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NON-MEDIATED ZERO VOLTAGE CONDUCTANCE OF HYDROPHOBIC IONS THROUGH BILAYER LIPID MEMBRANES

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SUMMARY

A charge pulse technique applied to the study of charge transfer at metal-solution interfaces has been used to determine the capacity and the conductance of a membrane bilayer at both zero time and zero voltage. The transport of hydrophobic ions across a glycerol-monooleate bilayer (tetraphenyl borate, picrate, dipicrylamine and tetraphenyl arsonium) has been investigated by this method. A theoretical approach to the problem has been proposed based on one analogous to that used for the compact double layer at metallic electrodes.

INTRODUCTION

Experimental studies of the transport of hydrophobic ions through bilayers membranes [1–5] are less numerous than those dealing with inorganic ion transport by carriers or channel-inducing materials. However, such studies are interesting as they lead to information concerning factors which affect mediated transports. Thus the limiting membrane conductance at zero time and zero current is an experimental datum, which can be related to certain physicochemical characteristics of the membrane-solution system at the equilibrium state, i.e. when values such as concentrations in the interfacial layers are exactly known.

Electrical resistance measurements necessarily imply the passage of current, which in membrane-solution systems induces an ionic flux capable of modifying the ionic cencentrations in the bilayer and in the aqueous interfacial layers. This is the reason why in practice one must apply a very low voltage during very short periods of time in order to reduce to a minimum the ion concentration variations.

As lipid bilayers can be considered equivalent to a resistance in parallel with a capacitance, the current which crosses the membrane during the first instants is mainly a capacitance-charging current. In order to deduce the initial value of the current when a voltage step is applied, it is necessary to maintain the voltage strictly constant and, furthermore, the variation of the intensity with time should depend on a simple law that allows accurate extrapolation to zero time De Levie et al [6] have used the sine wave impedance method which gives accurate results in the studies of electrochemical processes at metallic electrodes. This technique not only enables one to evaluate the membrane capacitance and resistance, but also gives information concerning the diffusion and adsorption of ions However, a series of measurements is necessary to obtain an impedance spectrum.

In this paper, we have undertaken a charge pulse study of the bilayer membrane conductance in the presence of hydrophobic ions The advantage of using this technique, which is commonly employed for the study of electrochemical processes with metallic electrodes, has been largely detailed by Fedberg and Kissel [7] The results given here by this relaxation method can be considered to correspond to the limiting value of the membrane conductance at both zero voltage and zero time, as the technique depends on the application during a few ms of a very small voltage, which in this work does not exceed 3 mV.

THEORETICAL

The small values for the membrane resistance obtained with hydrophobic ions, as compared to those where inorganic ions are present in the aqueous solutions [8], indicate that only the former ions can penetrate into the membrane. This fact is corroborated by the sign of the Nernstian transmembrane potential under zero current conditions [4].

The membrane model which is considered here is composed of three parts: a hydrocarbon layer of thickness d, situated between the planes $x = O$ and $x = d$ with, on both sides of this hydrocarbon leaflet, two interfacial layers made up by the polar groups of the lipid molecules and more or less orientated water molecules. At each interface one can define two planes of minimum approach for the permeant ions. Firstly, outside the hydrocarbon layer, plane 1 (abscissa $= x_1$) can be considered as the inner Helmholtz plane, with respect to the aqueous solution, at which permeant ions are absorbed, while secondly, inside the hydrocarbon medium, the plane 2 (abscissa x_2) can be assimilated at the same time to the inner and the outer Helmholtz plane with respect to the hydrocarbon phase, since the lipophilic ions are not hydrated. As in the case of the layer between the inner Helmholtz plane and that of the electrode at a metal solution interphase, the dielectric constant ε_L between the planes 1 and 2 is considered to be space independent [9] Inside the hydrocarbon phase, the standard chemical potential and the diffusion coefficient of the permeant ion are assumed to be space dependent and their values are symmetrical with respect to the central plane ($x = d/2$) [10]. The adsorption of ions from the aqueous solution to plane 1 will obey the same law as at an oil-water interface [11]. The number of ions in plane 1 (n_i^1) is related to the ionic concentrations in the bulk of the aqueous solution (c_i^w) by the well known equation.

$$n_i^1 = K f^w (f')^{-1} c_i^w \exp\left(-\frac{zF\Phi^1}{RT}\right) \qquad \text{with } K = \exp\left[\frac{\mu_i^{0w} - \mu_i^{01}}{RT}\right] \tag{1}$$

Where Φ^1 is the electric potential in the plane 1, if the potential in the bulk of the aqueous solution is taken as zero. f^w is the activity coefficient in the water phase and f' a parameter depending on $n_i{}^1$ which can be considered as a surface activity coefficient or as a Langmuirian correction factor. The limiting value of f' is unity when n'_i tends to zero. The passage of ions i from plane 1 to plane 2 can be assimilated to an ion-transfer reaction from an aqueous solution to one non-miscible with water [12]. If the existence of an energy barrier between these two planes is taken into account, the interfacial current and the potential difference $\Phi^2-\Phi^1$ are related by the following equation:

$$\frac{I}{zF} = k_f n_i^1 \exp\left[\frac{\alpha F(\Phi^1-\Phi^2)}{RT}\right] - k_b c_i^*(x_2) \exp\left[-\frac{(1-\alpha)F(\Phi^1-\Phi^2)}{RT}\right] \tag{2}$$

The asterisk refers to the hydrocarbon phase, k_f and k_b are the forward and the backward rate constants of the ion-transfer reaction from plane 1 to plane 2 while α is a transfer coefficient having the same significance as the one used in the case of electrochemical reactions at an electrode in a solution. The displacement of the permeant ion in the hydrocarbon phase between the planes x_2 and $d-x_2$ obeys the Nernst-Planck electrodiffusion law applied to a medium where the standard chemical potential and the coefficient diffusion are continuous functions of the distance. Assuming that there is only one ionic flux and that there is no possibility of charge accumulation between x_2 and $d-x_2$, the flux of permeant ions i will have, at a given instant, the same value at each point between x_2 and $d-x_2$. Thus in view of the constant field approximation, the flux of permeant ion between x_2 and $d-x_2$ will be given by the following relation:

$$J_i^* = \frac{\exp\left(\frac{\mu^{0*}_{i(x_2)}}{RT}\right)\left[c^*_{i(x_2)} \exp\left(\frac{zF\Phi_2}{RT}\right) - c^*_{i(d-x_2)} \exp\left(\frac{zF\Phi_{(d-x_2)}}{RT}\right)\right]}{\int_{x_2}^{d-x_2} \frac{1}{D_i^*} \exp\left(\frac{\mu^{0*}_{i(x)}}{RT} + \frac{zF\Phi_{(x)}}{RT}\right) \mathrm{d}x} \tag{3}$$

where D_i is the diffusion coefficient of ion i in the hydrocarbon layer. In view of this somewhat cumbersome equation, it is impossible to obtain a relatively simple relation between the limiting membrane conductance λ^0 at zero time and the corresponding voltage, except in certain special cases. For instance, when the ion-transfer reaction between plane 1 and 2 is a reversible process, the ion-transfer resistance can be neglected and in view of the symmetry of the system, the potential difference $\Phi_{(x_2)}-\Phi_{(d-x_2)}$ can be considered as equal to the voltage applied across the two aqueous solutions as long as the diffuse layer potential Φ^1 and the potential drop between the two planes of closest approach $(\Phi^2-\Phi^1)$ have symmetrical values at the two interfaces.

Under coulostatic conditions, if the composition of the membrane remains unchanged and the applied voltage is very small, Eqn 3 gives:

$$\lambda^0 = \beta c^*_{i(x_2)} \tag{4}$$

with

$$\beta \approx \frac{z^2F^2}{RT} \exp\left(\frac{\mu^{0*}_{i(x_2)}}{RT}\right)\left[\int_{x_2}^{d-x_2} \frac{1}{D_i^*} \exp\left(\frac{\mu^{0*}_{i(x)}}{RT}\right) \mathrm{d}x\right]^{-1}$$

If one takes into account the similarity between the charge transfer process at the metal-solution interface and the aqueous solution-water non-miscible solution interface, the idea of the reversibility of the ion transfer between planes 1 and 2, which in turn leads one to assume the continuity of the electrochemical potential at the membrane interface, is justified by the low values of the applied voltage and of the current crossing the membrane during the measurement

Hence in this particular case Eqn 2 yields.

$$c^*_{i(x_2)} = n_i^1 K' \exp\left[-\frac{zF(\Phi^2-\Phi^1)}{RT}\right] \tag{5}$$

with

$$K' = \frac{k_f}{k_b} = \exp\left[\frac{\mu_i^{01}-\mu^{0*}_{i(x_2)}}{RT}\right]$$

μ_i^{01} is the standard chemical potential of the ion on plane 1 while the total charge of the membrane is given by:

$$q^m = zF\int_{x_2}^{d-x_2} c^*_{i(x)}\,dx \tag{6}$$

If one takes into account that the composition of membrane, during the measurement, remains the same as at equilibrium, then with the constant field approximation, Eqn 6 can be written in the form

$$q^m = z\gamma c^*_{i(x_2)} \tag{7}$$

with:

$$\gamma = F\int_{x_2}^{d-x_2} \exp\left(\frac{\mu^{0*}_{i(x)}-\mu^{0*}_{i(x_2)}}{RT}\right)$$

A Gouy-Chapman diffuse layer stretches out from plane 1 toward the bulk of the aqueous solution, it has a total charge that compensates the charges distributed on plane 1 (σ^1) and half of the membrane charge. The classical Gouy-Chapman calculation gives a relation between Φ^1, and the total charge which compensates the diffuse space charge. In the case where a 1–1 electrolyte is in excess with respect to the permeant ion, Φ^1 is expressed as.

$$\Phi^1 = \frac{2RT}{F}\ln\left[\frac{q^{m/2}+\sigma^1}{B} + \sqrt{\left(\frac{q^{m/2}+\sigma^1}{B}\right)^2+1}\right] \tag{8}$$

with:

$$B = \frac{\varepsilon_w \kappa RT}{2\pi F} \tag{8'}$$

($1/\kappa$ = Debye length)

The layer between planes 1 and 2 possesses properties similar to those of the metal solution compact double-layer; in particular, the potential varies linearly and is due only to the oriented dipoles of the water molecules and lipid polar groups. The potential difference between plane 1 and 2 is thus:

$$\Phi^2 - \Phi^1 = \frac{4\pi\delta}{\varepsilon_L} \tag{9}$$

where δ is the average dipole moment distribution between planes 1 and 2.

EXPERIMENTAL

As indicated, the measurements are based on the application of a very brief coulostatic impulse (from 3 to 50 μs) which, by charging the membrane capacitance, gives rise to a potential difference never exceeding, in any case, more than 3 mV. The coulostatic impulse is obtained by means of a pulse generator in series with a resistance greater than 1 M Ω while the potential decrease with time after the impulse is recorded on a storage oscilloscope. During the pulse the variation of the current with time is given by.

$$I = \frac{1}{R_0} \cdot \frac{E}{R_0 + R_M} \left[R_0 + R_M \exp\left(- \frac{(R_0 + R_M)t}{C_M \cdot R_0 R_M} \right) \right] \tag{10}$$

Where R_0 is the resistance in series with the pulse generator, E is the voltage level of the pulse and R_M and C_M the resistance and the capacitance of the membrane respectively. If I_0 is the intensity of the current when $t = 0$, then for short times, Eqn 10 gives.

$$\frac{I_0 - I}{I_0} = \frac{t}{R_0 C_M} \tag{11}$$

The values of the bilayer capacitances usually do not differ very much and the duration of the pulse θ is chosen so that·

$$\frac{\theta}{R_0 C_M} < 10^{-3} \tag{12}$$

This means that during the pulse the current remains practically constant.

The variation of the potential difference between the two aqueous solutions, during the pulse, is given by:

$$V = I_0 R_M \left[1 - \exp\left(- \frac{t}{R_M C_M} \right) \right] \tag{13}$$

which when t is small reduces to:

$$V = \frac{I_0 t}{C_M} \tag{14}$$

Eqn 14 simply means that during the brief period of the impulse the current is essentially a capacitance charging current, the membrane behaving like a capacitor without a leak. The values of E, θ and R_0 are chosen so that the value of V at the end of the pulse should not be greater than 3 mV. In order to obtain the true value of C_M from Eqn 14 a preliminary measurement is sometimes necessary. After the pulse, the membrane capacitance is discharged through the two parallel resistances R_0 and R_M. From the recorded time-voltage curve, the membrane conductance is deduced from the value of the time constant τ·

$$\lambda^0 = \frac{C_M \; R_0 \cdot 1/\tau - 1}{R_0} \cdot S$$

where S is the membrane area.

A Philips (PM 5711) pulse generator and a Tektronix (549+W) storage oscilloscope with an imput impedance higher than $5 \cdot 10^4$ M Ω were used.

The method of Szabo et al. [13] was used for the preparation of the bilayer membranes on a ‘Teflon’ cup wall with a hole of 1 mm diameter The electrodes, which are carefully cleaned before each measurement, are constituted of silver plate of 1 cm^2 covered with silver chloride. The lipid bilayer is formed from a solution 7 mM glycerylmonooleate (Sigma) in n-decane. Sodium tetraphenylborate (Merck), tetraphenylarsonium (Fluka pa) and dipicrylamine (Fluka puriss) were used without further purification.

RESULTS

Fig. 1 shows the linear decrease with time of the logarithm of the potential difference between the two aqueous solutions after a coulostatic impulse. The shapes of the curves representing log λ^0 with the logarithm of the hydrophobic ion concentra-

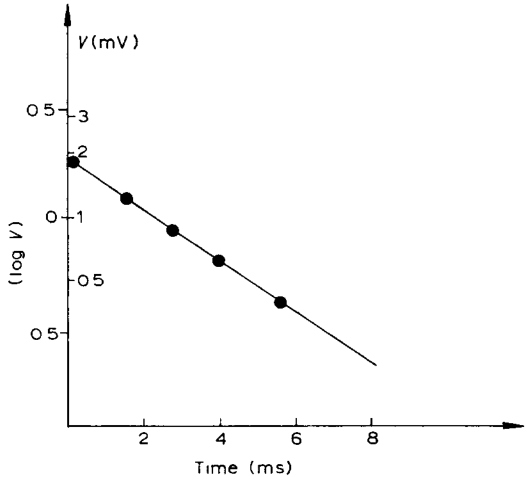

Fig 1 Decrease of the potential difference between the two aqueous solutions after the coulostatic impulse Composition of the aqueous solution sodium tetraphenylborate 3 10^{-4} M+NaCl 10^{-1} M

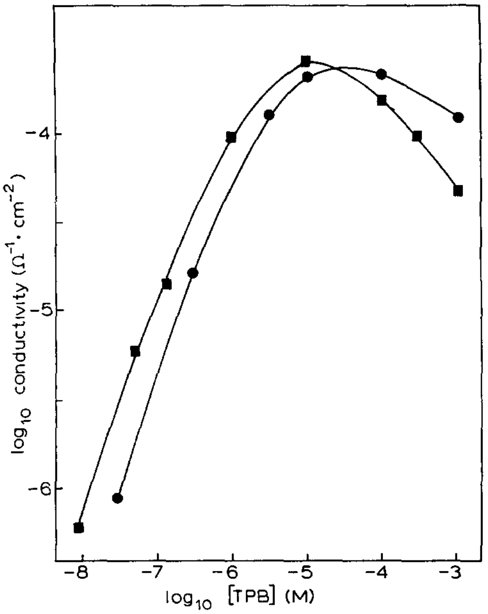

Fig 2 Variation of the membrane conductance with the concentration of tetraphenylborate ions (TPB) in presence of NaCl 10^{-1} M (■) and $5 \cdot 10^{-3}$ M (●).

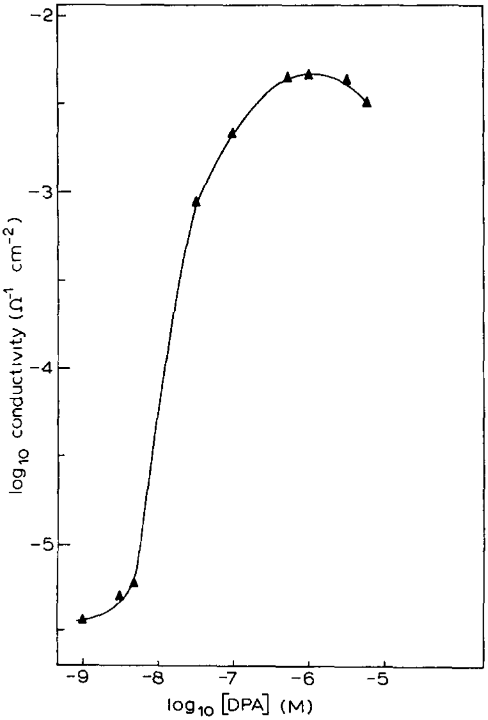

Fig. 3. Variation of the membrane conductance with the concentration of dipicrylamine ions (DPA) in presence of NaCl 10^{-1} M.

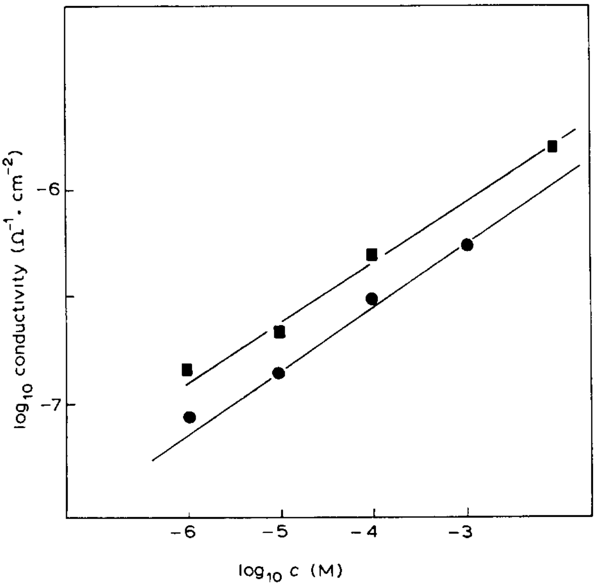

Fig. 4. Variation of the membrane conductance with the concentration of picrate ion (●) and of tetraphenylarsonium (■) in the presence of NaCl 10^{-1} M

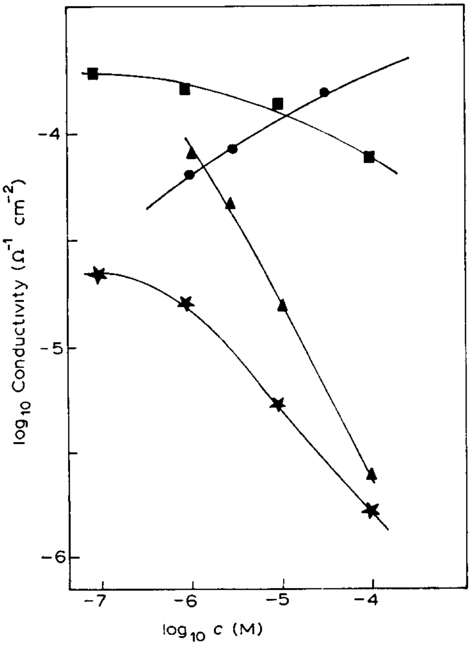

Fig 5 Variation of the membrane conductance with dodecyl sulphate ion concentration, with tetraphenylborate ions NaCl, 10^{-1} M. Concentration of tetraphenylborate ★, 3 10^{-7} M, ▲, 10^{-6} M; ■, 5 10^{-5} M; ●, $3 \cdot 10^{-4}$ M

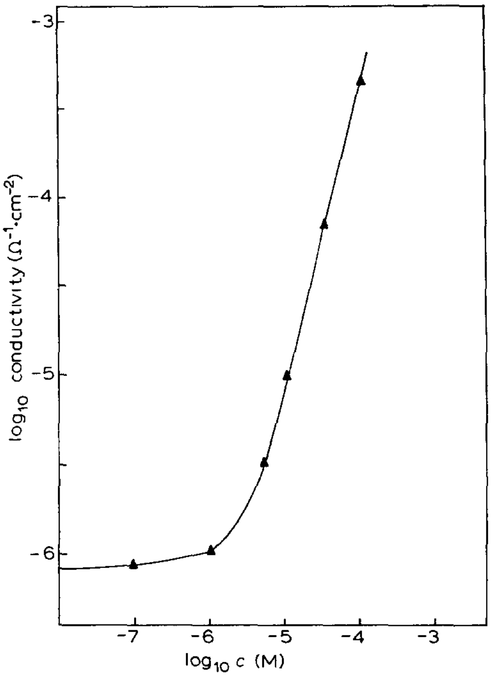

Fig. 6. Variation of the membrane conductance with the concentration of dodecyl sulphate ions with tetraphenylarsonium chloride 10^{-3} M+NaCl 10^{-1} M.

tion obtained with tetraphenylborate and dipicrylamine ions (Figs 2 and 3) are similar to those obtained by Ketterer et al. [5]. However, the conductance here depends on the ionic strength. With picrate and tetraphenylarsonium ions, the membrane conductance is lower and no longer passes through a maximum (Fig. 4)

Haydon and Myers [14] have shown that the dodecyl sulphate ion is strongly adsorbed at the interface of a similar membrane. The membrane conductance in the presence of this surface-active ion is very small (less than $2 \cdot 10^{-7}$ $\Omega^{-1} \cdot cm^{-2}$ in the 0–10^{-4} M concentration range). The effect of the adsorbed dodecyl sulphate ions on the membrane conductance in the presence of tetraphenylborate ions differs, dependding whether the concentration of the latter ion is greater or smaller than that corresponding to the conductance maximum. Fig. 5 indicates that for concentrations smaller than at this maximum, the addition of dodecyl suplhate ions decreases the conductance. This effect is in agreement with previous results [14–16], indicating a decrease of conductance when the membrane surface acquires a charge of the same sign as the permeant ion. On the other hand, for concentrations higher than the maximum conductance concentration, the addition of dodecyl sulphate ions leads to a slight increase in the conductance.

In the concentration range where the solubility product of tetraphenylarsonium dodecyl sulphate is not yet reached, the addition of dodecyl sulphate ions gives rise to a rapid increase of the membrane conductance (Fig. 6).

DISCUSSION

Up till now, only a kinetic approach has been used [4, 5, 16] to account for the conductance of bilayers in the presence of hydrophobic ions. Here an attempt is made to relate the limiting conductance to the electro-diffusion concept as applied to the hydrocarbon core of the membrane. While the previous kinetic treatments are general and may be applied both to low voltage and high voltage conditions, our study is strictly restricted to very small values of the applied voltage. One is thus entitled to consider that the ion adsorption on plane 1 and the ion transfer between planes 1 and 2 are both reversible processes. The limiting membrane conductance, which under these conditions is nothing more than the conductance of the hydrocarbon core at the equilibrium state, enables one to establish how the distribution of the permeant ions between the aqueous solution and the hydrocarbon core depends on the electrical double layer and the interfacial compact layer.

Thus, using Eqns 1, 4, 5, 7, 8 and 9, a general relation can be obtained relating the limiting membrane conductance λ^0 and c_i^w, the permeant ion concentration in the bulk solution

$$\lambda^0 = Mc_i^w \left[zv\lambda^0 + \frac{\sigma^1}{B} + \sqrt{\left(zv\lambda^0 + \frac{\sigma^1}{B} \right)^2 + 1} \right]^{-2z} \tag{16}$$

with

$$M = K \cdot K' \; f^w (f')^{-1} \exp\left(- \frac{zF4\pi\delta}{RT\varepsilon_L} \right)$$

and

$$v = \frac{\gamma}{2\beta B}$$

These relations cannot be used directly since no exact information is available concerning the charge distribution on plane 1 (σ^1), the coefficient f′ and the average dipole density δ in the compact layer. Nevertheless, the effect of the addition of dodecyl sulphate ion can be accounted for by Eqn 16 in the range of low permeant ion concentration where f′ and δ may be assumed to have constant value and where the surface charge, due to the adsorption of the added surface-active ion, can be varied independently of the charge of the hydrocarbon core When the permeant ion concentration is maintained constant, the addition of dodecyl sulphate ion to the aqueous solution induces a negative charge distribution on plane 1 Further more, when the charge of the permeant ion is also negative, there is a decrease in the conductance as can be observed with tetraphenylborate ion (Fig. 5) for concentrations lower than the concentration corresponding to the maximum conductance On the other hand, when the permeant ion is positive, the membrane conductance is increased, as in the case of tetraphenylarsonium ion (Fig. 6), where a strong synergetic effect is observed.

Let us consider the particular case where the hydrophobic ion is strongly adsorbed onto plane 1, so that the total charge inside the hydrophobic core is small with respect to the charge on plane 1 due to the adsorbed permeant ions. In this case the charge density of plane 1 is related to the number of permeant ions fixed on this plane (n'_i) by the evident relation:

$$\sigma^1 = zn^1_i$$

From equations (1) and (2), it appears that in the range where f′ remains constant in the particular case of univalent permeant ions, n^1_i tends to become proportional to the cubic root of c_i^w, whatever the sign of the ion. In this case, the slope of the log λ^0 —log c_i^w curve tends to 1/3 as can be observed with picrate and tetraphenylarsonium ions (Fig. 4) where the measured conductances are small compared to those obtained with tetraphenylborate and dipicrylamine ions. The large difference in the membrane conductance for tetraphenylborate and tetraphenylarsonium may be attributed, as suggested by Haydon and Hladky [16], to a positive value of the potential drop between plane 2 and 1, ($\Phi^2 - \Phi^1$), due to the average dipole density of water molecules located between two planes and of the polar group of the lipids. The low conductance observed for picrate ions in spite of their negative charge, may be due not only to a hydrophobic character less pronounced than for tetraphenylborate, but also to the possibility of the formation of hydrogen bonds with the glyceryl group of the lipid used.

One can now examine the more general case where the permeant ion is again adsorbed on plane 1 but the charge of the hydrocarbon core is not negligible with respect to that on plane 1.

The discussion is restricted to the case where the total charge of the membrane is small, justifying the use of the classical Gouy-Chapman approximation·

$$\frac{q^m}{2} + zn^1_i \approx \frac{BF\Phi^1}{2RT} \tag{17}$$

Introducing Eqn 17 into Eqn 1, and taking into account Eqns 4, 5 and 7, one may deduce, if one considers only the first two terms of the exponential series·

$$\lambda^0 = \frac{K \cdot f^w(f')^{-1} c_i^w}{\dfrac{1}{\beta K'} \exp \dfrac{zF4\pi\delta}{RT\varepsilon_L} + K \cdot f^w(f')^{-1} \dfrac{2z^2}{\beta B} \left[\dfrac{\gamma}{2} + \dfrac{1}{K'} \exp\left(\dfrac{zF4\pi\delta}{RT\varepsilon_L}\right)\right]}$$

In the range where δ and f' are constant, λ^0 appears to be proportional to c_i^w. This variation of the membrane conductance has been observed with tetraphenylborate ion (Fig. 2) at low concentrations. The same result has been obtained not only with tetraphenylborate ion but also with dipicrylamine by Ketterer et al. [5] in the case of a bilayer constituted by an other lipid

In this concentration range, the membrane conductance is higher for a 10^{-1} M NaCl solution than one $5 \cdot 10^{-3}$ M (Fig. 2). This effect of the ionic strength on the membrane conductance is in accordance with the variation which can be expected from Eqn 18 if one recalls the significance of parameter B.

It is not yet possible to explain the slope of the conductance curves obtained with dipicrylamine and with tetraphenylborate in the higher concentration range. Several factors may affect the parameters f' and δ; for example, δ depends not only on the orientation and on the dipolar moment of the polar lipid groups but also on the number of water molecules bound to these polar groups, and the latter may also influence the parameter f' Moreover, the possible adsorption of ions of the supporting electrolyte and their effective size have not been taken into consideration.

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