of diffusion-limited reactions, and therefore physically unreal. Analogous conclusions as to the plausibility of neglecting the contribution of the (P_{is}) mechanism were reached previously by Stark and Benz (1971) from considerations on the limiting current rather than the zero-current conductance. This result seems interesting

to us, because it can be used in appropriate cases to infer the nature of the mechanism of interfacial permeation, and because it provides support to the currently prevailing opinion that a heterogeneous reaction between ions and carriers is occurring indeed in the case of valinomycin and trinactin (Stark & Benz, 1971; Stark, Ketterer, Benz & Läuger, 1971; Ciani, Eisenman, Laprade & Szabo, 1973*a*; Hladky, Gordon & Haydon, 1974).

In previous papers, such as those just mentioned as well as in others (Läuger & Stark, 1970; Ciani, Laprade, Eisenman & Szabo, 1973*b*), a criterion for identifying the nature of the mechanism of interfacial permeation was deduced from theoretical models which neglected the effects of unstirred layers, and was based on a property of the dependence of the conductance on the concentration of ions rather than that of the carriers; more precisely, a deviation from linearity of the conductance at high concentrations of the permeant ions, not explainable in terms of aqueous association, was considered to be indicative of the presence of the interfacial reaction. However, Hladky (1972) has pointed out that the presence of unstirred layers makes it difficult to discriminate between the two mechanisms (P_{is}) and (R_{is}) on this basis. We have come to the same conclusion; it will be shown, in fact, that when the effects of diffusion and chemical reactions in the unstirred layers are taken into account, the same type of saturation, previously considered a unique characteristic of the interfacial reaction (R_{is}), may be expected also in the case of the (P_{is}) mechanism, and therefore is not a sufficiently discriminative criterion per se. The independent criterion presented in the Discussion section of this paper and based on the study of the dependence of the conductance on the concentration of carriers is probably more reliable since it was deduced from a more comprehensive model.

Description of the Model

The model assumes that the ion-carrier complexes are the only charged species present in the membrane. If J_{is}^{*}[1] denotes the net flux of complexes across the membrane, the total current density I is

$$I = zFJ_{is}^{*} \tag{1}$$

where F is the Faraday and z the valency of the complex.

Assuming that ion-permeation across the interfaces is due to a heterogeneous reaction between ions from the solutions and carriers from the membrane, rather than to partitioning of the complexes formed in solution,

1 We will use an asterisk to denote all the quantities pertaining to the membrane phase.

we will deduce here the results for the former mechanism and will defer the analysis of the latter to the Discussion. Two such mechanisms are represented schematically in Fig. 1 *a* and *b*, and will be briefly referred to as (R_{is}) and (P_{is}), respectively.

For a clearer description of the model and the assumptions underlying it, we consider separately the equations of transport in the unstirred layers, across the interfaces and in the membrane interior.

The Unstirred Layers

The concept of "aqueous unstirred layer" (*see* Vetter, 1967) is a useful approximation to account for the fact that in nonequilibrium situations, such as when current is passed through the membrane, the convective movement caused by stirring of the solutions is never effective enough to equalize the aqueous concentrations up to the interfaces, but always leaves unaffected a region adjacent to the membrane. Even though the transition between the bulk stirred solution and this static region must be gradual, the simplest way to describe its effect is to assume the existence of a convection-free aqueous layer of a given thickness, where the ionic fluxes are governed by electrodiffusion. We postulate, however, that in our case the applied potential drops entirely within the low dielectric interior of the membrane, so that the transport in the unstirred layers is determined by diffusion alone. Considering unidirectional fluxes perpendicular to the membrane and assuming ideal behavior in the aqueous phases for both the carriers *s* and the complexes *is* as well as constant activity coefficient for the permeant ion *i*[2], the flux equations for these three species will be

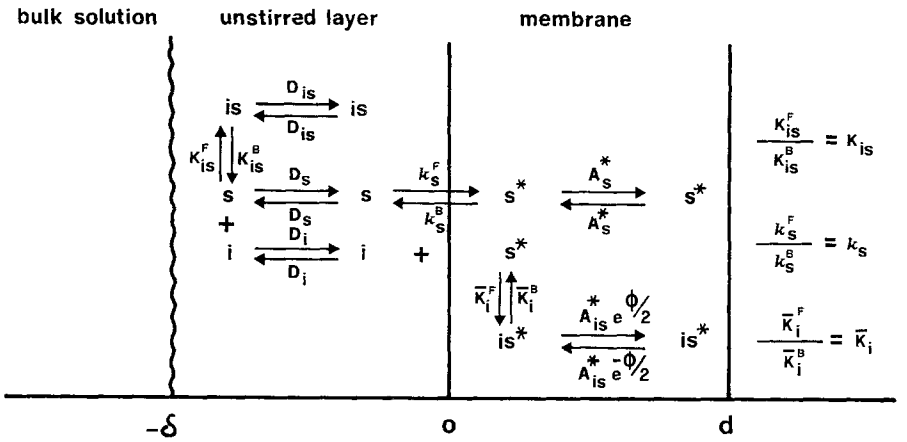
$$J_i = -D_i \frac{dC_i}{dx} \qquad -\delta \leqq x \leqq 0;\ d \leqq x \leqq d+\delta \tag{2}$$

$$J_s = -D_s \frac{dC_s}{dx} \qquad -\delta \leqq x \leqq 0;\ d \leqq x \leqq d+\delta \tag{3}$$

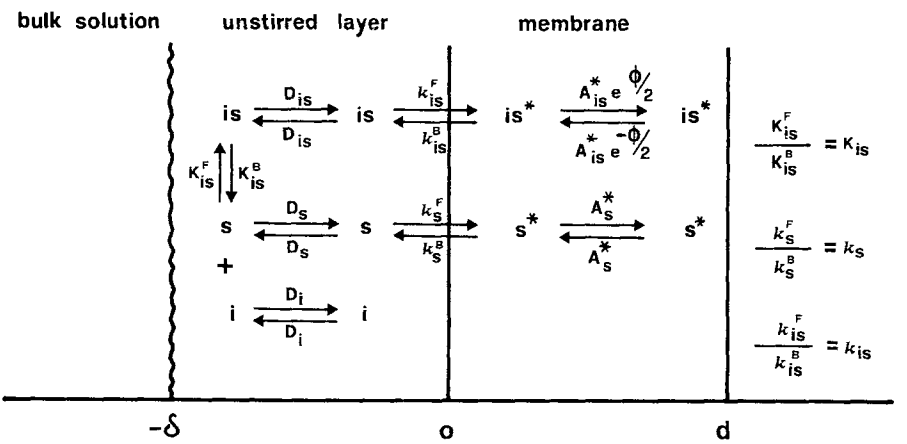
$$J_{is} = -D_{is} \frac{dC_{is}}{dx} \qquad -\delta \leqq x \leqq 0;\ d \leqq x \leqq d+\delta. \tag{4}$$

0 and d are the co-coordinates of the interfaces, and δ is the thickness of the unstirred layers. Since the three species *i*, *s* and *is* are linked by the chemical reaction of complex formation and dissociation, the three fluxes

2 This is a plausible approximation if the ionic strength in the aqueous layer is kept constant by addition of a sufficiently high concentration of an impermeant electrolyte (e.g. LiCl).



(a)



(b)

Fig. 1. Model for the carrier-mediated ionic transport. (*a*) Refers to the "heterogeneous reaction" mechanism of interfacial ion permeation (R_{is}); (*b*) to the "partitioning" of the complexes formed in the aqueous solution (P_{is})

J_i, J_s and J_{is} will be, in general, functions of x; if $K_{is}^F \gamma_i^{\pm} c_i(x) c_s(x)$ denotes the rate of formation and $K_{is}^B c_{is}(x)$ the rate of dissociation of the complexes in moles per unit time and volume, the mass conservation equations give at steady-state

$$-\frac{dJ_i}{dx} = -\frac{dJ_s}{dx} = \frac{dJ_{is}}{dx} = K_{is}^F \gamma_i^{\pm} c_i(x) c_s(x) - K_{is}^B c_{is}(x) \quad \begin{matrix} -\delta \leqq x \leqq 0 \\ \delta \leqq x \leqq d+\delta \end{matrix} . \tag{5}$$

K_{is}^F and K_{is}^B are the rate constants of formation and dissociation of the complexes in the aqueous phase and $\gamma_i^{\pm}$ is the activity coefficient of the ion, assumed to be equal to the mean activity coefficient of the electrolyte. Combining Eq. (5) with the three flux Eqs. (2), (3) and (4) we obtain the following nonlinear differential equations in the variables $c_i(x)$, $c_s(x)$ and $c_{is}(x)$:

$$\begin{aligned} D_i \frac{d^2 c_i}{dx^2} &= D_s \frac{d^2 c_s}{dx^2} = -D_{is} \frac{d^2 c_{is}}{dx^2} \\ &= K_{is}^F \gamma_i^{\pm} c_i(x) c_s(x) - K_{is}^B c_{is}(x) \quad \begin{matrix} -\delta \leqq x \leqq 0 \\ d \leqq x \leqq d+\delta \end{matrix} . \end{aligned} \tag{6}$$

For small displacements from equilibrium, such as when low currents are passed through the membrane, these equations can be linearized and integrated analytically according to a procedure described in Appendix B.

At equilibrium ($J_i = J_s = J_{is} = 0$), Eq. (5) gives

$$\left[\frac{c_{is}}{a_i c_s}\right]_{eq} = \frac{K_{is}^F}{K_{is}^B} = K_{is} \tag{7}$$

where the activity a_i is equal to $\gamma_i^{\pm} c_i$ and K_{is} is the equilibrium constant of the association reaction. Since the total concentration of carriers

$$c_s^T = c_s + c_{is} \tag{8}$$

is a known variable, rather than c_s and c_{is}, it is convenient to express these last two quantities in terms of c_s^T and a_i. From Eqs. (7) and (8) we deduce easily

$$c_s = \frac{c_s^T}{1 + K_{is} a_i}; \qquad c_{is} = \frac{K_{is} c_s^T a_i}{1 + K_{is} a_i}. \tag{9}$$

The Membrane-Solution Interfaces

Consistently with Fig. 1*a*, which represents the (R_{is}) mechanism, two processes are postulated to occur at the membrane-solution interfaces:

1) The "partitioning of the neutral carrier", by which the carrier molecules are exchanged between the aqueous and the membrane phase. Using the formalism of "chemical kinetics", the rightward flux of neutral carriers across the left interface will be given by

$$J_s^{\text{int}}(0) = k_s^F c_s(0) - k_s^B c_s^*(0). \tag{10}$$

$c_s(0)$ and $c_s^*(0)$ are the interfacial concentrations of neutral carriers outside and inside the membrane, respectively; the rate constants, k_s^F and k_s^B, are related to the partition coefficient k_s by

$$\left[\frac{c_s^*(0)}{c_s(0)}\right]_{\text{eq}} = \frac{k_s^F}{k_s^B} = k_s. \tag{11}$$

2) The "heterogeneous reaction" of complex formation, by which an ion from the solution reacts with a carrier from the membrane to form a charged ion-carrier complex in the membrane. Denoting the rate constants of association and dissociation by $\bar{K}_i^F$ and $\bar{K}_i^B$, respectively, the rate of complex formation at the left interface will be given by

$$\frac{d\lambda(0)}{dt} = \bar{K}_i^F \gamma_i^{\pm} c_i(0) c_s^*(0) - \bar{K}_i^B c_{is}^*(0) \tag{12}$$

where $d\lambda(0)/dt$ is meant to be expressed in moles per unit time and surface. At equilibrium

$$\left[\frac{c_{is}^*(0)}{c_i(0)\, c_s^*(0)}\right]_{\text{eq}} = \gamma_i^{\pm} \frac{\bar{K}_i^F}{\bar{K}_i^B} = \gamma_i^{\pm} \bar{K}_i. \tag{13}$$

Note that the rate of the interfacial reaction given by Eq. (12) coincides with the net flux of ions i across the left interface $J_i^{\text{int}}(0)$, and is related to the current density by

$$\frac{d\lambda(0)}{dt} = J_i^{\text{int}}(0) = \frac{I}{zF}. \tag{14}$$

The Membrane Phase

It has been shown previously (Haydon & Hladky, 1972; Hladky, 1972; Ciani *et al.*, 1973*b*) that the formal expression for the zero-current conductance in the constant field approximation is by and large independent of the particular equation of transport used to describe the transmembrane fluxes[3]. For example, two alternative approaches, such as those

3 This would probably not be true if we used the formalism of the irreversible thermodynamics to allow for the interaction between the fluxes of the neutral carriers and of the charged complexes.

based on the Nernst-Planck equation or the Eyring absolute rate reaction theory, and corresponding to the cases in which the membrane is viewed either as a flat or a sharp energy barrier, lead to entirely equivalent results. We will use the Eyring formalism, because it is the least cumbersome, and will also schematize the membrane as a single activation energy barrier for both the neutral carriers and the charged complexes.

The fluxes of the carrier and the complexes will then be described by the two following equations

$$J_s^* = A_s^* [c_s^*(0) - c_s^*(d)] \tag{15}$$

and

$$J_{is}^* = A_{is}^* [e^{\phi/2}\, c_{is}^*(0) - e^{-\phi/2}\, c_{is}^*(d)] \tag{16}$$

where ϕ is the transmembrane potential in RT/zF units,

$$\phi = \frac{zF}{RT} V \tag{17}$$

and A_s^*, A_{is}^* are the rate constants of crossing the interior of the membrane.

Theoretical Results

A general expression for the zero-current conductance, independent of the particular mechanism of permeation across the interface, is deduced in Appendix A and is given by

$$G_{oi} = \frac{z^2 F^2}{RT} \frac{A_{is}^*\, c_{is}^*(\mathrm{eq})}{1 - 2zF \Delta_{is}^*[0]\, A_{is}^*}. \tag{18}$$

$c_{is}^*(\mathrm{eq})$ is the equilibrium concentration of complexes at either interface and $\Delta_{is}^*[0]$ represents the deviation from equilibrium, caused by the unit current density, of the concentration of complexes at the left interface, namely

$$\Delta_{is}^*[0] = \lim_{I \to 0} \frac{c_{is}^*(0) - c_{is}^*(\mathrm{eq})}{I} = \left[\frac{d\, c_{is}^*(0)}{dI}\right]_{I=0}. \tag{19}$$

The explicit value of $\Delta_{is}^*[0]$ depends on the mechanism of interfacial permeation, as well as on the processes in the unstirred layers. For the (R_{is}) mechanism the expression is calculated in Appendix B; the general result is complicated, but can be approximated in most practical cases by

$$\Delta_{is}^*[0] = -\frac{1}{zF} \left\{ \frac{1}{\bar{K}_i^B} + \frac{\bar{K}_i \gamma_i^{\pm}}{2A_s^*} c_i + \frac{\bar{K}_i \gamma_i^{\pm} k_s \delta}{D_i} c_s \right\}. \tag{20}$$

Substituting Eq. (20) in Eq. (18) and expressing $c_{is}^{*}(\text{eq})$ in terms of c_s and c_i by use of Eqs. (13) and (11), we find

$$G_{oi}=\frac{z^2 F^2}{RT}\cdot\frac{k_s L_i c_i c_s}{1+N_i c_i+P_i c_s} \tag{21}$$

where

$$L_i=\frac{\bar{K}_i \gamma_i^{\pm} A_{is}^{*}}{1+2A_{is}^{*}/\bar{K}_i^{B}} \tag{22}$$

$$N_i=\frac{L_i}{A_s^{*}} \tag{23}$$

and

$$P_i=\frac{2k_s L_i \delta}{D_i}. \tag{24}$$

At concentrations of carriers sufficiently high that $1+N_i c_i \ll P_i c_s$

$$G_{oi}^{\text{sat}}\simeq\frac{z^2 F^2}{RT}\frac{k_s L_i}{P_i}c_i=\frac{z^2 F^2}{RT}\cdot\frac{D_i}{2\delta}c_i. \tag{25}$$

Eq. (21) reduces to previously derived expressions for the conductance (Markin *et al.*, 1969; Läuger *et al.*, 1970; Ciani *et al.*, 1973*a*, *b*) when

$$P_i c_s \ll 1+N_i c_i. \tag{26}$$

It will be shown, however, that for the most permeant ions and for sufficiently high concentrations of the carrier, relationship (26) is not satisfied, so that the term $P_i c_s$ must be taken into account in the description of the steady-state conductance. Eq. (24) shows that, when D_i is known, the ratio between the experimentally measurable parameters P_i and $k_s L_i$ gives the thickness of the stagnant layer δ. We will calculate this ratio and will compare it with the value of δ measured by an independent method.

Experimental Results

Materials and Methods

Glyceryl-monooleate purchased from Sigma (St. Louis, Missouri) was diluted in *n*-decane to form a solution of 2.5 % by weight. Bilayer membranes were formed across a 1-mm^2 hole in a teflon chamber. Valinomycin was purchased from Calbiochem (Los Angeles, California), while trinactin was a generous gift from Hans Bickel of CIBA.

A Keithley electrometer (Model 602) and a Hewlett Packard strip-chart recorder were used to measure the transmembrane current following a procedure analogous to that described by Szabo, Eisenman and Ciani (1969). The electrodes were chlorided silver plates of about

5 cm^2 area. Stirring of the solutions at the rate of 225 rpm was obtained by coupling two teflon-covered magnets (1 cm long) with an external rotating magnetic field.

The measurements of the membrane conductance as a function of carrier concentration were performed adding small amounts of a concentrated ethanolic solution of the antibiotic on both sides; in most cases the entire conductance-concentration curve could be deduced from measurements on the same membrane. The diameter of the membrane was measured and controlled continuously during the experiment with a micrometric ocular. To avoid possible ionic strength effects on the conductance, as well as to maintain the resistance of the solutions far below that of the membrane, the total ion concentration was raised to 1 M by adding appropriate amounts of the highly impermeant electrolyte LiCl, except in the case when NH_4^+ was used as the transported ion.

For the measurements of the time-dependent current decay following a voltage step, a different teflon chamber was used with the two sides of the partition converging to the hole with identical inclination. The time course of the current was recorded on a Varian 2100 XY recorder, using a sweep rate of 1 cm/sec. To increase the unstirred layer thickness, so as to provide better conditions for the analysis of the time dependence of the current, the stirring rate was reduced to 50 rpm.

In order to gain independent information on the range of solubility of valinomycin in the aqueous solutions, optical density (OD) measurements were made with a Zeiss PMQ II spectrophotometer.

The optical density of ionic solutions containing various amounts of valinomycin was determined by comparison with that of the corresponding valinomycin-free solutions. The ionic composition of the solutions was the same as that used for the conductance measurements. The concentration of valinomycin was increased by adding small amounts of a stock ethanolic solution (10^{-4} M). Ethanol for UV spectrophotometry purchased from Carlo Erba (Milan, Italy) was employed. In each measurement the same amount of ethanol was added also to the reference cell. After addition of valinomycin the solutions were vigorously shaken. OD was determined before and after centrifugation at 4500 rpm ($\simeq 2500 \times g$). All the experiments were carried out at the wavelength of 220 nm.

Steady-State Zero-Current Conductance as a Function of Carrier Concentration

The steady-state conductance in the ohmic region was measured applying low voltages ($V < 10$ mV) to membranes formed across a hole 1 mm in diameter[4] and interposed between solutions of identical composition. The principal features of the relation between the conductance G_{oi} and

4 Benz *et al.* (Benz, Stark, Janko & Läuger, 1973) report that for membranes of such a small area the specific conductance in the presence of valinomycin increases with the diameter, probably because of the exchange of neutral carriers between the membrane and the surrounding torus. In our experiments the membrane area, even though quite small (0.8 mm), did not vary from one experiment to the other by more than 15 %, and we never found inconsistent values for the steady-state specific conductance. Nevertheless, the presence of a flux of carriers between the membrane and the torus might very well seem to invalidate the results of the theoretical model which assumes rigorous equilibrium at zero current. In Appendix C we show, however, that a steady flux of neutral carriers from the membrane to the torus modifies the expression of the conductance only to the extent of requiring the partition coefficient k_s to be replaced by a slightly more complex combination of parameters, but has no influence whatsoever on the validity of Eqs. (21–26).

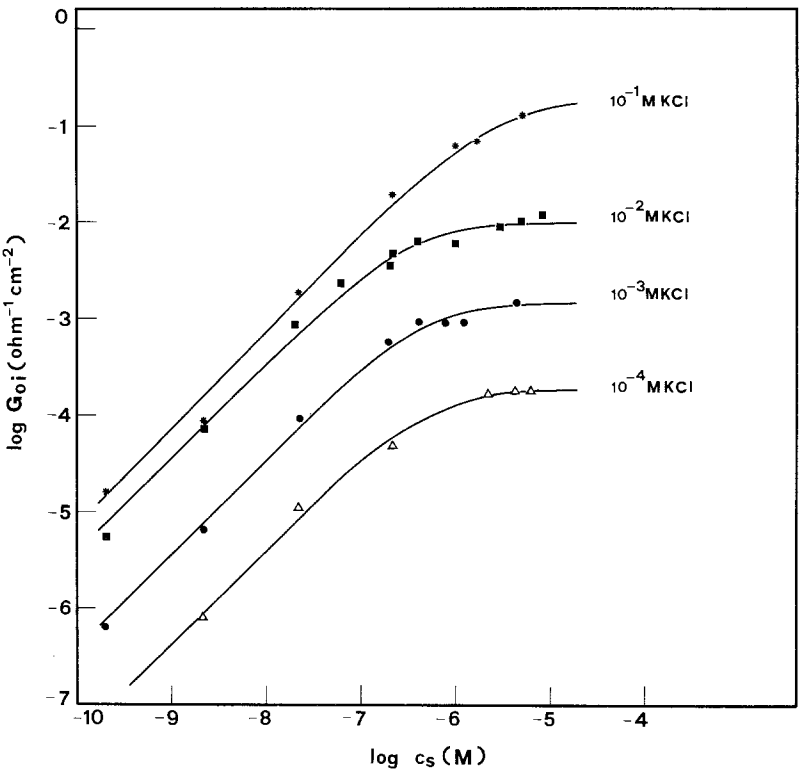


Fig. 2. Membrane conductance G_{oi} as a function of valinomycin concentration, in a log-log scale, for the indicated concentration of KCl. The ionic strength was held constant (1 M) by addition of LiCl. $T = 20$ °C

the concentration of carriers c_s can be observed already in Fig. 2, where the four series of experimental points refer to measurements carried out at different concentrations of KCl. For low values of c_s the plot of log G_{oi} *vs.* log c_s is a straight line with slope of one; at higher values (10^{-7} M), however, a bending approaching to saturation can be observed. The slope of unity in the initial part of the diagram implies a linear dependence of G_{oi} on c_s, in agreement with data reported previously by several authors (*see*, e.g., Gotlieb, Buzhinsky & Lev, 1968). However, the saturation of the conductance which is seen at higher concentrations when the system is in a true stationary state[5] has not, to our knowledge, been carefully analyzed

5 Our steady-state measurements were taken after waiting at least 10 min after addition of the antibiotic to the continuously stirred aqueous solutions. In the course of each experiment the potential applied to the membrane was periodically inverted. After these sudden changes, the behavior of the current was characterized by an initial peak, decreasing slowly to a steady-state level; such steady-state was used to calculate the conductance. If one plots the conductance as calculated from the peak values, an almost linear relation between G_{oi} and c_s is found at least up to 5×10^{-6} M. Fig. 3 illustrates this feature comparing the peak conductance with the corresponding steady-state values for 10^{-2} M KCl.

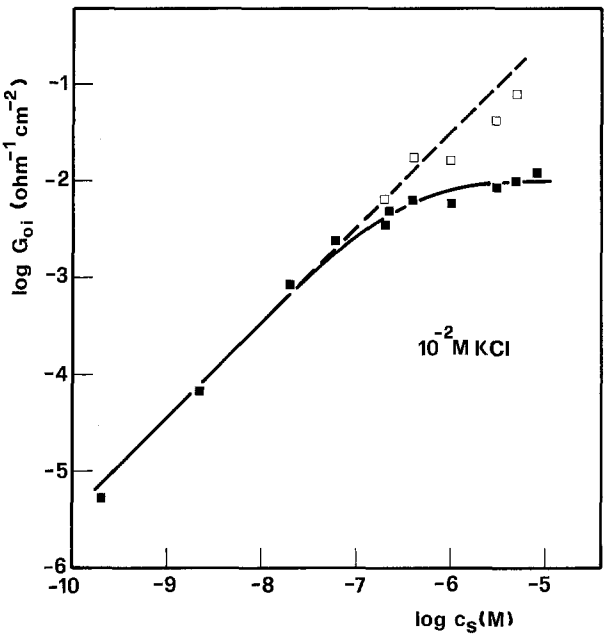


Fig. 3. The steady-state conductance (■) compared with the corresponding conductance (□) measured at $t=0$ after application of a voltage step. KCl $=10^{-2}$ M and ionic strength $=1$ M by addition of LiCl

before. Such behavior can be interpreted as due to unstirred layers and is clearly expected from Eq. (21) when the term $P_i\, c_s$ is comparable or greater than $1+N_i\, c_i$. Consistently with Eq. (25), the limiting values for the conductance shown in Fig. 2 are proportional to the concentration c_i of the transported ion.

Table 1 gives the values of the three parameters $k_s L_i$, N_i and $P_i/2$ extracted from the best fitting of the experimental points of Fig. 2 with Eq. (21). In the same Table are given also the values for the thickness of the unstirred layers, as deduced from $P_i/k_s L_i$ and using 1.75×10^{-5} cm^2/sec

Table 1. Values of the parameters and of the unstirred layer thickness, δ, deduced from the best fitting of the experimental points of Fig. 2 with Eq. (21)

c_i (M)	log G_{oi} (Ω^{-1} cm^{-2}) $c_s \to \infty$	$N_i \times 10^2$ m^3/moles	$(k_s L_i) \times 10^2$ m^4/sec moles	$\left(k_s L_i \frac{\delta}{D_i}\right) \times 10^{-3}$ m^3/moles	δ(m) $\times 10^4$
10^{-1}	−0.7	4.2±0.9	1 ±0.1	1 ±0.4	2 ±1
10^{-2}	−2	4 ±1	1.3±0.1	2.5±0.3	3.3±0.6
10^{-3}	−2.8		1 ±0.1	1.3±0.2	2.3±0.5
10^{-4}	−3.7		1.1±0.1	1.1±0.2	1.8±0.4

δ has been calculated taking $D_i = 1.75 \times 10^{-9}$ m^2/sec. The carrier was valinomycin.

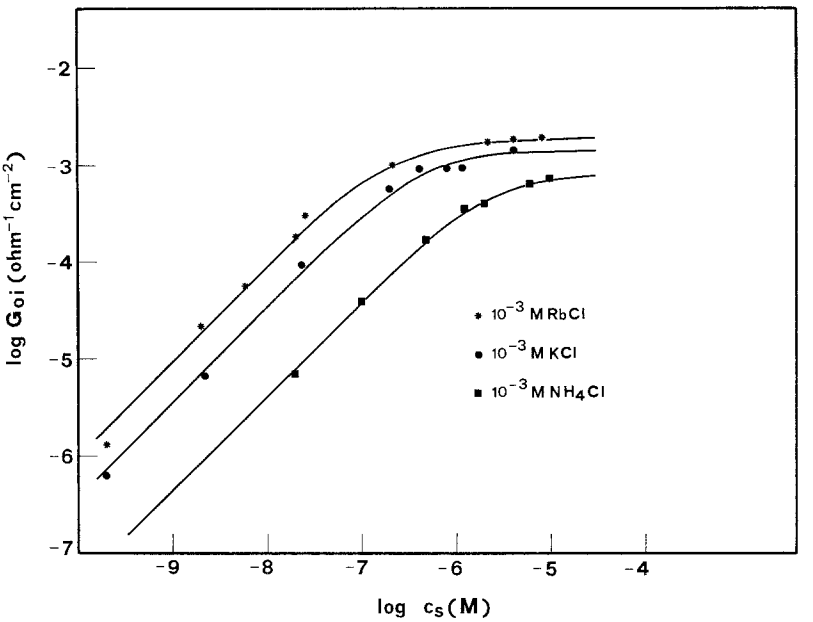


Fig. 4. Membrane conductance as a function of valinomycin concentration, in a log-log scale, for various 10^{-3} M salt solutions. For the RbCl and KCl curves the ionic strength was raised to 1 M by addition of LiCl. $T=24$ °C in the RbCl and NH_4Cl experiments

for the aqueous diffusion coefficient of potassium. We will see that these numbers are in agreement with those obtained independently by a method to be discussed later.

Note that the two curves of Fig. 2 corresponding to the KCl concentrations of 10^{-2} and 10^{-1} M start bending at higher concentrations than the first two. This is consistent with the fact that deviation from linearity is expected when

$$c_s \simeq \frac{1+N_i c_i}{P_i} \tag{27}$$

and that, for the value of N_i given in Table 1, $N_i c_i$ becomes of the order of unity at $c_i \simeq 10^{-2}$ M.

Fig. 4 compares the behavior of the membrane conductance as a function of valinomycin concentration for the three ions Rb^+, K^+ and NH_4^+. The relevant feature of this set of curves, obtained for equal ionic concentrations, is the convergence to approximately the same saturation value, despite the different conductances in the linear region. This result is consistent with Eq. (25), since the aqueous diffusion coefficients of the three ions are about the same and also the thickness of the stagnant layers is expected to be independent of the particular ionic species dissolved.

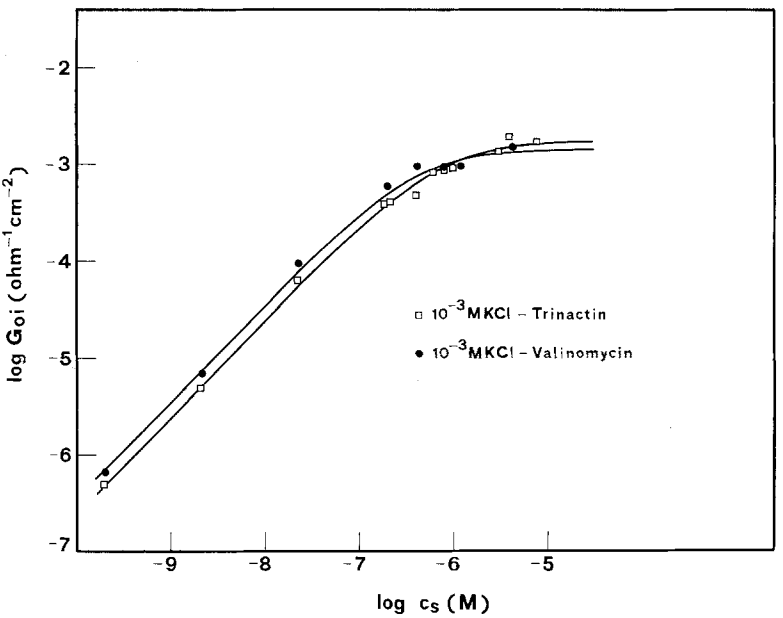


Fig. 5. Conductance behavior for two different carriers, valinomycin and trinactin, at 10^{-3} M KCl. The ionic strength was raised to 1 M by addition of LiCl. $T=20$ °C

The fact that the carrier concentration at which the saturation becomes appreciable increases in the sequence Rb^+, K^+ and NH_4^+ is clearly expected from condition (27), considering that $P_i=2k_s L_i \delta/D_i$ decreases in the same sequence, as is shown in Table 2. Fig. 5 illustrates the behavior of the conductance for the two different carriers valinomycin and trinactin at 10^{-3} M KCl. Also in the case of trinactin the curve bends, tending to a plateau; the saturation value deduced from the best fit of the data with Eq. (21) is, within experimental errors, equal to that of the curve for valinomycin. This is expected from Eq. (25), since the limiting value for the conductance is independent of the nature of the carrier.

Table 2. Values of the parameters and of the unstirred layer thickness δ deduced from the best fitting of the experimental points of Figs. 3 and 4 with Eq. (21). The values of the diffusion coefficients employed in the evaluation of δ are indicated

Ion	Antibiotic	c_i (M)	Ionic strength (M)	$D_i \times 10^9$ m^2/sec	log G_{oi} (Ω^{-1}cm^{-2}) $c_s \to \infty$	$(k_s L_i) \times 10^2$ m^4/sec moles	$\left(k_s L_i \frac{\delta}{D_i}\right) \times 10^{-3}$ m^3/moles	δ(m) $\times 10^4$
Rb^+	Valinomycin	10^{-3}	1	1.75	−2.7	2.7±0.2	2.8±0.4	1.8±0.5
K^+	Valinomycin	10^{-3}	1	1.75	−2.8	1 ±0.1	1.3±0.2	2.3±0.5
NH_4^+	Valinomycin	10^{-3}	10^{-3}	1.85	−3.1	0.108±0.005	0.24±0.02	4 ±0.5
K^+	Trinactin	10^{-3}	1	1.75	−2.8	0.64 ±0.003	0.69±0.07	1.9±0.3

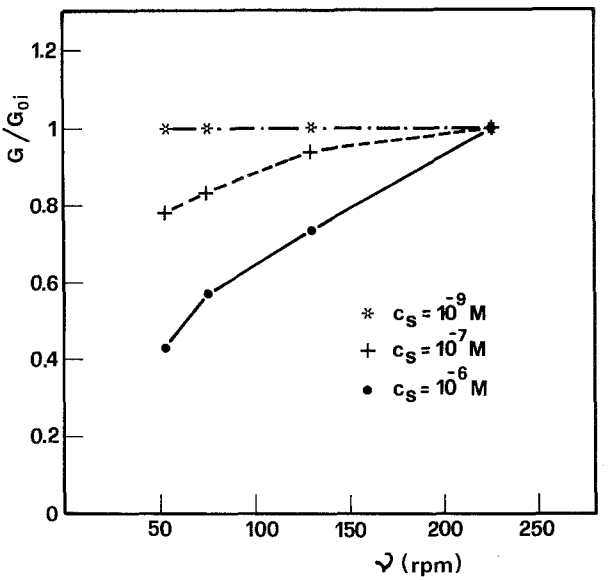


Fig. 6. The ratio G/G_{oi} between the conductance G at a given rate of stirring and the conductance G_{oi} at 225 rpm versus the frequency of stirring ν at the indicated valinomycin concentration. The KCl concentration was 10^{-3} M and the ionic strength was raised to 1 M by addition of LiCl

Dependence of the Conductance on the Rate of Stirring

Further evidence that at high values of the carrier concentration the rate-limiting step for ionic transport is diffusion across the unstirred layers can be given by an analysis of the effect of stirring on the steady-state conductance level. In fact, since the rate of stirring affects the thickness of the unstirred layers, it should also affect the current density in the range of carrier concentrations where diffusion across the unstirred layers becomes rate limiting.

Fig. 6 illustrates this effect; at low carrier concentrations (e.g. 10^{-9} M), where transport is expected to be controlled by the movement of the complexes across the membrane interior, the rate of stirring has no influence on the conductance. On the other hand, at higher concentrations, such as 10^{-7} M and 10^{-6} M, variations of the conductance of the order of 60% were determined.

Time Dependence of the Current Density after a Voltage Step

An additional test that diffusion across the unstirred layers is rate limiting at high concentrations of valinomycin can be given by the analysis of the time dependence of the transmembrane current after the application of a voltage step. LeBlanc (1969) and Neumcke (1971*a*) have shown that when

the rate of transport is determined by the unstirred layers, the time dependence of the current after a voltage step is not a simple exponential. For unstirred layers of finite thickness and in the limit of membranes with infinitely high conductivity Neumcke gives the following expression for the current

$$I(t)=zF\sqrt{\frac{D_i}{\pi t}}(c_i(d)-c_i)\left\{1+2\sum_{\nu=1}^{\infty}\exp\left[-2\nu^2\frac{\tau_s}{t}\right]\right\} \tag{28}$$

where c_i and $c_i(d)$ are the ionic aqueous concentrations in the bulk solutions and at the right interface, respectively, and $\tau_s=\delta^2/2D_i$. Considering that for $t<\tau_s$ the sum of the series on the right-hand side is negligible compared to unity, recalling that for unstirred layer limited currents

$$I(\infty)=zF\frac{D_i}{\delta}[c_i(d)-c_i] \tag{29}$$

we find for $t<\tau_s$

$$\frac{I(t)}{I(\infty)}=\frac{\delta}{\sqrt{\pi D_i t}}. \tag{30}$$

Eq. (30) has been shown to fit accurately the data for the time dependence of the bilayer conductance in the presence of the highly permeant anion Tph B^- (Neumcke, 1971*a*) and it might be applicable also to our system when the ion fluxes become unstirred layer limited. To see whether this is indeed the case at high c_s, various experiments have been performed applying voltage steps of different values across membranes interposed between KCl solutions in the presence of valinomycin ($c_{KCl}=10^{-3}$ M; $c_s=5\times10^{-6}$ M). In all cases the $t^{-1/2}$ dependence of the current was observed for $t<10$ sec. Note that this range satisfies the conditions $t<\tau_s$; in fact, substituting the following experimental values: $D_i=1.75\times10^{-5}$ cm^2/sec and $\delta\simeq0.35$ mm, one finds $\tau_s=35$ sec.

A different teflon chamber was used in these experiments with symmetrical geometry on the two sides of the hole; moreover, the rate of stirring was reduced to 50 rpm in order to increase δ and thus extend the time range where the $t^{-1/2}$ dependence for the conductance is expected.

Fig. 7 plots $I(t)/I(\infty)$ as a function of $t^{-1/2}$ in the range between 2 and 10 sec for a voltage step of 50 mV. The $t^{-1/2}$ dependence is satisfied, but neither the slope (which is one-half of the theoretical) nor the intercepts with the coordinate axes correspond to the values deducible from Eq. (30). Improvements of the experimental conditions, such as the use of shorter holes, might aid in resolving the discrepancy, but the limits of applicability of the theory should also be considered.

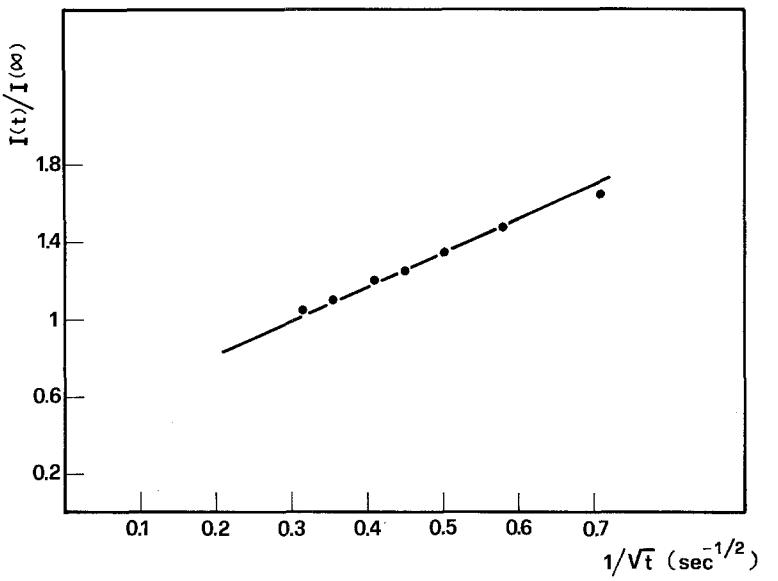


Fig. 7. The ratio $I(t)/I(\infty)$ between the current density $I(t)$ at the time t and the steady-state current density $I(\infty)$ as a function of $1/\sqrt{t}$. A voltage step, $V=50$ mV, was applied across the lipid film. The ionic solution contained 10^{-3} M KCl and the ionic strength was raised to 1 M by addition of LiCl. The antibiotic concentration was $c_s=5\times10^{-6}$ M

Solubility of Valinomycin as Determined by Optical Density Measurements

All the experimental results described in the previous sections are consistent with the interpretation of the saturation of the conductance in terms of unstirred layer effects, and seem rather conclusive against the possibility of its primary cause being insolubility of valinomycin in the aqueous solutions, at least up to 10^{-6} M. Nevertheless, at higher concentrations the two phenomena may overlap and it seemed therefore interesting to see whether any additional information on the solubility of valinomycin could be deduced by an independent method. To this end we carried out optical density measurements following the method described previously. The results are shown in Figs. 8 and 9, where the OD is plotted as a function of the concentration of valinomycin. The experimental points have been fitted by a straight line according to Beer's law

$$\mathrm{OD}=\log\frac{I_{\mathrm{inc}}}{I_{\mathrm{tr}}}=\varepsilon l c_s \tag{31}$$

where $I_{\mathrm{inc}}/I_{\mathrm{tr}}$ is the ratio of the intensity of the incident to the transmitted light, ε is the molar extinction coefficient, l is the thickness of the sample and c_s is the concentration of valinomycin.

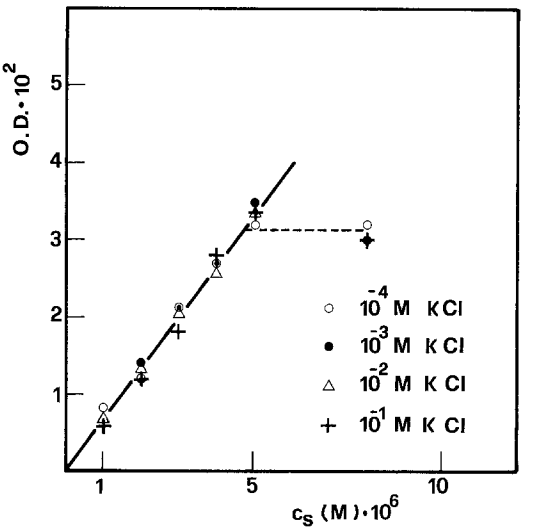


Fig. 8. Optical density, OD, versus valinomycin concentration for the indicated ionic solutions, maintained at 1 M ionic strength by addition of LiCl. All the points have been fitted by the same straight line. Wave length $\lambda = 220$ nm. The points on the far right indicate the OD values after centrifugation for the nominal concentration of valinomycin indicated by the corresponding abscissa. Assuming that these solutions are saturated, the abscissa of the intercept between the dashed line and the straight line fitting the experimental results should give the limit of solubility of valinomycin

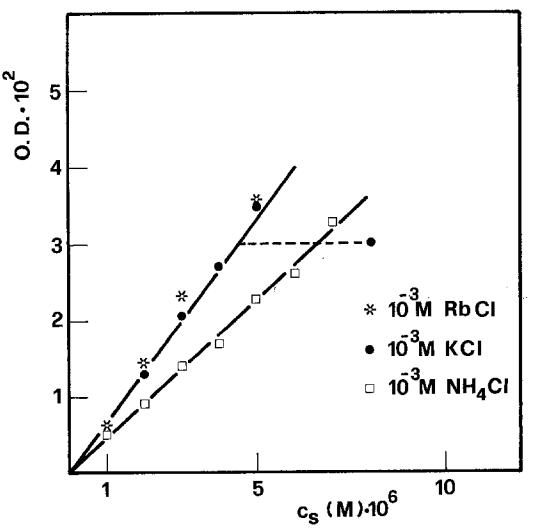


Fig. 9. Optical density versus valinomycin concentration for the indicated ionic solutions. For RbCl and KCl the ionic strength was held constant (1 M) by addition of LiCl. Wave length $\lambda = 220$ nm. The meaning of the point on the far right is the same as those of Fig. 8

For the points on the straight line no difference was found in OD before and after centrifugation. At 4500 rpm ($2500 \times g$) the linearity between OD and c_s in both Figures shows no evidence for precipitation of valinomycin up to concentrations of the order of 5×10^{-6} M for all the solutions studied; while in the case of NH_4Cl, where the ionic strength was not kept constant, the solubility may be even higher. The broken lines of Figs. 8 and 9 indicate points where the valinomycin solution is certainly saturated. For these points the OD values shown in the Figures and referring to measurements performed on centrifuged solutions are considerably lower than those obtained prior to centrifugation. This finding can probably be ascribed to suprasaturation or to the presence of aggregates which are removed by centrifugation.

An Independent Method to Determine the Thickness of the Unstirred Layers

In this section we describe an independent method for measuring the thickness of the unstirred layers and compare the values so obtained with those deduced from the conductance experiments. The procedure consists of raising the concentration of the permeant ion in one solution and following the time course of the zero-current potential. Suppose that initially ($t \leqq 0$) the concentration of the two solutions are equal

$$c^{(1)} = c^{(2)} \equiv c \tag{32}$$

and that at $t = 0$ a certain amount of the permeant ion is added to the left solution so as to increase its bulk concentration from c to c'. Because of diffusion through the unstirred layer, the concentration at the interface, $c(0, t)$, will increase slowly with a time course deducible from the integration of the time-dependent diffusion equation[6]. For a certain interval of time t after addition of the salt, sufficiently short that $\Delta c(0, t)/c = [c(0, t) - c]/c \simeq 10^{-2}$, the time dependence of the concentration can be described approximately by the solution of the diffusion equation valid for a semi-infinite medium ($x > -\delta$), where the boundary condition $c(-\delta, t) = c'$ remains unvaried; this is

$$c(x, t) = (c' - c)\left[1 - \operatorname{erf}\frac{x + \delta}{2\sqrt{D_i t}}\right] + c \tag{33}$$

6 Rigorously, one should use the electrodiffusion equation, since addition of the electrolyte to the bulk solution may produce a diffusion potential in the unstirred layer. This effect is assumed to be small, mainly because of the high ionic strength (1 M LiCl) of the solution.

where

$$\operatorname{erf} y = \int_0^y e^{-\eta^2} d\eta. \tag{34}$$

At the left interface

$$c(0, t) = (c' - c)\left[1 - \operatorname{erf} \frac{\delta}{2\sqrt{D_i t}}\right] + c. \tag{35}$$

Assuming negligible contribution of the diffusion potential across the unstirred layer, the zero-current membrane potential will be given by the Nernst equation

$$\phi(t) = -\ln \frac{c(0, t)}{c} \tag{36}$$

or, for $(c(0, t) - c)/c < 1$

$$\phi(t) = -\frac{c(0, t) - c}{c}. \tag{37}$$

Inserting Eq. (35) in Eq. (37) we obtain a relation between the transmembrane potential, time and the thickness of the stagnant layers, δ.

The measured values of t to be inserted in Eq. (35) were corrected for the time needed by the bulk stirred solution to equilibrate with the added salt. Measurements were made in aqueous solutions of 10^{-3} M KCl, 10^{-2} M KCl and 10^{-3} M RbCl, maintaining the ionic strength constant (1 M) by addition of LiCl, and using valinomycin (10^{-6} M) to induce cation selectivity in the membranes. The salt concentration was increased adding small amounts of concentrated solutions of KNO_3 or $RbNO_3$. Owing to the low permeability of the membrane to LiCl, the presence of this salt does not affect appreciably the validity of Eq. (36). Because of the different inclination of the surfaces of the diaphragm near the hole (cone-shaped on one side and coplanar with the membrane on the other), the thicknesses of the two unstirred layers were found to be different. On the cone-shaped side, where the aqueous region near the membrane is expected to be less effected by stirring, the thickness δ_1 was considerably larger than on the opposite side ($\delta_1 \simeq 0.6$ mm; $\delta_2 < 0.1$ mm). The larger value of δ could be determined quite accurately by the procedure described above, while the measurement of the smaller one with the same method presented some difficulties, due to the faster rising time of the potential and the error caused by the indeterminacy of the initial time $t = 0$; therefore, only an upper limit could be estimated for δ_2. Comparing the values indicated in Table 3 with those of Tables 1 and 2, it can be seen that the mean values of δ_1 and δ_2 are in fairly good agreement with those deduced from conductance measurements.

Table 3. Anterior, posterior and mean value of the unstirred layer thickness δ as deduced by the direct method

Ion	c_i (M)	Ionic strength (M)	δ_1 (m) $\times 10^4$	δ_2 (m) $\times 10^4$	$\bar{\delta}$ (m) $\times 10^4$
K^+	10^{-3}	1	7	<1	<4
K^+	10^{-2}	1	6	<0.5	<3
Rb^+	10^{-3}	1	6	<0.5	<3

It can be noted that there is an inconsistency between the assumption of symmetry made in the theoretical analysis and the experimental finding of unequal thicknesses of the unstirred layers. We have shown, however *(unpublished results)*, that extending the analysis to include different thicknesses of the unstirred layers has no other effect on Eq. (20) than that of replacing δ with the mean value $(\delta_1 + \delta_2)/2$.

Discussion

Some Inferences on the Mechanisms of Interfacial Permeation from the Dependence of the Zero-Current Conductance on Carrier Concentration

We have mentioned already that at least two distinct mechanisms for ion permeation across the interfaces, called briefly (R_{is}) and (P_{is}) and illustrated in parts (*a*) and (*b*) of Fig. 1, respectively, are in principle possible and equally plausible. Here we present an argument, based on the behavior of the conductance, in support of the opinion that (R_{is}) is a closer approximation to physical reality than (P_{is}); we will show that, for physically reasonable values of the rate constants of ion-carrier complexation in the aqueous phases, the saturation of the conductance as a function of carrier concentration, predicted by Eq. (21) and found experimentally in our system, would not be expected to be observable in the case of the (P_{is}) mechanism. An analogous conclusion as to (R_{is}) being the real mechanism of interfacial permeation, based on a comparison between predicted and measured limiting currents, was reached previously by Stark *et al.* (1971). For the (P_{is}) mechanism the conductance can be written again in the form of Eq. (18), where the general expression for $\Delta_{is}^*[0]$ deduced from the model is derived in Appendix B and is given by Eq. (40B). As shown in the same Appendix, that equation can be approximated in most practical cases by

$$\Delta_{is}^*[0] = -\frac{1}{zF}\left\{\frac{1}{k_{is}^B} + \frac{k_s^B + 2A_s^*}{2k_s k_s^B A_s^*} k_{is} K_{is} \gamma_i^{\pm} c_i + k_{is}\left[\frac{A}{K_{is}^B} + \frac{\delta K_{is}\gamma_i^{\pm}}{D_i} c_s\right]\right\}. \quad (38)$$

k_{is}^B denotes the rate at which the complexes cross the interfaces from the membrane into the aqueous phases (*see* Fig. 1 *b*), and k_{is} is the partition coefficient of the complexes. The quantity denoted by A is defined as

$$A=\left[K_{is}^F\gamma_i^{\pm}\left(\frac{c_i}{D_s}+\frac{c_s}{D_i}\right)+\frac{K_{is}^B}{D_{is}}\right]^{1/2}. \tag{39}$$

As to the dependence on c_i and c_s, Eq. (38) is formally similar to Eq. (20), which refers to the (R_{is}) mechanism, except for the presence of the term A/K_{is}^B. If this term were sufficiently small that

$$\frac{A}{K_{is}^B}\ll\frac{\delta K_{is}\gamma_i^{\pm}}{D_i}c_s \tag{40}$$

a saturation of the conductance at high values of c_s, due to the last term Eq. (38), would be expected also for the (P_{is}) mechanism. Aside from the fact that relation (40) is certainly not valid for sufficiently small values of c_s, we want to show that it cannot be satisfied in our systems even for the highest experimentally attainable values of c_s. Using the explicit definition of A given by Eq. (39), relation (40) can be rewritten in the form

$$K_{is}^F\gamma_i^{\pm}\gg\frac{D_i^2}{\delta^2 c_s^2}\left(\frac{c_i}{D_s}+\frac{c_s}{D_i}+\frac{1}{D_{is}K_{is}\gamma_i^{\pm}}\right). \tag{41}$$

To estimate the right-hand side of this relation, the major uncertainty concerns the value of K_{is}. According to certain measurements based on fluorescence (Feinstein & Felsenfeld, 1971), as well as on conductance experiments (Ciani *et al.*, 1973 *a*, p. 155), the value of K_{is} for trinactin and potassium is of the order of $0.5\,\text{M}^{-1}$. Values of the same order of magnitude for valinomycin, potassium and rubidium have been reported again by Feinstein & Felsenfeld (1971), as well as more recently by Benz *et al.* (1973). Therefore, for $K_{is}=0.5\,\text{M}^{-1}$, $c_i=10^{-3}\,\text{M}$, $c_s=10^{-6}\,\text{M}$, $\delta=2\times10^{-2}\,\text{cm}$, $D_i=2\times10^{-5}\,\text{cm}^2/\text{sec}$ and $D_s=5\times10^{-6}\,\text{cm}^2/\text{sec}$, relation (41) gives

$$K_{is}^F\gamma_i^{\pm}\gg4\times10^{11}\,\text{M}^{-1}\,\text{sec}^{-1}. \tag{42}$$

Since this value is considerably greater than the upper physical limit of $K_{is}^F\gamma_i^{\pm}$, corresponding to diffusion limited reactions $(5\times10^8-10^{10}\,\text{M}^{-1}\cdot\text{sec}^{-1}$; *see* Diebler *et al.*, 1969), it is reasonable to conclude that relationship (40) cannot be satisfied and, therefore, that the saturation of the conductance at high carrier concentrations would not be expected to be observable in the case of the (P_{is}) mechanism. This result leads to the conclusion that for our system the "heterogeneous reaction" mechanism is more consistent with the interpretation of the experimental data.

Some Comparative Observations on the Present and Previous Theoretical Results for the Conductance in the Case of the (P_{is}) Mechanism

Previous results, based on models which neglected the effects of the unstirred layers (Läuger & Stark, 1970; Ciani *et al.*, 1973*a*, *b*), suggested that some hints as to the mechanism of interfacial permeation could be deduced from the dependence of the conductance on the concentration of the permeant ion c_i, since a saturation of the conductance as a function of c_i was expected for the (R_{is}) mechanism, but not for (P_{is}). More precisely, when the effects of the unstirred layers are neglected and equilibrium is assumed in the aqueous phases up to the interfaces, $\Delta_{is}^*[0]$ for the (P_{is}) mechanism is found to be

$$\Delta_{is}^*[0] = -\frac{1}{zF k_{is}^B} \tag{43}$$

whereas for (R_{is})

$$\Delta_{is}^*[0] = -\frac{1}{zF}\left\{\frac{1}{\bar{K}_i^B} + \frac{\bar{K}_i \gamma_i^{\pm}}{k_s^B + 2A_s^*} c_i\right\}. \tag{44}$$

This led to the inference that a saturation of the conductance for high ion concentrations, expected from Eq. (44), but not from Eq. (43), could be used as a criterion to distinguish between (P_{is}) and (R_{is}). From the more general treatment given here it is seen that this criterion is not unambiguous, since an ion concentration-dependent term appears in Eq. (38), which refers to (P_{is}), as well as in Eq. (20), which refers to (R_{is}).

The physical meaning of the ion concentration-dependent term in Eq. (20), as well as of the corresponding one in Eq. (38), is related to the limitation imposed on the conductance by the back diffusion of the neutral carriers. The difference between the two mechanisms is that, while for (R_{is}) the carrier may remain confined within the membrane, in the case of (P_{is}) it must cross the interfaces. Consistently with this, Eq. (38) gives infinity and G_{oi} vanishes when $k_s^B = 0$.[7]

Appendix A

Derivation of Eq. (18)

The purpose of this Appendix is to derive the general expression for the zero-current conductance given by Eq. (18). For small applied voltages, Eq. (16) can be approximated by

7 Rigorously, even if $k_s^B = 0$, the conductance should not fall to zero, because a depletion of carriers at the interfaces can be compensated also by carriers diffusing from the aqueous solution. This possibility is neglected in the approximation of Eq. (38), but is contemplated in the rigorous expression for $\Delta_{is}^*[0]$ given by Eq. (40B).

$$J_{is}^{*} = A_{is}^{*}\left\{c_{is}^{*}(0) - c_{is}^{*}(d) + \frac{\phi}{2}\left[c_{is}^{*}(0) + c_{is}^{*}(d)\right]\right\}. \tag{1 A}$$

Recalling the definition of $\Delta_{is}^{*}[0]$ given by Eq. (19), we can write for small current densities

$$c_{is}^{*}(0) = c_{is}^{*}(0)_{\mathrm{eq}} + \Delta_{is}^{*}[0]\, I. \tag{2 A}$$

At the right interface we will have analogously

$$c_{is}^{*}(d) = c_{is}^{*}(d)_{\mathrm{eq}} + \Delta_{is}^{*}[d]\, I. \tag{3 A}$$

Since the system is symmetrical at equilibrium, we have

$$c_{is}^{*}(0)_{\mathrm{eq}} = c_{is}^{*}(d)_{\mathrm{eq}} \equiv c_{is}^{*}(\mathrm{eq}). \tag{4 A}$$

Moreover, the value of $c_{is}^{*}(d)$ for a given current density is clearly equal to the value of $c_{is}^{*}(0)$ for the same current flowing in the opposite direction. This implies

$$\Delta_{is}^{*}[0] = -\Delta_{is}^{*}[d]. \tag{5 A}$$

Substituting the right-hand sides of Eqs. (2 A) and (3 A) in (1 A), and making use of Eqs. (4 A) and (5 A) as well as of Eqs.(1) and (17), we find after simple rearrangements

$$G_{oi} = \lim_{I \to 0} \frac{I}{V} = \frac{z^2 F^2}{RT} \frac{A_{is}^{*} c_{is}^{*}(\mathrm{eq})}{1 - 2 z F A_{is}^{*} \Delta_{is}^{*}[0]}. \tag{6 A}$$

Eq. (6 A) is rather general, but the explicit expression of $\Delta_{is}^{*}[0]$ will depend on the particular mechanism of permeation across the interfaces, as well as on the physical processes in the unstirred layers.

Appendix B

Derivation of the Explicit Expression for the Conductance in the Cases of the (R_{is}) and (P_{is}) Mechanisms

The purpose of this Appendix is to evaluate the explicit expression for the term $\Delta_{is}^{*}[0]$ in the two cases of the (R_{is}) and (P_{is}) mechanisms.

Since we consider only small currents and small deviations from equilibrium, we can simplify the calculations by expanding all the quantities in powers of I and retaining only the terms linear in I (or V). So, if I is a small current density flowing across the membrane, the concentrations of the species i, s and is in the left unstirred layer can be written

$$c_i(x) = c_i + \delta_i(x) I; \quad c_s(x) = c_s + \delta_s(x) I; \quad c_{is}(x) = c_{is} + \delta_{is}(x) I; \quad -\delta \leqq x \leqq 0 \tag{1 B}$$

where c_i, c_s and c_{is} are meant to represent the concentrations at equilibrium. At the left interface, inside the membrane, we will have

$$c_s^*(0)=k_s c_s+\delta_s^*(0)I; \qquad c_{is}^*(0)=\overline{K}_i\gamma_i^{\pm}\, c_i c_s+\Delta_{is}^*[0]I. \tag{2B}$$

All the five quantities $\delta_i(x)$, $\delta_s(x)$, $\delta_{is}(x)$, $\delta_s^*(0)$ and $\Delta_{is}^*[0]$ are derivatives of concentrations with respect to the current density, calculated at $I=0$. Following the same argument which led us to Eq. (5A), it should be clear that each of these is antisymmetrical with respect to the center of the membrane ($x=d/2$); for instance

$$\delta_s^*(0)=-\delta_s^*(d). \tag{3B}$$

This result is important because it relates, in an extremely simple way, the properties of the left and right interfaces and unstirred layers, and will relieve us from the necessity of considering them separately. Eq. (6), which results from combination of the flux equations and the equations of conservation of mass, can be conveniently rewritten as

$$D_i\frac{d^2c_i}{dx^2}+D_{is}\frac{d^2c_{is}}{dx^2}=0 \tag{4B}$$

$$D_s\frac{d^2c_s}{dx^2}+D_{is}\frac{d^2c_{is}}{dx^2}=0 \tag{5B}$$

$$\frac{d^2c_i}{dx^2}=\frac{K_{is}^F\gamma_i^{\pm}}{D_i}c_i(x)c_s(x)-\frac{K_{is}^B}{D_i}c_{is}(x). \tag{6B}$$

Using the definitions of (1B), Eqs. (4B) and (5B) become

$$D_i\frac{d^2\delta_i(x)}{dx^2}+D_{is}\frac{d^2\delta_{is}(x)}{dx^2}=0 \tag{7B}$$

$$D_s\frac{d^2\delta_s(x)}{dx^2}+D_{is}\frac{d^2\delta_{is}(x)}{dx^2}=0. \tag{8B}$$

Substituting now Eq. (1B) in Eq. (6B), recalling that the term $K_{is}^F\gamma_i^{\pm}\,c_ic_s-K_{is}^Bc_{is}$ vanishes identically because the concentrations c_i, c_s and c_{is} are those of equilibrium, and neglecting the term in I^2, we find

$$\frac{d^2\delta_i(x)}{dx^2}=\frac{K_{is}^F\gamma_i^{\pm}}{D_i}[c_i\delta_s(x)+c_s\delta_i(x)]-\frac{K_{is}^B}{D_i}\delta_{is}(x). \tag{9B}$$

A first integration of Eq. (7B) gives

$$D_i\frac{d\delta_i(x)}{dx}+D_{is}\frac{d\delta_{is}(x)}{dx}=\text{const.} \tag{10B}$$

If we observe now that the current density in the unstirred layers is given by

$$\frac{I}{zF}=J_i+J_{is} \tag{11B}$$

and if we recall Eqs. (2), (4) and (1B), it should be clear that the constant in (10B) is equal to $-1/zF$; therefore

$$D_i\frac{d\delta_i(x)}{dx}+D_{is}\frac{d\delta_{is}(x)}{dx}=-\frac{1}{zF}. \tag{12B}$$

Assuming that the concentrations remain unperturbed at the edges of the unstirred layers far from the interfaces ($x=-\delta$) so that

$$\delta_i(-\delta)=\delta_s(-\delta)=\delta_{is}(-\delta)=0 \tag{13B}$$

integration of Eq. (12B) gives

$$\delta_{is}(x)=-\frac{D_i}{D_{is}}\delta_i(x)-\frac{x+\delta}{zFD_{is}}. \tag{14B}$$

Integrating now Eq. (8B) and using Eq. (14B) to express $\delta_{is}(x)$ as a function of $\delta_i(x)$, we get

$$\delta_s(x)=\frac{D_i}{D_s}\delta_i(x)+\left[\delta_s(0)-\frac{D_i}{D_s}\delta_i(0)\right]\left(1+\frac{x}{\delta}\right). \tag{15B}$$

If we substitute the right-hand sides of Eqs. (14B) and (15B) in Eq. (9B) we obtain a linear differential equation of the second order in $\delta_i(x)$ with constant coefficients; integration by standard procedures gives

$$\delta_i(x)=\left[\delta_i(0)+\frac{\Omega}{A^2}\right]\frac{\sinh\left[A(x+\delta)\right]}{\sinh A\delta}-\frac{\Omega}{A^2}\left(1+\frac{x}{\delta}\right) \tag{16B}$$

where A is defined in Eq. (39) and

$$\Omega=\frac{K_{is}\gamma_i^{\pm}c_i}{D_i}\left[\delta_s(0)-\frac{D_i}{D_s}\delta_i(0)\right]+\frac{\delta K_{is}^B}{zFD_iD_{is}}. \tag{17B}$$

Eq. (16B) gives the explicit dependence of $\delta_i(x)$ on x. The analogous explicit functions for $\delta_{is}(x)$ and $\delta_s(x)$ are obtained by substituting Eq. (16B) in Eqs. (14B) and (15B).

It is useful for the purpose of this Appendix to consider the aqueous fluxes of the three species i, s and is near the interfaces, namely $J_i(0)$, $J_s(0)$ and $J_{is}(0)$. Using the definitions of Eq. (1B), Eqs. (2–4) can be rewritten as

$$\frac{J_i}{I} = -D_i \frac{d\delta_i(x)}{dx}; \quad \frac{J_s}{I} = -D_s \frac{d\delta_s(x)}{dx}; \quad \frac{J_{is}}{I} = -D_{is} \frac{d\delta_{is}(x)}{dx}. \tag{18B}$$

These derivatives can be calculated straightforwardly using Eq. (16B), as well as Eqs. (15B) and (14B), in which $\delta_i(x)$ has been expressed in terms of Eq. (16B). From Eqs. (14B–18B) it follows that the quantities $J_i(0)/I$, $J_s(0)/I$ and $J_{is}(0)/I$ are linear functions of the two unknown quantities $\delta_i(0)$ and $\delta_s(0)$; omitting, for brevity, to write down their explicit expressions, we denote them by

$$\frac{J_i(0)}{I} = F_i[\delta_i(0), \delta_s(0)]; \quad \frac{J_s(0)}{I} = F_s[\delta_i(0), \delta_s(0)];$$
$$\frac{J_{is}(0)}{I} = F_{is}[\delta_i(0), \delta_s(0)]. \tag{19B}$$

We can proceed now to consider the two interfacial mechanisms (R_{is}) and (P_{is}).

(R_{is}) mechanism. In the case of the (R_{is}) mechanism the complexes do not cross the interfaces. It is then very reasonable to assume that also the aqueous flux of the complexes vanishes near the interfaces; from Eqs. (19B) we have

$$F_{is}[\delta_i(0), \delta_s(0)] = 0. \tag{20B}$$

Let us now consider the heterogeneous reaction at the interfaces; combining Eqs. (12) and (14), using (1B) and (2B) to express $c_i(0)$, $c_s^*(0)$ and $c_{is}^*(0)$ in terms of $\delta_i(0)$, $\delta_s^*(0)$ and $\Delta_{is}^*[0]$, and neglecting the term in I^2, we find

$$\bar{K}_i^F \gamma_i^{\pm} k_s c_s \delta_i(0) + \bar{K}_i^F \gamma_i^{\pm} c_i \delta_s^*(0) - \bar{K}_i^B \Delta_{is}^*[0] = \frac{1}{zF}. \tag{21B}$$

Eqs. (20B) and (21B) are two relationships in the unknowns $\delta_i(0)$, $\delta_s(0)$, $\delta_s^*(0)$ and $\Delta_{is}^*[0]$. In order to evaluate $\Delta_{is}^*[0]$, which is the purpose of the Appendix, we need two more equations; these are obtained by imposing the condition that the total flux of carriers in the unstirred layers must be equal to the total flux of carriers across the interfaces as well as to that across the membrane interior, namely

$$J_s + J_{is} = J_s^{\text{int}} + J_{is}^{\text{int}} = J_s^* + J_{is}^*. \tag{22B}$$

Since $J_s + J_{is}$ in the unstirred layers is constant, we can take its value at $x = 0$; but $J_{is}(0) = 0$ as indicated in Eq. (20B); therefore

$$J_s + J_{is} = J_s(0). \tag{23B}$$

On the other hand, $J_{is}^* = I/zF$ and in the case of the (R_{is}) mechanism, $J_{is}^{int} = 0$, so that the two relationships in Eq. (22B) become

$$J_s(0) = J_s^{int} = J_s^* + \frac{I}{zF}. \tag{24B}$$

With the help of Eqs. (19B), (10), (15), (1B), (2B) and (3B), Eq. (24B) becomes

$$F_s[\delta_i(0), \delta_s(0)] = k_s^F \delta_s(0) - k_s^B \delta_s^*(0) = 2A_s^* \delta_s^*(0) + \frac{1}{zF}. \tag{25B}$$

Finally, solving Eqs. (20B), (21B) and (25B) for $\Delta_{is}^*[0]$, we find

$$\Delta_{is}^*[0] = -\frac{1}{zF}\left\{\frac{1}{\bar{K}_i^B} + \frac{\bar{K}_i \gamma_i^{\pm} c_i}{2A_s^*}\xi_1 + \frac{\bar{K}_i \gamma_i^{\pm} k_s \delta c_s}{D_i}\xi_s\right\} \tag{26B}$$

where

$$\xi_1 = \frac{Q\left[k_s^F + \frac{D_s}{\delta}\right] + R\frac{D_s}{\delta} + S\frac{D_i}{\delta}}{(Q+R)\left[k_s^F + \frac{D_s}{\delta} + \frac{k_s^B D_s}{2A_s^*\delta}\right] + S\frac{D_i}{\delta}\left(1 + \frac{k_s^B}{2A_s^*}\right)} \tag{27B}$$

$$\xi_2 = \frac{Q\left[k_s^F + \frac{D_s}{\delta}\left(1 + \frac{k_s^B}{2A_s^*}\right)\right] + 2S\frac{D_i}{\delta}}{(Q+R)\left[k_s^F + \frac{D_s}{\delta} + \frac{k_s^B D_s}{2A_s^*\delta}\right] + 2S\frac{D_i}{\delta}\left(1 + \frac{k_s^B}{2A_s^*}\right)} \tag{28B}$$

$$Q = 1 + \frac{K_{is}^B}{D_{is}}\frac{f[A\delta]}{A^2}; \qquad R = \frac{K_{is}^F \gamma_i^{\pm} c_s}{D_i}\frac{f[A\delta]}{A^2}; \qquad S = R\frac{c_i}{c_s}. \tag{29B}$$

A is defined in Eq. (39), and

$$f[A\delta] = A\delta \coth A\delta - 1. \tag{30B}$$

The quantities ξ_1 and ξ_2, appearing in the rigorous expression for $\Delta_{is}^*[0]$ and defined in Eqs. (27B) and (28B), are rather complicated, but become equal to unity when

$$\frac{D_s}{\delta} \ll 2k_s A_s^* \tag{31B}$$

and

$$Q \gg R; \qquad Q \gg 2S\frac{D_i}{\delta k_s A_s^*}. \tag{32B}$$

Recalling the definitions of Q, R and S given in Eq. (29 B), it can be seen that the two relationships (32 B) are certainly satisfied if

$$K_{is}\gamma_i^{\pm} c_s \ll \frac{D_i}{D_{is}} \quad \text{and} \quad K_{is}\gamma_i^{\pm} c_i \ll \frac{\delta k_s A_s^*}{D_{is}}. \tag{33 B}$$

Using any of the data given in the literature for parameters such as $K_{is}\gamma_i^{\pm}$, k_s, A_s^* referred to either valinomycin or trinactin, it can be seen that the relationships (31 B) and (32 B) are amply satisfied. We therefore conclude that ξ_1 and ξ_2 can be approximated by unity and that the rigorous expression for $\Delta_{is}^*[0]$ given in Eq. (26 B) can be approximated by Eq. (20).

(P_{is}) *mechanism.* Since in the case of the (P_{is}) mechanism no chemical reactions occur at the interfaces, the fluxes of the three species i, s and is must be continuous, namely

$$J_i(0)=0; \quad J_{is}(0)=J_{is}^{\text{int}}; \quad J_s(0)=J_s^{\text{int}}. \tag{34 B}$$

Expressing J_{is}^{int} and J_s^{int} in terms of the rate constants k_{is}^F, k_{is}^B and k_s^F, k_s^B, respectively, and using the definitions (1 B) and (2 B) we get

$$\frac{J_{is}^{\text{int}}}{I}=k_{is}^F\,\delta_{is}(0)-k_{is}^B\,\Delta_{is}^*[0]; \quad \frac{J_s^{\text{int}}}{I}=k_s^F\,\delta_s(0)-k_s^B\,\delta_s^*(0). \tag{35 B}$$

Recalling Eq. (19 B), the three Eqs. (34 B) become

$$\begin{aligned} &F_i[\delta_i(0),\delta_s(0)]=0\\ &F_s[\delta_i(0),\delta_s(0)]=k_s^F\,\delta_s(0)-k_s^B\,\delta_s^*(0)\\ &F_{is}[\delta_i(0),\delta_s(0)]=k_{is}^F\,\delta_{is}(0)-k_{is}^B\,\Delta_{is}^*[0]. \end{aligned} \tag{36 B}$$

Moreover, the interfacial flux of neutral carriers must be equal to the corresponding transmembrane flux J_s^*,

$$J_s^{\text{int}}=J_s^*. \tag{37 B}$$

Recalling Eqs. (15), (2 B) and (3 B) as well as (35 B), Eq. (37 B) becomes

$$k_s^F\,\delta_s(0)-k_s^B\,\delta_s^*(0)=2A_s^*\,\delta_s^*(0). \tag{38 B}$$

Eqs. (36 B) and (38 B) constitute a system of four linear equations in the five unknowns $\delta_i(0)$, $\delta_s(0)$, $\delta_{is}(0)$, $\delta_s^*(0)$ and $\Delta_{is}^*[0]$. The additional relationship needed to evaluate $\Delta_{is}^*[0]$ is provided by Eq. (14 B), which gives for $x=0$

$$\delta_{is}(0)=-\frac{D_i}{D_{is}}\,\delta_i(0)-\frac{\delta}{zFD_{is}}. \tag{39 B}$$

Solving Eqs. (36 B), (38 B) and (39 B) for $\Delta_{is}^{*}[0]$ we find

$$\Delta_{is}^{*}[0] = -\frac{1}{zF k_{is}^{B}} \left\{ 1 + \frac{k_{is}^{F}[2k_s^F A_s^* + a_{22}(k_s^B + 2A_s^*)]}{(a_{11}\, a_{22} - a_{12}\, a_{21})(k_s^B + 2A_s^*) + 2a_{11}\, k_s^F A_s^*} \right\} \tag{40 B}$$

with

$$a_{11} = \frac{1}{\delta} \left\{ D_{is} + \frac{f[A\delta]\, K_{is}^{B}}{A^2 + f[A\delta]\, K_{is}^{F} \gamma_i^{\pm}\, c_s/D_i} \right\} \tag{41 B}$$

$$a_{22} = \frac{1}{\delta} \left\{ D_s + \frac{f[A\delta]\, K_{is}^{F} \gamma_i^{\pm}\, c_i}{A^2 + f[A\delta]\, K_{is}^{F} \gamma_i^{\pm}\, c_s/D_i} \right\} \tag{42 B}$$

$$a_{12} = a_{22} - D_s/\delta \tag{43 B}$$

$$a_{21} = a_{11} - D_{is}/\delta. \tag{44 B}$$

A and $f[A\delta]$ are defined in Eqs. (36) and (30 B), respectively.

Eq. (40 B) is rather complicated, but it can be simplified considerably with the help of approximations which are very likely satisfied in the case of the common macrocyclic antibiotics, including trinactin and valinomycin. For instance, in the discussion of relation (38) we had mentioned already that the highest estimates for the equilibrium constant K_{is} are of the order of 1 M^{-1}. If for K_{is}^{F} we take values ranging from those expected for diffusion-limited reactions (10^{10} M^{-1} sec^{-1}) to even six orders of magnitude lower, and if we use realistic values for δ, D_i, D_s and D_{is} (e.g. $\delta = 2 \times 10^{-2}$ cm; $D_i = 2 \times 10^{-5}$ cm^2/sec, $D_s = D_{is} = 5 \times 10^{-6}$ cm^2/sec) we find

$$\frac{D_s}{\delta} \ll a_{22}; \qquad \frac{D_{is}}{\delta} \ll a_{11}; \qquad A\delta \gg 1. \tag{45 B}$$

Recalling Eqs. (43 B–45 B) and (30 B) the following approximations can be made:

$$a_{11}\, a_{22} - a_{12}\, a_{21} = 0; \qquad f[A\delta] \simeq A\delta. \tag{46 B}$$

Inserting Eq. (46 B) in Eq. (40 B), and neglecting the term D_{is}/δ in the definition of a_{11}, compatibly with relation (45 B), we obtain finally the approximated expression for $\Delta_{is}^{*}[0]$ given by Eq. (38).

Appendix C

In all the experiments described in this paper we used membranes of approximately 0.8 mm diameter. While offering the advantage of extremely high stability, membranes of such small areas give rise to other difficulties already mentioned in footnote 5; namely, when valinomycin is introduced in the aqueous solutions, the rate of exchange of the neutral

carrier between the aqueous phases and the membrane is comparable to that between the membrane and the torus, with the result that the specific membrane conductance depends on the area. However, we want to show in this Appendix that, provided the membrane area stays constant, the flux of neutral carriers between the membrane and the torus, considered as an empty reservoir of carriers, has negligible effects on the theoretical results used for the interpretation of the conductance experiments (Eqs. 21–26).

If we assume that for the time of the experiment the concentration of carriers in the torus is practically negligible and that the concentration of carrier c_s^* is uniform over the area of the membrane, the flux of carriers from the membrane to the torus will be

$$J_s^{MT} = 2\pi r k^{MT} c_s^* \tag{1C}$$

where k^{MT} is an appropriate rate constant and $2r$ is the membrane diameter. At steady-state and zero net current, and assuming negligible effects of aqueous complexation, the rate of accumulation of neutral carrier in the torus, expressed by Eq. (1C), is related to the flux through the left unstirred layer and across the left interface by

$$\pi r^2 \frac{D_s}{\delta}[c_s - c_s(0)] = \pi r^2 [k_s^F c_s(0) - k_s^B c_s^*(0)] = \pi r k^{MT} c_s^*(0). \tag{2C}$$

Solving for $c_s(0)$ and $c_s^*(0)$ we find

$$c_s(0)_{st} = \frac{c_s}{1 + \dfrac{k^{MT} k_s \delta}{r D_s \left(1 + \dfrac{k^{MT}}{r k_s^B}\right)}}; \qquad c_s^*(0)_{st} = \frac{k_s c_s}{1 + \dfrac{k^{MT}}{r}\left[\dfrac{1}{k_s^B} + \dfrac{k_s \delta}{D_s}\right]}. \tag{3C}$$

The interfacial concentration of complexes at the interfaces at zero current will be

$$c_{is}^*(0)_{st} = \bar{K}_i c_i c_s^*(0)_{st} \tag{4C}$$

where $c_s^*(0)_{st}$ is given by Eq. (3C). When current flows across the membrane, and if we neglect complex formation in the aqueous phases, the steady-state equations for the total flux of carriers are

$$\begin{aligned} \frac{D_s}{\delta}[c_s - c_s(0)] &= k_s^F c_s(0) - k_s^B c_s^*(0) \\ &= \frac{k^{MT}}{r} c_s^*(0) + A_s^*[c_s^*(0) - c_s^*(d)] + \frac{I}{zF}. \end{aligned} \tag{5C}$$

For small currents we can use the same procedure as in the two previous Appendices and define correspondingly

$$\begin{aligned} c_i(0) &= c_i + \delta_i(0)\, I; & c_s(0) &= c_s(0)_{st} + \delta_s(0)\, I \\ c_s^*(0) &= c_s^*(0)_{st} + \delta_s^*(0)\, I; & c_{is}^*(0) &= c_{is}^*(0)_{st} + \Delta_{is}^*[0]\, I. \end{aligned} \tag{6C}$$

Substituting Eq. (6C) in Eq. (5C) we find after obvious cancellations

$$-\frac{D_s}{\delta}\,\delta_s(0) = k_s^F\,\delta_s(0) - k_s^B\,\delta_s^*(0) = \frac{k^{MT}}{r}\,\delta_s^*(0) + 2A_s^*\,\delta_s^*(0) + \frac{1}{zF}. \tag{7C}$$

From the continuity of the electric current through the unstirred layers and across the interfaces we find

$$\frac{D_i}{\delta}\,[c_i - c_i(0)] = \bar{K}_i^F\,\gamma_i^{\pm}\,c_i(0)\,c_s^*(0) - \bar{K}_i^B\,c_{is}^*(0) = \frac{I}{zF}. \tag{8C}$$

Combining Eq. (6C) with Eq. (8C) and neglecting the terms in I^2, we find

$$-\frac{D_i}{\delta}\,\delta_i(0) = \bar{K}_i^F\,\gamma_i^{\pm}\,c_i\,\delta_s^*(0) + \bar{K}_i^F\,\gamma_i^{\pm}\,c_s^*(0)_{st}\,\delta_i(0) - \bar{K}_i^B\,\Delta_{is}^*[0] = \frac{1}{zF}. \tag{9C}$$

Solving Eqs. (7C) and (9C) for $\Delta_{is}^*(0)$ we get

$$\begin{aligned} &-zF\,\Delta_{is}^*[0] \\ &= \frac{1}{\bar{K}_i^B} + \frac{\bar{K}_i\,\gamma_i^{\pm}\,c_i}{2A_s^* + \dfrac{k^{MT}}{r} + \dfrac{1}{\dfrac{k_s\,\delta}{D_s} + \dfrac{1}{k_s^B}}} + \frac{\bar{K}_i\,\gamma_i^{\pm}\,k_s\,c_s}{D_i\left[1 + \dfrac{k^{MT}}{r}\left(\dfrac{1}{k_s^B} + \dfrac{k_s\,\delta}{D_s}\right)\right]}. \end{aligned} \tag{10C}$$

If we now substitute Eqs. (10C), (4C) and (3C) in the expression of the conductance, Eq. (18), and if we make the approximation

$$2A_s^* \gg \frac{1}{\dfrac{k_s\,\delta}{D_s} + \dfrac{1}{k_s^B}} \tag{11C}$$

we obtain an expression which can be written in the same form as Eq. (21), provided that k_s is replaced everywhere by

$$k_s\left[1 + \frac{k^{MT}}{r}\left(\frac{1}{k_s^B} + \frac{k_s\,\delta}{D_s}\right)\right]^{-1}$$

and that N_i is defined as

$$N_i = \frac{L_i}{A_s^* \left(1 + \frac{k^{MT}}{2rA_s^*}\right)}. \tag{12C}$$

The plausibility of approximation (11C) can be deduced from the following considerations: it should be clear that Eq. (11C) is certainly satisfied if

$$A_s^* \gg \frac{D_s}{2\delta k_s}. \tag{13C}$$

From relaxation experiments Stark *et al.* (1971) deduced that

$$2\frac{A_s^*}{d} \simeq 2 \times 10^4 \text{ sec}^{-1} \tag{14C}$$

where d is the membrane thickness. If $d \simeq 7 \times 10^{-7}$ cm, we find

$$A_s^* \simeq 7 \times 10^{-3} \text{ cm sec}^{-1}. \tag{15C}$$

On the other hand, for $D_s \simeq 10^{-6}$ cm^2/sec, $\delta \simeq 2 \times 10^{-2}$ cm and $k_s \simeq 10^4$, we find

$$\frac{D_s}{2\delta k_s} \simeq 5 \times 10^{-9} \text{ cm sec}^{-1}. \tag{16C}$$

Comparing Eq. (15C) with Eq. (16C) it is clear that the approximations (13C) and (11C) are amply satisfied.

This work was supported by a grant from the C.N.R. of Italy, USPHS Grant No. NS09931 and NSF Grant No. GB30835. The technical assistance of Mr. C. Cugnoli has been greatly appreciated.

References

Benz, R., Stark, G., Janko, K., Läuger, P. 1973. Valinomycin-mediated ion transport through neutral lipid membranes: Influence of hydrocarbon chain length and temperature. *J. Membrane Biol.* **14**:339

Ciani, S.M., Eisenman, G., Laprade, R., Szabo, G. 1973*a*. Theoretical analysis of carrier-mediated electrical properties of bilayer membranes. *In:* Membranes—A Series of Advances. G. Eisenman, Editor. Vol. 2, p. 61. Marcel Dekker, Inc., New York

Ciani, S., Laprade, R., Eisenman, G., Szabo, G. 1973*b*. Theory for carrier-mediated zero-current conductance of bilayers extended to allow for nonequilibrium of interfacial reactions, spatially dependent mobilities and barrier shape. *J. Membrane Biol.* **11**:255

Diebler, H., Eigen, M., Ilgenfritz, G., Maas, G., Winkler, R. 1969. Kinetics and mechanism of reactions of main group metal ions with biological carriers. *Pure Appl. Chem.* **20**:93

Feinstein, M.B., Felsenfeld, H. 1971. The detection of ionophorous antibiotic-cation complexes in water with fluorescent probes. *Proc. Nat. Acad. Sci.* **68**:2037

Gotlieb, V.A., Buzhinsky, E.P., Lev, A.A. 1968. Nature of the cationic specificity of bimolecular phospholipid membranes with valinomycin introduced to them. *Biophysics* **13**(3):675

Haydon, D.A., Hladky, S.B. 1972. Ion transport across thin lipid membranes: A critical discussion of mechanisms in selected systems. *Quart. Rev. Biophys.* **5**:187

Hladky, S.B.1972. The steady-state theory of the carrier transport of ions. *J. Membrane Biol.* **10**:67

Hladky, S.B. 1973. The effect of stirring on the flux of carriers into black lipid membranes. *Biochim. Biophys. Acta* **307**:261

Hladky, S.B., Gordon, L.G.M., Haydon, D.A. 1974. Molecular mechanisms of ion transport in lipid membranes. *Ann. Rev. Phys. Chem* **25**:11

Läuger, P., Stark, G. 1970. Kinetics of carrier mediated ion transport across lipid bilayer membranes. *Biochim. Biophys. Acta* **211**:75

LeBlanc, O.H., Jr. 1969. Tetraphenylborate conductance through lipid bilayer membranes. *Biochim. Biophys. Acta* **993**:350

LeBlanc, O.H., Jr. 1971. The effect of uncouplers of oxidative phosphorylation on lipid bilayer membranes: Carbonylcyanide *m*-chlorophenylhydrazone. *J. Membrane Biol.* **4**:227

Markin, V.S., Pastushenko, V.F., Kristalik, L.I., Liberman, E.A., Topaly, V.P. 1969. Membrane potential and short circuit current in artificial phospholipid membranes in the presence of agents uncoupling oxidative phosphorylation. *Biofizika* **14**:462

Neumcke, B. 1971*a*. Diffusion polarization at lipid bilayer membranes. *Biophysik* **7**:95

Neumcke, B. 1971*b*. Diffusion polarization at lipid bilayer membranes in the presence of a homogeneous chemical reaction in the solutions. *Life Sci.* **1**:85

Stark, G., Benz, R. 1971. The transport of potassium through lipid bilayer membranes by the neutral carriers valinomycin and monactin. Experimental studies to a previously proposed model. *J. Membrane Biol.* **5**:133

Stark, G., Ketterer, B., Benz, R., Läuger, P. 1971. The rate constants of valinomycin-mediated ion transport through thin lipid membranes. *Biophys. J.* **11**:981

Szabo, G., Eisenman, G., Ciani, S. 1969. The effects of the macrotetralide actin antibiotics on the electrical properties of phospholipid bilayer membranes. *J. Membrane Biol.* **1**:346

Vetter, K.J. 1967. Electrochemical Kinetics. Theoretical Aspects. Academic Press, New York