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IONIC PERMEATIVE PROPERTIES OF LIPID BILAYER MEMBRANES

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SUMMARY

Relations are derived for the isotopic permeability coefficient, electrical resistance and the diffusion potential for a simple model membrane in which the flows of the mobile species, *i.e.* two ions and water, are mutually coupled. Results are presented for membrane resistance and diffusion potential for a lipid bilayer system. In terms of the chosen membrane model any coupling between ion and water flow in the bilayer is shown to be negligible. Some simple relationships between the phenomenological coefficients, descriptive of the flows, are obtained and discussed in terms of possible membrane models.

INTRODUCTION

Much of the research on lipid bilayer membranes has been aimed at eliciting permeation characteristics from measurements of the flow rate of radio-isotopes, of the electrical resistance and of the diffusion potentials developed under concentration gradients.

The first part of this report contains derivations of thermodynamic relations suitable for such data in the case of a simple model membrane in which the flows of the mobile species, *i.e.* two ions and water, are mutually coupled. This is followed by the presentation of results for membrane resistance and diffusion potential, which were obtained for a lipid bilayer system in a number of electrolyte solutions and for a wide range of concentrations. The membranes investigated were fairly large (approx. 40 mm^2) and were made from bulk mixtures containing egg lecithin and cholesterol dissolved in a chloroform–methanol–tetradecane–heptane solvent mixture.

When the results are interpreted in terms of the chosen membrane model it is shown that any coupling between ion and water flow is negligible. This enables some simple relationships between the phenomenological coefficients, descriptive of the flows, to be obtained. These are discussed in terms of some possible coupling mechanisms between the positive and negative ionic fluxes.

THEORETICAL

Introduction

In the present section expressions are obtained, following the methods of others[1-4], which involve the coefficients of membrane permeability which characterise

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(a) tracer flow, (b) voltage–current characteristics and (c) concentration potentials.

The system considered is isothermal and consists of a membrane separating two compartments of well-mixed electrolyte solutions. The membrane is regarded as containing a matrix of parallel pathways which have identical transport properties. All flows are treated as steady in time and in the x direction which is taken as perpendicular to the membrane. The planes $x = 0$ and $x = \delta$ define the internal and external membrane boundaries respectively.

The basic equations are the expression for the negative gradient in electrochemical potential for the ith diffusing species

$$-\frac{d\mu_i}{dx} = -RT\frac{d(\ln a_i)}{dx} - \bar{v}_i\frac{dP}{dx} - \frac{d(z_iFV)}{dx} \tag{1}$$

the phenomenological equation

$$-\frac{d\mu_i}{dx} = R_{ii}\Phi_i + \sum_{j \neq i} r_{ij}\Phi_j \tag{2}$$

and the Onsager relation

$$r_{ij} = r_{ji} \tag{3}$$

where R denotes the gas constant, T temperature, a chemical activity, $\bar{v}$ partial molar volume, P pressure, z valence, Φ flux, F Faraday's constant, V electric potential and R_{ii} and r_{ij} are phenomenological coefficients.

The signs of the coefficients R_{ii} and r_{ij} have been discussed by KEDEM AND KATCHALSKY[3]. The coefficients R_{ii} are always positive. In the most common case the coefficients r_{ij} are frictional in origin and are negative. However, r_{ij} may become positive in the case of carrier transport.

In the following treatment the convention is adopted that the total flux of a particle species is the algebraic sum of the one-way fluxes. Thus effluxes are regarded as positive quantities and influxes as negative.

Tracer flow

It is desired to obtain an expression for the isotopic permeability coefficient of a permeative species for the case when the membrane separates two identical electrolyte solutions, there being no applied potentials or hydrostatic pressure differences.

The subscripts p, n and w will be used when quantities relate to the positive ions, negative ions and water respectively. The symbol * will be used when quantities relate to a labelled isotope. The symbols $\Phi_{1,p}$ and $\Phi_{2,p}$ will be used to denote the influx and efflux of the positive ions.

Considering first the general case where there is a net flow of positive ions, the net flux Φ_p is the sum of the one-way fluxes, *i.e.*

$$\Phi_p = \Phi_{1,p} + \Phi_{2,p} \tag{4}$$

The unidirectional fluxes are estimated using radioactive tracers. In the case of perfect mixing the deduced flux ratio satisfies[4,5]

$$\frac{\Phi_{1,p}}{\Phi_{2,p}} = -\exp\left(-\frac{\Phi_p}{RT}\int_0^\delta (R_{pp} - r_{pp}^*)dx\right) \tag{5}$$

where r_{pp}^* is the resistance coefficient between the labelled and unlabelled ions. Eqns. 4 and 5 yield

$$\Phi_{1,p}\left\{1-\exp\left(+\frac{\Phi_p}{RT}\int_0^\delta (R_{pp}-r_{pp}^*)\mathrm{d}x\right)\right\} = \Phi_p \tag{6}$$

Since for sufficiently small x, $e^x \simeq 1 + x$ Eqn. 6 gives for $\Phi_p \to 0$

$$\Phi_{1,p} = -\frac{RT}{\int_0^\delta (R_{pp}-r_{pp}^*)\mathrm{d}x} \tag{7}$$

whence

$$P_{is} = \frac{RT}{c(\delta)\int_0^\delta (R_{pp}-r_{pp}^*)\mathrm{d}x} \tag{8}$$

is the expression for the isotopic permeability coefficient under conditions where the total species flux is zero and where there is perfect mixing.

When stirring is non-ideal local variations in isotope concentration may occur in the "stagnant layers" near the membrane boundaries[6, 7] and render unknown the specific activity of the test species at the boundaries. It is fairly easy to show that the preceding treatment is valid for this case except that the integration limits in Eqns. 5, 6, 7 and 8 are then X_1 and X_2 where the planes $x = X_1$, and $x = X_2$ define the loci of those points in the inner and outer compartments respectively where local concentrations first attain the values of the bulk concentrations. Thus for this case the formula for the permeability coefficient becomes

$$P_{is} = \frac{RT}{c(X_2)\int_{X_1}^{X_2} (R_{pp}-r_{pp}^*)\mathrm{d}x} \tag{9}$$

Eqns. 8 and 9 are the required expressions and analogous relations apply for the negative ions and for water. Whereas the coefficient R_{ii} is always positive the coefficient r_{ii}^* may be positive or negative* subject to the restriction** that

$$R_{ii} - r_{ii}^* > 0 \tag{10}$$

The voltage-current expression

In the presence of applied voltages the positive and negative ions will drift in opposing directions. The membrane resistance will be influenced by such factors as a coupling between the ionic fluxes or between their fluxes and that of water.

From Eqn. 2

$$-\frac{\mathrm{d}\mu_p}{\mathrm{d}x} = R_{pp}\Phi_p + r_{pn}\Phi_n + r_{pw}\Phi_w \tag{11a}$$

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* The possible signs of r_{ii}^* have been discussed by KEDEM AND ESSIG[4].

** This renders the permeability coefficient positive.

$$-\frac{\mathrm{d}\mu_\mathrm{n}}{\mathrm{d}x} = r_\mathrm{np}\Phi_\mathrm{p} + R_\mathrm{nn}\Phi_\mathrm{n} + r_\mathrm{nw}\Phi_\mathrm{w} \tag{11b}$$

$$-\frac{\mathrm{d}\mu_\mathrm{w}}{\mathrm{d}x} = r_\mathrm{wp}\Phi_\mathrm{p} + r_\mathrm{wn}\Phi_\mathrm{n} + R_\mathrm{ww}\Phi_\mathrm{w} \tag{11c}$$

It will be supposed in the following that the adjacent electrolyte solutions are uni–univalent, have equal concentrations and that hydrostatic pressure gradients exist.

Integration of Eqns. 11a, 11b yields, after use of Eqn. 1*

$$z_\mathrm{p}FV_\mathrm{m} - \int_{X_1}^{X_2} \bar{v}_\mathrm{p}\mathrm{d}P = \Phi_\mathrm{p} \int_{X_1}^{X_2} R_\mathrm{pp}\mathrm{d}x - \Phi_\mathrm{n} \int_{X_1}^{X_2} r_\mathrm{pn}\mathrm{d}x + \Phi_\mathrm{w} \int_{X_1}^{X_2} r_\mathrm{pw}\mathrm{d}x \tag{12a}$$

$$z_\mathrm{n}FV_\mathrm{m} - \int_{X_1}^{X_2} \bar{v}_\mathrm{n}\mathrm{d}P = \Phi_\mathrm{p} \int_{X_1}^{X_2} r_\mathrm{np}\mathrm{d}x + \Phi_\mathrm{n} \int_{X_1}^{X_2} R_\mathrm{nn}\mathrm{d}x + \Phi_\mathrm{w} \int_{X_1}^{X_2} r_\mathrm{nw}\mathrm{d}x \tag{12b}$$

Henceforth the integration limits will be understood to be X_1 and X_2. The total current density J is given by

$$J = F(\Phi_\mathrm{p} - \Phi_\mathrm{n}) \tag{13}$$

Substitution for Φ_n from Eqn. 13 into Eqns. 12a, 12b gives

$$z_\mathrm{p}FV_\mathrm{m} - \int \bar{v}_\mathrm{p}\mathrm{d}P = \Phi_\mathrm{p} \int (R_\mathrm{pp} + r_\mathrm{pn})\mathrm{d}x - \frac{J}{F}\int r_\mathrm{pn}\mathrm{d}x + \Phi_\mathrm{w} \int r_\mathrm{pw}\mathrm{d}x \tag{14a}$$

$$z_\mathrm{n}FV_\mathrm{m} - \int \bar{v}_\mathrm{n}\mathrm{d}P = \Phi_\mathrm{p} \int (R_\mathrm{nn} + r_\mathrm{np})\mathrm{d}x - \frac{J}{F}\int R_\mathrm{nn}\mathrm{d}x + \Phi_\mathrm{w} \int r_\mathrm{nw}\mathrm{d}x \tag{14b}$$

Elimination of Φ_p from Eqns. 14a, 14b leads to the following expression for the membrane potential, in terms of electric current, water flux and pressure difference.

$$\begin{aligned} V_\mathrm{m} = {} & \frac{J}{F^2}\,\frac{A_\mathrm{p}\int R_\mathrm{nn}\mathrm{d}x - A_\mathrm{n}\int r_\mathrm{pn}\mathrm{d}x}{z_\mathrm{p}\int (R_\mathrm{nn} + r_\mathrm{np})\mathrm{d}x - z_\mathrm{n}\int (R_\mathrm{pp} + r_\mathrm{pn})\mathrm{d}x} + \\ & + \frac{1}{F}\,\frac{A_\mathrm{n}\int \bar{v}_\mathrm{p}\mathrm{d}P - A_\mathrm{p}\int \bar{v}_\mathrm{n}\mathrm{d}p}{z_\mathrm{p}\int (R_\mathrm{nn} + r_\mathrm{np})\mathrm{d}x - z_\mathrm{n}\int (R_\mathrm{pp} + r_\mathrm{pn})\mathrm{d}x} + \\ & + \frac{\Phi_\mathrm{w}}{F}\,\frac{A_\mathrm{n}\int r_\mathrm{pw}\mathrm{d}x - A_\mathrm{p}\int r_\mathrm{nw}\mathrm{d}x}{z_\mathrm{p}\int (R_\mathrm{nn} + r_\mathrm{np})\mathrm{d}x - z_\mathrm{n}\int (R_\mathrm{pp} + r_\mathrm{pn})\mathrm{d}x} \end{aligned} \tag{15}$$

where

$$A_\mathrm{p} = \int (R_\mathrm{pp} + r_\mathrm{pn})\mathrm{d}x;\ A_\mathrm{n} = \int (R_\mathrm{nn} + r_\mathrm{np})\mathrm{d}x$$

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* Continuity of μ at an interface is presupposed.

With regard to the term involving Φ_w in Eqn. 15, the osmotic permeability coefficient for water in lecithin–cholesterol membranes probably exceeds $2 \cdot 10^{-3}$ μ/sec per atm (refs. 7–9). The membranes dealt with in EXPERIMENTAL could be bowed to a hemisphere under a pressure difference of about $2 \cdot 10^{-5}$ atm so that the volume flow of water per cm^2 of hemispherical membrane probably exceeded $4 \cdot 10^{-12}$ ml/sec. The corresponding value for the water flux is about $0.2 \cdot 10^{-12}$ mole/cm^2 per sec. This compares with a total ionic flux (J/F) of $0.2 \cdot 10^{-13}$ mole/cm^2 per sec when a potential difference of 20 mV is applied, the membrane resistance being typically about $1 \cdot 10^7$ $\Omega \cdot$cm^2.

The calculations of the previous paragraph suggest that when small voltages (< 20 mV) are applied to hemispherical membranes the contributions to the total current due to the applied voltage and due to the coupling of ion movement to the water flux might be of similar magnitudes. If this were the case, then the term which involves Φ_w in Eqn. 15 would be comparable with the term involving J, and the V–J curve would bypass the origin.

By contrast, a V–J plot passing through the origin for a hemispherical membrane would be interpreted on the present treatment as implying that the water-flux and pressure terms in Eqn. 15 are negligible compared with the current term. For this case the voltage–current relation becomes by use of Eqn. 3

$$V_m = \frac{J}{F^2} \frac{\int R_{nn} dx \int R_{pp} dx - \left(\int r_{np} dx\right)^2}{z_p \int (R_{nn} + r_{np}) dx - z_n \int (R_{pp} + r_{pn}) dx} \tag{16}$$

The diffusion potential

It will be supposed in the present section that the membrane separates two solutions of the same electrolyte, but of different concentrations, and that there are no hydrostatic pressure gradients. In the steady state

$$\Phi_p = \Phi_n \tag{17}$$

Eqns. 11a, 11b and 17 yield

$$\Phi_p \int (R_{pp} + r_{pn}) dx = -RT \ln \frac{a_{p,2}}{a_{p,1}} + z_p F V_m - \Phi_w \int r_{pw} dx$$

$$\Phi_n \int (R_{nn} + r_{np}) dx = -RT \ln \frac{a_{n,2}}{a_{n,1}} + z_n F V_m - \Phi_w \int r_{nw} dx$$

where $a_{p,2}$, $a_{p,1}$, $a_{n,2}$, $a_{n,1}$ are the chemical activities at $x = X_2$, $x = X_1$. Taking ratios and assuming an equality between activity and concentration, we obtain for the steady state

$$\frac{\int (R_{pp} + r_{pn}) dx}{\int (R_{nn} + r_{np}) dx} = \frac{RT \ln \frac{c_2}{c_1} - z_p F V_m + \Phi_w \int r_{pw} dx}{RT \ln \frac{c_2}{c_1} - z_n F V_m + \Phi_w \int r_{nw} dx}$$

where c_2 and c_1 denote the solute concentration at $x = X_2$ and $x = X_1$, respectively. Rearrangement of terms and use of Eqn. 3 gives

$$V_m = \frac{RT}{F} \frac{\int R_{nn}\mathrm{d}x - \int R_{pp}\mathrm{d}x}{z_p \int (R_{nn} + r_{np})\mathrm{d}x - z_n \int (R_{pp} + r_{pn})\mathrm{d}x} \ln \frac{c_2}{c_1} + \frac{\Phi_w}{F} \frac{A_n \int r_{pw}\mathrm{d}x - A_p \int r_{nw}\mathrm{d}x}{z_p \int (R_{nn} + r_{np})\mathrm{d}x - z_n \int (R_{pp} + r_{pn})\mathrm{d}x} \tag{18}$$

Eqn. 18 expresses the diffusion potential as a function of the concentration ratio of the adjacent electrolyte solutions and of the net water flux in the membrane. When the coupling between ion and water flow is negligible then

$$V_m = \frac{RT}{F} \frac{\int R_{nn}\mathrm{d}x - \int R_{pp}\mathrm{d}x}{z_p \int (R_{nn} + r_{np})\mathrm{d}x - z_n \int (R_{pp} + r_{pn})\mathrm{d}x} \ln \left(\frac{c_2}{c_1}\right) \tag{19}$$

Small membrane potentials satisfying

$$| V_m | \ll \frac{RT}{F} \ln \frac{c_2}{c_1} \tag{20}$$

are then obtained only when

$$\int R_{nn}\mathrm{d}x \simeq \int R_{pp}\mathrm{d}x \tag{21}$$

It follows from Eqn. 19, since R_{nn} and R_{pp} are positive while z_n is negative and r_{np} may be negative, that if $-\int r_{np}\,\mathrm{d}x$ exceeds $\int R_{pp}\,\mathrm{d}x$ or $\int R_{nn}\,\mathrm{d}x$*, the membrane potential may exceed what has been conventionally regarded as the theoretical maximum *i.e.*

$$V_m(\max) = \pm \frac{RT}{zF} \ln \frac{c_2}{c_1} \tag{22}$$

This difference in predicted maxima arises because the derivation leading to Eqn. 22 assumes that only conservative forces determine the steady-state potential. However, it seems that if the concentration of the more permeable species is the larger in the membrane, then in the steady state the centre of mass of the less permeable species will be the faster moving. The more permeable species can thus also experience a non-conservative (frictional) force directed towards the compartment of lower concentration.

It is convenient to introduce the term admittance ratio of positive to negative ions defined by

$$1 \Big/ \int R_{pp}\mathrm{d}x \quad : \quad 1 \Big/ \int R_{nn}\mathrm{d}x$$

It appears that a large admittance ratio is not justifiably inferred from Eqn. 18 in

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* Subject to the condition $R_{nn}R_{pp} \geq r_{np}^2$ which is a consequence of positive entropy production[3].

the case of large membrane potentials, since the water-flux term might be predominant. One method for choosing between these possibilities[10] is to examine the effect on membrane potential of changes in the osmotic pressure gradient by the use of solutes which by themselves do not produce significant membrane potentials.

Suitability of the theory for bilayer membranes

In the treatments for the voltage–current relation and the diffusion potential it has been assumed that the current is carried by two ions, and that water is the only other permeative species present. The treatments are thus inapplicable to bilayer systems if conduction is electronic, as has been suggested[11] for membranes containing lecithin or oxidised cholesterol, and which are generated in solutions containing I^-. The treatments are also inapplicable to bilayer systems if conduction is by more than two ions as in the case of mixed electrolyte solutions or solutions of a single electrolyte but of sufficiently small ionic strength so that the ions of the water as well as those of the dissolved electrolyte contribute significantly to charge transport. It seems, therefore, that the most likely bilayer systems for which the treatments might be suitable are those where no I^- are present and where the concentration of electrolyte exceeds 10–100 μM.

EXPERIMENTAL

Measurement of electrical resistance and diffusion potential

Current pulses from a current generator of variable internal resistance ($1 \cdot 10^5$–$1 \cdot 10^9\ \Omega$) were passed through the membrane *via* Pt/Pt black electrodes immersed in the aqueous phases. The transmembrane potential was detected with Ag/Ag halide electrodes placed in close proximity to the membrane and connected to a differential electrometer amplifier. A continuous record of the transmembrane potential and the membrane current was obtained by connecting a two-channel chart recorder to the electrometer output and across a $1 \cdot 10^4\ \Omega$ resistor in series with the current electrodes.

The cell itself was clamped to an antivibration mounting and the whole apparatus placed in a Faraday cage to reduce pick-up of stray signals.

The diffusion potential was measured using Ag/Ag halide electrodes connected to a d.c. amplifier the output of which was displaced on a chart recorder. Concentration gradients were established by generating the membranes in a 10 μM electrolyte solution and then adding known amounts of 10 μM and 1 M solutions of the same electrolyte, simultaneously to the two cell compartments, using coupled syringes. The potential difference across the membrane was calculated by subtracting the theoretical value for the potential developed at the electrodes *i.e.* $(RT/F)\ \ln\ (c_1/c_2)$ from the total measured potential.

Unless otherwise stated all measurements were carried out at room temperature which was 20–22°.

RESULTS

The voltage–current characteristics were approximately linear up to the breakdown potential. The variation of the membrane resistance with the nature and concentration of the electrolyte is shown in Fig. 1. The measured values were variable by a factor of 10. It can be seen that when the anion is I^- the resistance is markedly concentration dependent and decreases from a value of around $4 \cdot 10^7\ \Omega \cdot cm^2$ in 10 μM solutions to about $1 \cdot 10^3\ \Omega \cdot cm^2$ in 1 M solutions. In other cases the resistance is only a slowly varying function of concentration and in 1 M solutions has a value of around $8 \cdot 10^6\ \Omega \cdot cm^2$, when the anion is Cl^- or Br^-, and $2 \cdot 10^7\ \Omega \cdot cm^2$ when the anion is F^-.

The voltage–current curve continued to pass through the origin when the membrane was bowed to a hemisphere. In terms of the stated model any coupling between the ionic fluxes and that of water may therefrore be neglected.

The breakdown potential generally lay between 125 and 175 mV, and appeared to be independent of the nature of the bathing medium, provided that there were no I^- present. However, in LiI, NaI and KI solutions and in mixed 0.1 M NaCl, 0.1 M NaI solutions the breakdown potentials were generally in the range 250–350 mV.

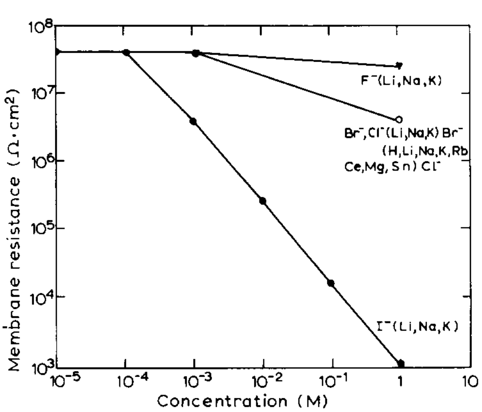
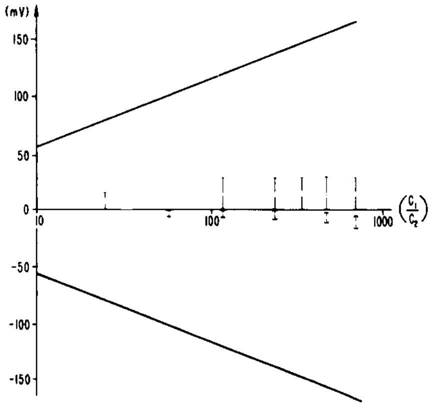


Fig. 1. Variation of membrane resistance with nature and concentration of electrolyte.



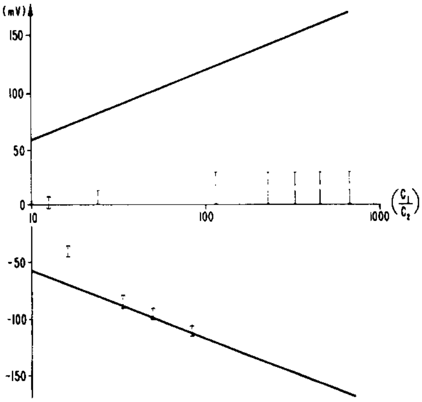


Fig. 2. Diffusion potential measurements for LiCl, NaCl, KCl, RbCl (upper bars), and $CaCl_2$, $MgCl_2$, $SrCl_2$ (lower bars). The upper and lower straight lines satisfy $V_m = \pm (RT/F) \ln (c_2/c_1)$.

Fig. 3. Diffusion potential measurements for NaCl, NaBr (middle and lower bars), and NaI (upper bars). The upper and lower straight lines satisfy $V_m = \pm (RT/F) \ln (c_2/c_1)$.

The measurements for the diffusion potential as a function of the concentration ratio when the lower concentration was 10 μM are shown in Figs. 2 and 3. In these diagrams the upper and lower straight lines satisfy $V_m = \pm (RT/F) \ln (c_2/c_1)$.

In Fig. 2 are shown the results which were obtained for the Cl^- solutions of the monovalent cations. These do not suggest a selectivity between the Li^+, Na^+, K^+ and Rb^+. However, the small positive potentials appear to indicate that the cations have slightly larger permeabilities than the Cl^-.

Fig. 2 also shows the results which were obtained for $CaCl_2$, $MgCl_2$ and $SrCl_2$. The low values for the membrane potential indicate that the transference numbers for the divalent ions and for the Cl^- are approximately equal.

Fig. 3 shows the results obtained for NaBr, NaCl and NaI. It can be seen that the results for NaBr and NaCl are very similar and differ from those for NaI. In this last case the admittance ratio appears to favour the cation, when the variable concentration is about 0.1 mM, and the I^- at higher concentrations.

To check whether the iodide results were due to solvent drag effects, solutions of 1 mM NaI and 10 mM NaI were established adjacent to a membrane. The steady-state membrane potential was 58 mV. NaCl solutions of different concentrations were then added to the compartments until the osmotic pressure gradient was nullified. The final membrane potential was 56 mV. These results are similar to those of LAUGER *et al.*[10], who used membrane mixtures which contained lecithin but no cholesterol and appear to rule out the possibility that the membrane potential arises from solvent drag effects (see page 453).

DISCUSSION

In the first part of this paper expressions have been derived for the permeability coefficient of a labelled isotope, the voltage–current relation and diffusion potential for a membrane, in which the permeative species are two ions and water, their fluxes being mutually coupled. Experiments have been suggested which should enable the importance of terms involving the water flux to be gauged. When these terms are negligible the expressions for the voltage–current characteristics and diffusion potential simplify (see Eqns. 16 and 19) and involve only three phenomenological coefficients *i.e.* R_{pp}, R_{nn} and r_{np}. Since the isotopic permeability coefficient for the positive ions (see Eqn. 9) involves R_{pp} and r_{pp}^* (and for the negative ions R_{nn} and r_{nn}^*) it seems that, except in special cases, permeability, voltage-current and diffusion potential measurements, by themselves do not yield sufficient information for evaluating the individual phenomenological coefficients.

The experimental results indicating the low permeability as evidenced by the electrical resistance measurements and poor selectivity for Na^+, K^+ and Cl^- and the "apparent" high permeability for I^- are similar to findings by ANDREOLI *et al.*[13] for lecithin-decane membranes, HOWARD AND BURTON[9] for lecithin–cholesterol–decane membranes and LAUGER *et al.*[10] for lecithin–decane membranes. It seems likely that all these systems are similar with respect to the poor selectivity found for H^+, Li^+, Na^+, K^+, Br^- and Cl^-, the equality of transference numbers for Ca^{2+}, Mg^{2+}, Sr^{2+} and Cl^-, the diminution in I^- permeability at concentrations less than 0.1 mM, and the enhanced dielectric strength of membranes formed in I^- solutions. This latter effect might be related to the formation of the lecithin iodine complexes reported recently by ROSENBERG AND JENDRASIAK[11].

The voltage–current curves for hemispherical membranes pass through the origin. On the model membrane this implies that the coupling to water flow may be neglected so that the V–J relation is given by Eqn. 16 and the diffusion potential by Eqn. 19. Since the diffusion potentials were generally small, satisfying when no I^- were present Eqn. 20, it follows that $\int R_{pp} \simeq \int R_{nn}\, dx$. Using Eqn. 16 the term $\int (R_{ii} - r_{np})\, dx$, where i denotes either p or n, may therefore be evaluated using the rough resistance values $4\cdot10^3\,\Omega\cdot m^2$ in 0.1 mM (F^-, Br^- and Cl^-) solutions, $2\cdot10^3\,\Omega\cdot m^2$ in 1 M (F^-) solutions and $8\cdot10^2\,\Omega\cdot m^2$ in 1 M (Br^- and Cl^-) solutions. This yields the values $8\cdot10^{13}$ Joules·sec·m²/mole² in 0.1 mM solutions, $4\cdot10^{13}$ Joules·sec·m²/mole² in 1 M (F^-) solutions and $2\cdot10^{13}$ Joules·sec·m²/mole² in 1 M (Br^- and Cl^-) solutions.

Some discussion on possible flow mechanisms follows. The degree of coupling q between the ionic flows may be written as[14]

$$q = -r_{pn}/\sqrt{R_{pp}R_{nn}} \simeq -r_{pn}/R_{ii}$$

where i denotes either p or n. If the ions were solubilised in separate regions of the membrane then the coupling would be weak *i.e.* $|r_{pn}| \ll R_{ii}$. Since $R_{ii} = F/c_iU_i$, where U denotes effective mobility[15], the calculations of the preceding paragraph would then indicate that the product (concentration × mobility), in the membrane, shows only a slight dependence on both the concentration and nature of the external electrolyte. The absence of a strong concentration effect appears understandable in terms of a saturation effect. However, a stronger dependence of R_{ii} on the nature of the permeating ion might be expected for a solubility membrane in which the ions move separately.

An alternative type of transport which superficially at least seems more satisfying is that where there is a strong positive coupling between the ionic fluxes[14] such as might occur if the flows take place in the same regions. High electrical resistances and poor ionic selectivity would be expected as the limiting case of ion pair flow is approached.

Studies on theoretical models are continuing.

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