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# The Effects of a Cyclic Polyether on the Electrical Properties of Phospholipid Bilayer Membranes

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*Summary.* The cyclic polyether XXXII, a neutral, lipid soluble molecule, produces large increases in the conductance of bilayer membranes formed from a variety of lipids. The conductance increases linearly with the concentration of alkali metal cation but with the square, and at higher concentrations the cube, of the polyether concentration. This implies that two or three polyether molecules combine with a single cation to carry it across the membrane. In the presence of XXXII the bilayer is permeable solely to cations and the membrane potential is described by an equation of the Goldman-Hodgkin-Katz type. The permeability ratios determined from potential measurements are independent of salt concentration, decrease in the sequence $Cs > Rb > K > NH_4 > Na > Li$ (1.0, 0.25, 0.15, 0.075, 0.007, 0.0013) and are equal to the conductance ratios at low (e.g. $10^{-3}$ M) salt concentration. At higher salt concentrations, the permeability and conductance ratios are not equal and maxima in the conductance *vs.* salt concentration curves are observed. Both these phenomena are postulated to be caused by the formation of relatively impermeant 1:1 polyether cation complexes in the aqueous phase. The 1:1 aqueous association constants deduced from bilayer measurements decrease in the sequence $K > Rb > Na > NH_4 > Cs > Li$ (120, 34, 26, 19, 12, 4 liters per mole) and agree quantitatively with the literature values for the more water soluble polyether XXXI, which lacks only the *t*-butyl groups of XXXII.

The antibiotics nonactin and valinomycin function as neutral carriers of ions by binding to a cation and increasing its solubility in the interior of a bilayer membrane (e.g. Eisenman, Szabo, McLaughlin & Ciani, 1972). This increase in solubility is facilitated both by the inherent lipophilic nature of the carrier and by the ability of the carrier to screen the charge and increase its effective radius, which reduces the Born energy required to take the ion into the low dielectric interior of the membrane (e.g. Parsegian, 1969).

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Any lipophilic molecule which binds to and screens an ion in this manner should therefore be capable of functioning as a neutral carrier.

The cyclic polyethers (Pedersen, 1967; for a recent review *see* Christensen, Hill & Izatt, 1971) are synthetic molecules which form strong complexes with the alkali metal cations, the aqueous association constants for these complexes being several orders of magnitude higher than for valinomycin and nonactin. The association constants of the antibiotics with potassium, for example, are less than 0.1 liter per mole (Stark & Benz, 1971) whereas the association constants of the 18-crown-6 polyethers with potassium are about 100 liters per mole (Frensdorff, 1971*a*). In view of these high association constants, the first report of their effects on bilayers was somewhat discouraging (Eisenman, Ciani & Szabo, 1968; Tosteson, 1968). The polyether bis-cyclohexyl-18-crown-6, which is diagrammed in Fig. 1 and referred to hereafter as XXXI following Pedersen's (1967) nomenclature, was found to produce an increase in the conductance of phospholipid bilayers, but was much less effective than either nonactin or valinomycin. This can be understood, as will be discussed in more detail below, in terms of the polyether XXXI having a lower partition coefficient into the membrane phase than the antibiotics and, furthermore, not being able to wrap around an ion and screen it as well as these molecules. The cyclic polyether XXXII (bis-*t*-butyl-cyclohexyl-18-crown-6, *see* Fig. 1) will be shown to partition more strongly into an organic phase than XXXI and, as expected from the carrier model, to enhance the conductance of bilayer membranes at a lower concentration than does XXXI.

For a simple neutral carrier like nonactin or valinomycin, a knowledge of the relative equilibrium constants for the formation of the permeant 1:1 antibiotic-cation complexes has been shown to be sufficient to predict the relative permeability and conductance changes these molecules produce on bilayer membranes when the rate-limiting step for ion permeation is the movement of the complex through the interior of the membrane (Eisenman *et al.*, 1972). In this case, the permeability, conductance, and salt extraction ratios were all predicted and found to be identical for a given pair of cations. For the polyether XXXI, however, the permeability and conductance ratios not only disagreed with one another, but also with the salt extraction equilibrium data (Eisenman *et al.*, 1968). The results to be described for the polyether XXXII suggest that the carrier model must be extended in the case of the polyethers to take into account both equilibria known to occur in the aqueous phase (Frensdorff, 1971*a*) and the formation of higher order complexes in the membrane phase. All the data we have obtained with the polyethers are compatible with such an extended model.

## Materials and Methods

The formulas and structure of the two cyclic polyethers used in these experiments are illustrated in Fig. 1. The polyethers were generously supplied by C. Pedersen and K. Frensdorff at du Pont. For permeability measurements, aqueous solutions of the polyethers were prepared on the day of their use from small volumes of concentrated stock solutions in ethanol. For conductance measurements, small volumes of XXXII in ethanol were added directly to the chamber containing the bilayer and salt solutions. (The ethanol content of the aqueous solution used in the experiments never exceeded 0.5%, a concentration which was found to have no effect on the electrical properties of the bilayer.) Pyrex-distilled, deionized, then redistilled water was used to prepare all aqueous solutions.

Membranes were formed from mixtures of *n*-decane and the neutral lipid 7-dehydrocholesterol (7 DC, Sigma Chemical Co., St. Louis, Mo.), the neutral but amphoteric lipid phosphatidyl ethanolamine (PE, Supelco Inc., Bellefonte, Pa.), the negatively charged lipid phosphatidyl glycerol (PG, Supelco) and a mixture of neutral and negatively charged lipids obtained by the procedure of Szabo, Eisenman and Ciani (1969*a*) from asolectin (Associated Concentrates Incorporated, New York). The chamber used in the bilayer experiments (Teflon, with a hole about 1 $mm^2$ in area in the partition) and the recording apparatus (AgCl electrodes leading to a Keithley 602 electrometer) are also fully described in this previous publication.

All experiments were conducted at room temperature (22 ± 1 °C). The aqueous solutions used for the experiments were unbuffered (pH about 5.5). Disposable Pasteur pipettes were used to squirt a small volume of lipid ($<2$ μliters) onto the hole and membranes were formed by passing an air bubble from the pipette over the aperture. This technique introduces only a small known quantity of lipid into the chamber, an important consideration since a larger amount would have decreased appreciably the aqueous concentration of XXXII, which partitions strongly in favor of the lipid phase. The area

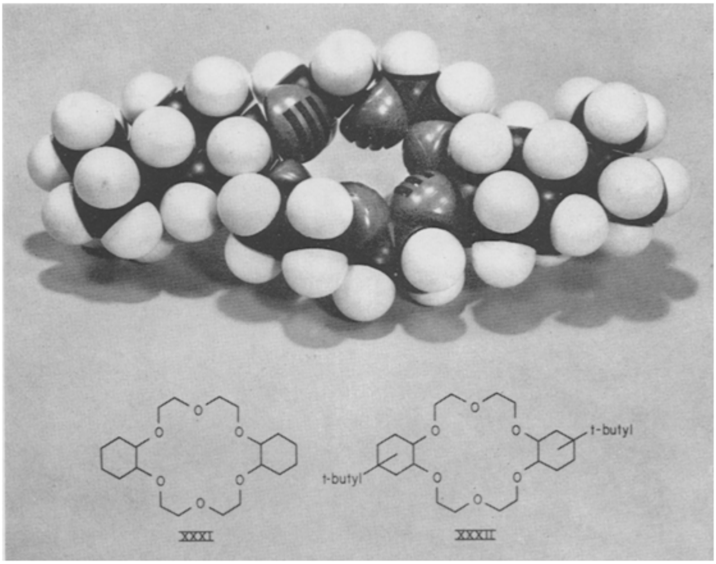

Fig. 1. A CPK model of the cyclic polyether XXXII. The formulas of the two 18-crown-6 compounds used in this study are also illustrated

of the bilayer membrane was measured by means of an eyepiece reticule and all conductance measurements were expressed in units of mho cm$^{-2}$. Small corrections were made, when necessary, for the resistance of the aqueous solutions.

The conductance produced by the polyether XXXII was observed to increase as much as a factor of two when the aqueous solutions were stirred. A similar effect was noted for the macrotetralide actins (Szabo *et al.*, 1969*a*), but in neither case did the permeability ratio depend on stirring. The effect is presumably due to a depletion of the aqueous concentration of neutral carrier in the immediate vicinity of the membrane because of partition into the torus. All conductance measurements reported here were made immediately after or during a rapid stirring of the solutions.

The partition coefficient of the polyether XXXII between water and hexane was determined in the following manner. One milliliter of hexane containing a known concentration of XXXII was shaken with 5 milliliters of water. The concentration of polyether in the aqueous phase was then measured and the partition coefficient deduced by dividing these two concentrations (the large partition coefficient of the polyether in favor of the hexane phase implies the concentration of XXXII in the hexane phase did not change appreciably). The concentration of polyether in water was determined by adding KCl and picric acid to the aqueous solution and then measuring spectrophotometrically the amount of picrate taken up as a counter ion to the polyether potassium complex when shaken with dichloromethane. An empirical calibration curve obtained from aqueous solutions containing a known concentration of polyether allowed us to deduce the aqueous concentration of polyether initially present.

## Results

### *A. Permeability and Conductance Measurements at Low Salt Concentrations*

*Conductance Measurements.* Fig. 2 illustrates the effects of the cyclic polyether XXXII on the conductance of a phospholipid bilayer. A bilayer was formed from a *n*-decane solution of asolectin in a $10^{-3}$ M solution of the indicated alkali metal chloride and the conductance $G$ determined by dividing the steady-state current produced by the applied potential of 10 mV [1]. Equal aliquots of a concentrated ethanolic solution of the polyether were then added to both aqueous phases and the conductance recorded after each addition.

Note in Fig. 2 that when the polyether concentration ($C_s^{\mathrm{Tot}}$) is zero ($\log C_s^{\mathrm{Tot}} = -\infty$) the conductance of the asolectin membranes is extremely low ($5 \times 10^{-8} < G < 10^{-9}$ mho cm$^{-2}$) and does not depend markedly on which cation is present. This low conductance and relative lack of intercationic selectivity is characteristic of all the phospholipid membranes we have examined to date. The addition of the polyether to the aqueous phases

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[1] The current-voltage curve was linear to about 20 mV, both in the presence and absence of the polyether. For higher applied voltages, the current increased more rapidly than the voltage. This was also the case for membranes in the presence of the polyether XXXI (Szabo, 1969) and the neutral carriers nonactin (Szabo *et al.*, 1969*a*) and valinomycin (e.g. Stark & Benz, 1971).

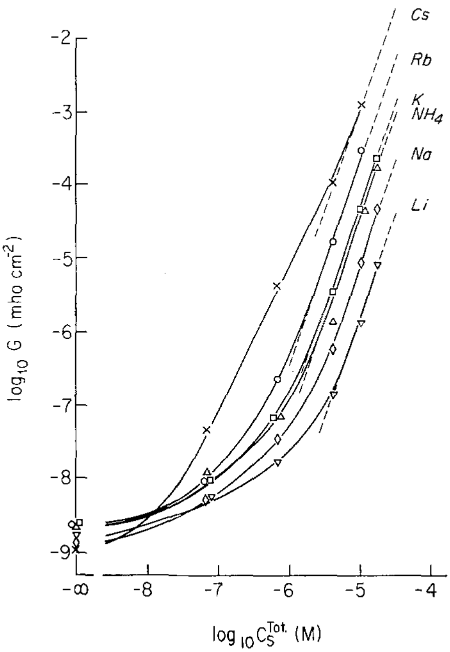

Fig. 2. The effect of the polyether XXXII on the conductance of an asolectin bilayer. The conductance measured in the limit of zero applied voltage $G$ is plotted against the total concentration of polyether in the aqueous phases $C_s^{\mathrm{Tot}}$. The membranes are bathed in a $10^{-3}$ M solution of the indicated alkali metal chloride. The dashed lines have been drawn with a slope of 3 to illustrate the cubic dependence of conductance on polyether concentration

causes the conductance to increase markedly. In the presence of CsCl, for example, the conductance increased six orders of magnitude as the polyether concentration was increased to $10^{-5}$ M.

Perhaps the most striking feature of Fig. 2 is the absence of a simple linear relationship between the conductance $G$ and the polyether concentration $C_s^{\mathrm{Tot}}$. Instead, for a polyether concentration less than $5 \times 10^{-6}$ M, the conductance increases with the square of the polyether concentration in the presence of cesium (the slope is 2 on a log-log plot in this region) and above this polyether concentration the conductance increases with the cube of the polyether concentration for all the cations. (The dashed lines have been drawn with a slope of 3.) These observations may be contrasted with the simple effects that macrotetralide actin (e.g. Eisenman *et al.*, 1972) and valinomycin (e.g. Stark & Benz, 1971) antibiotics produce on the membrane conductance. For these carriers the conductance is proportional, over a wide range of concentrations, to the first power of both antibiotic and salt concentration (provided the ionic strength is held constant if the mem-

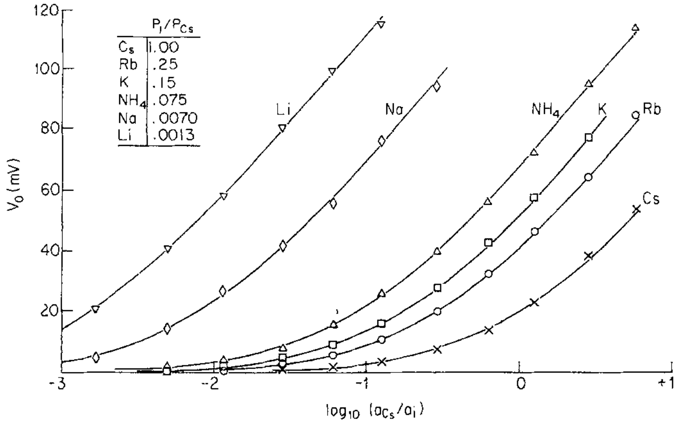

Fig. 3. The membrane potential $V_0$ produced by the addition of $CsNO_3$ to one side of an asolectin membrane bathed in aqueous solutions containing $10^{-5}$ M XXXII and $10^{-3}$ M of the indicated alkali metal chloride. The lines through the experimental points were drawn according to the Goldman-Hodgkin-Katz Eq. (1) with the indicated values of the permeability ratios

brane is charged). The simplest, although not the only, interpretation of the square and cubic relationship observed in Fig. 2 is that two or three polyethers are combining with a cation to solubilize it in the membrane.

Another interesting feature of Fig. 2 is that the conductance produced by the polyether depends on which alkali metal cation is present in the aqueous phase: the conductances of membranes formed in $10^{-3}$ M salt solutions decrease in the sequence $Cs > Rb > K > NH_4 > Na > Li$ at any polyether concentration above $10^{-7}$ M. To simplify the quantitative analysis of the data, however, permeability and conductance ratios will only be compared at a polyether concentration of $10^{-5}$ M, where there is a cubic dependence of $G$ on $C_s^{\mathrm{Tot}}$ for all ions. The exact ratios of the conductances at this polyether concentration are given in Table 1.

*Permeability Measurements.* As might be expected from the conductance data of Fig. 2, a bilayer membrane formed from any of the phospholipids examined here exhibits little selectivity among the alkali metal cations as deduced from potential measurements. Furthermore, when the lipids used to form the bilayer are neutral, little selectivity between anions and cations is observed (Hopfer, Lehninger & Lennarz, 1970). In the presence of the cyclic polyether XXXII, however, the membrane becomes permselective for cations and exhibits a marked intercationic selectivity, as illustrated in Fig. 3.

Table 1. Identity between conductance ratios and permeability ratios for the polyether XXXII at $10^{-3}$ M salt concentration

| Ion | $G_i/G_{Cs}$[a] | $P_i/P_{Cs}$[b] |
|---|---|---|
| Cs | 1.00 | 1.00 |
| Rb | 0.20 | 0.25 |
| K | 0.044 | 0.15 |
| $NH_4$ | 0.035 | 0.075 |
| Na | 0.0077 | 0.007 |
| Li | 0.0013 | 0.0013 |

[a] From Fig. 2.
[b] From Fig. 3.

The permeability ratios indicated in the insert of Fig. 3 were obtained in the following manner. A bilayer was formed in a solution containing the chloride salt of an alkali metal cation ($10^{-3}$ M) and the polyether XXXII ($10^{-5}$ M). Small volumes of a concentrated solution of $CsNO_3$ were then added to one side of the chamber and the steady-state or zero current membrane potential recorded. These potentials, corrected for a small change in the potential of the AgCl electrode caused by the effect of $CsNO_3$ on the activity coefficient of the chloride ion, were then plotted in Fig. 3 as a function of log $a_{Cs}/a_i$, where $a_{Cs}$ is the activity of the cesium ion and $a_i$ is the activity of the other alkali metal cation initially present in the solution. The curved lines in Fig. 3 have been drawn through the points according to Eq. (1):

$$V_0 = \frac{RT}{F} \ln \frac{a_i + (P_{Cs}/P_i)\, a_{Cs}}{a_i} \qquad (1)$$

where $V_0$ is the membrane potential, $R$ the gas constant, $F$ the Faraday, $T$ the absolute temperature and $P_{Cs}/P_i$ the permeability ratio. Eq. (1) is derived in the Appendix on the basis of a carrier model and is formally equivalent to the Goldman-Hodgkin-Katz equation (Goldman, 1943; Hodgkin & Katz, 1949). The ability of this equation to describe the experimental data indicates that the membranes are permeable only to cations (the limiting slopes all have the ideal value of 2.3 $RT/F = 58$ mV per decade at 22 °C) and that the permeability ratios are independent of membrane potential, at least up to 120 mV. The insert of Fig. 3 illustrates the permeability ratios used in Eq. (1) to fit the experimental data, and these values are reproduced in Table 1 for comparison with the conductance ratios measured at the same salt and polyether concentrations.

Table 1 illustrates that both the permeability and conductance ratios decrease in the lyotropic sequence and that the conductance ratios are equal to the permeability ratios for Li, Na and Rb (relative to Cs) within experimental error. The discrepancies between the permeability and conductance ratios for K/Cs and $NH_4$/Cs are probably real but not large and will be discussed in more detail later.

### *B. Permeability Ratios at Higher Salt Concentrations*

Potential measurements similar to those illustrated in Fig. 3 were made with initial salt concentrations of $10^{-2}$ and 1 M. The data, obtained as in Fig. 3 with a polyether concentration of $10^{-5}$ M in the aqueous phases, could all be fitted with Eq. (1), which contains only one adjustable parameter, the permeability ratio. The ratios derived from such measurements are summarized in Fig. 4, which plots on a logarithmic scale the permeability ratios against the salt concentrations at which the measurements were made. Note that with only two exceptions ($NH_4$ and Li) the permeability ratios are constant over a range of salt concentration of three orders of magnitude. The slight change in the $NH_4$ selectivity with concentration is probably real but the apparent increase of Li selectivity at the lowest salt concentration is almost certainly due to traces of more permeant contaminant ions such as H and $NH_4$ no longer being negligible. For this reason, the point is bracketed and the line joining it to the presumably correct, and at higher concentrations constant, $P_{Li}/P_{Cs}$ ratio is broken.

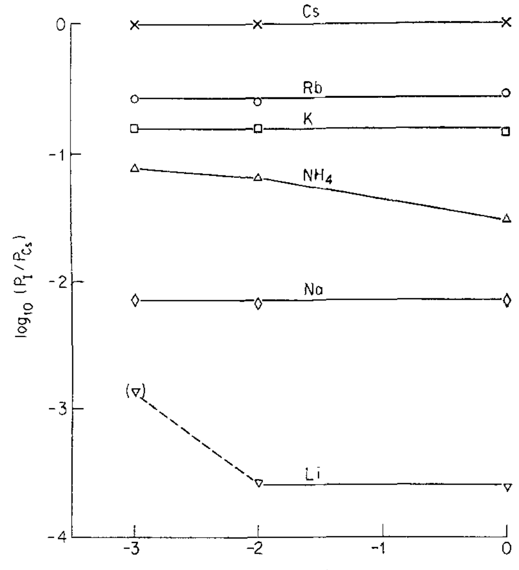

Fig. 4. The membrane permeabilities, relative to Cs, of the alkali metal cations at various salt concentrations $C_i$ when the solutions contain $10^{-5}$ M XXXII

The results of the above permeability experiments may be summarized by stating that when a bilayer is formed in a solution containing the chloride salts of the alkali metal cations and $10^{-5}$ M XXXII, it becomes permselective to cations, the permeability ratios decrease in the lyotropic sequence, Cs > Rb > K > Na > Li (1 : 0.24 : 0.15 : 0.007 : 0.00025) and are independent of both salt concentration and membrane potential.

### *C. Conductance Measurements at Higher Salt Concentrations*

*Dependence of Conductance on Polyether Concentration.* Fig. 5 summarizes conductance measurements made at four different salt concentrations. The form of the conductance *vs.* polyether concentration curves obtained in $10^{-3}$ M salt solutions (Fig. 5*a*) were commented on above and the curves

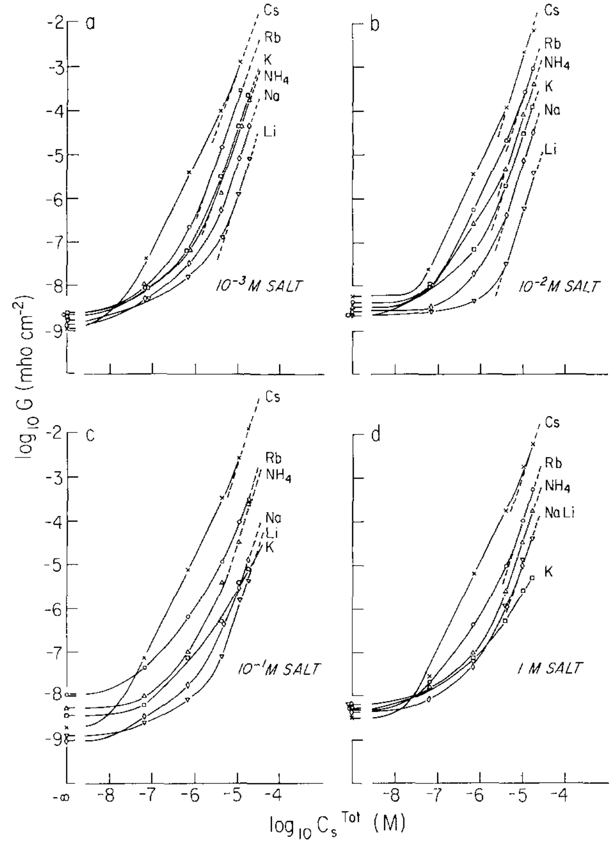

Fig. 5. The dependence of the conductance $G$ on the concentration of XXXII in the aqueous phase for an asolectin membrane in various alkali metal chloride solutions. The dashed lines have been drawn with a slope of 3

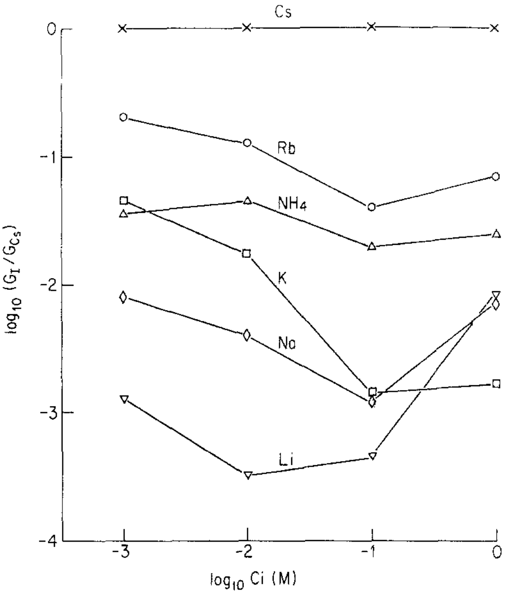

Fig. 6. The membrane conductances, relative to Cs, of the alkali metal cations plotted as a function of salt concentration. The aqueous solutions also contain $10^{-5}$ M XXXII

obtained in $10^{-2}$ M salt solutions (Fig. 5*b*) are virtually identical in shape. For all cations, the conductance depends on the cube of the polyether concentration (the slopes of the $G$ *vs.* $C_s^{\mathrm{Tot}}$ curves are 3 on a log-log plot) in the region of concentration around $10^{-5}$ M and a region of the Cs and Rb curves where the conductance depends on the square of the polyether concentration is also clearly visible (Fig. 5*b*).

The conductance ratios, however, have changed slightly. In particular, the K curve has shifted from above to below the $NH_4$ curve (compare Fig. 5*a* and *b*). This relative downward trend of the K curve continues as the salt concentration is increased (Fig. 5*c*) until in the 1 M salt solution (Fig. 5*d*) the K curve lies below those for the other cations. The slope of the potassium conductance *vs.* polyether concentration curve also decreases with increasing salt concentration and in the 1 M salt solution is less than 2 (Fig. 5*d*). For all other ions, a cubic dependence of conductance on polyether concentration is observed at $C_s^{\mathrm{Tot}} = 10^{-5}$ M regardless of the salt concentration. Other, less obvious changes in the conductance ratios have also occurred as the salt concentration was increased. These are best examined with the aid of Fig. 6.

*The Conductance Ratios at Higher Salt Concentration.* In Fig. 6 the conductance ratios, relative to cesium at a polyether concentration of $10^{-5}$ M,

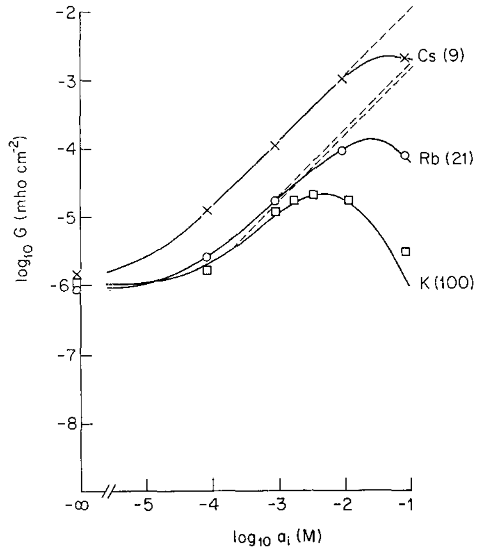

Fig. 7. Dependence of the conductance of an asolectin bilayer on salt activity in the presence of $10^{-5}$ M XXXII. The ionic strength is held constant at $10^{-1}$ M with LiCl. (*See* text for detailed description)

are plotted as a function of the salt concentration, as was done in Fig. 4 with the permeability ratios. The permeability ratios were essentially independent of salt concentration[2]; the conductance ratios obviously are not (compare Figs. 4 and 6). As will be examined in greater detail in the Discussion, the conductance ratios will depend on the concentration of relatively impermeant 1:1 polyether cation complexes which form in the aqueous phase, and reduce the free concentration of polyether. Indeed, the data of Fig. 6 can be used to calculate such aqueous association constants.

*Dependence of Conductance on Salt Concentration.* Fig. 7 illustrates the dependence of membrane conductance $G$ on the activity of the permeant ion $a_i$ when the polyether concentration is $10^{-5}$ M and the ionic strength is maintained constant at 0.1 M using the relatively impermeant electrolyte, lithium chloride. (As indicated by the points at the far left of Fig. 7, the conductance of a bilayer in a solution containing $10^{-1}$ M LiCl and $10^{-5}$ M XXXII is only about $10^{-6}$ mho cm$^{-2}$.) Fig. 7 illustrates that for low salt concentrations the conductance increases linearly with the activity of the permeant ion,

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[2] The ratio of the Cs to Li permeability decreased in the $10^{-3}$ M solutions. This loss of selectivity in the presence of a low concentration of lithium is also apparent in the conductance ratios of Fig. 6, presumably for the same reason—namely, trace contaminants of permeant ions combining with the polyether and carrying more current than $10^{-3}$ M of the highly impermeant Li$^+$.

as indicated by the dashed lines of unit slope. When combined with the observation that the conductance depends on the cube of the polyether concentration in the range around $C_s^{\text{Tot}} = 10^{-5}$ M, this result suggests the permeant species is the 3:1 polyether-cation complex. Note, however, that the $G$ *vs.* $a_i$ curves pass through maxima at higher salt concentrations and that the position of the maxima depends on which permeant ion $I^+$ is present.

Regardless of the mechanism of ion permeation in the presence of the polyether, the conductance depends on the first power of the salt concentration (at low salt concentration) and on the third power of the polyether concentration (at high polyether concentration) so we may describe the conductance $G$ by the following empirical relation,

$$G = (\gamma + \delta_i a_i) C_s^3 \tag{2}$$

where $\gamma$ is a constant denoting the conductance due to the lithium and $\delta_i$ is another constant due to the permeant ion $I^+$ ($i =$ Cs, Rb or K in Fig. 7). If a significant degree of ion association occurs in the aqueous phase, as is known to occur for the analogous polyether XXXI (Frensdorff, 1971*a*; Izatt, Nelson, Rytting, Haymore & Christensen, 1971) then

$$I^+ + S \underset{K_{is}^+}{\rightleftharpoons} IS^+; \qquad K_{is}^+ = \frac{C_{is}}{C_i C_s} \tag{3}$$

where $I^+$ denotes the cation, $S$ the polyether, $IS^+$ the polyether cation complex and $K_{is}^+$ the aqueous association constant. The free concentration of polyether in the aqueous phase will then be related to the total concentration by the expression[3]:

$$C_s = C_s^{\text{Tot}}/(1 + K_{is}^+ a_i). \tag{4}$$

By assuming the 1:1 complexes are relatively impermeable and combining Eqs. (2) and (4) the following expression is obtained:

$$G = \frac{(\gamma + \delta_i a_i) C_s^{\text{Tot}^3}}{(1 + K_{is}^+ a_i)^3}. \tag{5}$$

Eq. 5 indicates that the conductance is expected to increase linearly with the activity of permeant ion $a_i$ at low salt concentrations ($K_{is}^+ \ a_i \ll 1$), then deviate from linearity, pass through a maxima (at $a_i = 1/2K_{is}^+$) and then finally decrease with an inverse square dependence on the salt concentration. This is the behavior illustrated in Fig. 7 and, in fact, Eq. (5) has been used to draw the curves through the experimental points. The constant $\gamma$ was

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[3] We consider the degree of complexation between the polyether and Li to be negligible.

determined from the points at the far left-hand side of Fig. 7, and is about the same for all ions. The constants $\delta_i$ are determined from the intercepts of the linear region of the corresponding curve and are different for each permeant ion. The only adjustable parameter to describe the nonlinear portion of the curve is the association constant in the aqueous phase $K_{is}^{+}$ and a best fit to the data by eye was obtained with the values indicated in Fig. 7 (i.e., 100 liters per mole for K, 21 for Rb and 9 for Cs). For the present, Eq. (5) may be regarded as merely the simplest equation which describes empirically the experimental behavior of the conductance concentration curves. The values deduced for the constants $K_{is}^{+}$, however, indicate that the interpretation of these parameters in terms of aqueous phase association constants is correct, for they agree quantitatively with those measured directly for the analogous polyether XXXI (Frensdorff, 1971*a*; Izatt *et al.*, 1971), as will be examined further in the Discussion.

### D. *Effect of Lipid Composition on the Conductance Produced by the Polyether*

All conductance and permeability measurements presented above were made on membranes formed from a mixture of phospholipids obtained from asolectin. This mixed lipid is known to contain a substantial quantity of negatively charged phosphatidyl inositides, and bilayers formed from it appear to have a charge density comparable in magnitude to those formed from either pure phosphatidyl inositol or phosphatidyl glycerol (McLaughlin, Szabo, Eisenman & Ciani, 1970, *cf.* Fig. 2). The argument could be raised that the cubic dependence of conductance on polyether concentration observed on asolectin membranes is caused by either some specific interaction of the polyether with these lipids or is in some way contingent on the presence of negatively charged phospholipids. The action of the polyene antibiotics, for example, is dependent on the presence of sterols (e.g. Cass, Finkelstein & Krespi, 1970).

To examine this possibility experimentally, we have studied the dependence of conductance on polyether concentration with bilayers formed from *n*-decane solutions of three different lipids; phosphatidyl glycerol (PG), which bears one negative charge per phospholipid molecule, phosphatidyl ethanolamine (PE), which is amphoteric but bears virtually no net charge at neutral pH, and 7-dehydrocholesterol (DC), which is a truly neutral molecule. The results of conductance measurements made on bilayers formed in a $10^{-1}$ M solution of cesium chloride are presented in Fig. 8.

It is apparent that the level of the conductance induced by the polyether depends markedly on the nature of the phospholipid used to form the mem-

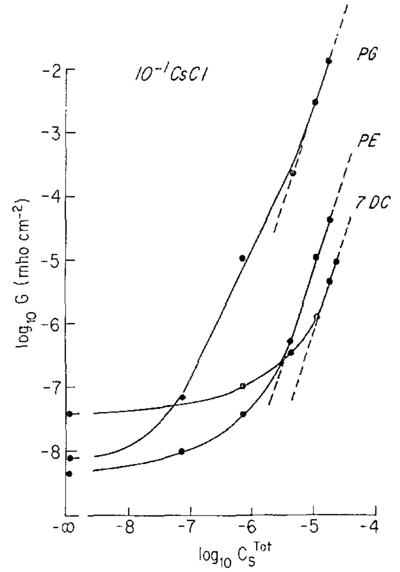

Fig. 8. Dependence of conductance on polyether concentration in the presence of $10^{-1}$ M CsCl when the bilayers have been formed from phosphatidyl glycerol (PG), phosphatidyl ethanolamine (PE) and 7-dehydrocholesterol (7-DC). The dashed lines have been drawn with the slope of 3

brane[4], but the feature of Fig. 8 we wish to stress is that for all lipids there is a cubic dependence of conductance on polyether concentration in the region about $C_s^{\mathrm{Tot}} = 10^{-5}$ M. Furthermore, as was seen with asolectin membranes (*see* Fig. 5), there is a region between $10^{-7}$ and $5 \times 10^{-6}$ M for the PG curve where the conductance depends on the square of the polyether concentration.

The data presented in Fig. 8 thus indicate that the cubic (and square) dependence of conductance on polyether concentration is not contingent on the nature of the polar head group, being observed for neutral, amphoteric,

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[4] The difference between the effectiveness of the polyether XXXII in enhancing the conductance of a negatively charged membrane (such as PG) and a neutral membrane (such as 7-DC) can be understood in terms of the negative potential developed at the membrane solution interface by a charge on the polar head group, as was discussed in detail elsewhere (McLaughlin *et al.*, 1970). The potential produced by the charges on the head groups was shown to be accurately described by the simple theory of the diffuse double layer (*see also* McLaughlin, Szabo & Eisenman, 1971). The addition of monovalent ions to the solution bathing a negatively charged membrane screens the charges, lowers the magnitude of the surface potential and should therefore decrease the conductance produced by the polyether XXXII in exactly the same manner as it does for the simple neutral carrier nonactin. It does (*unpublished observations*). This is why the ionic strength was held constant when examining the dependence of conductance on salt concentration in Fig. 7.

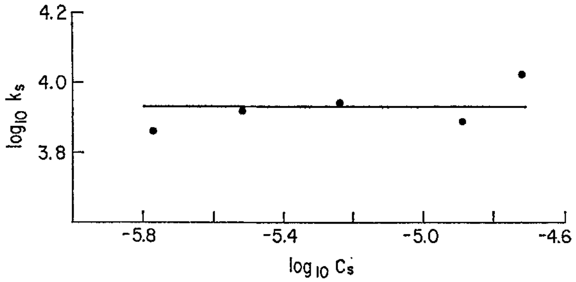

Fig. 9. The partition coefficient of the neutral polyether XXXII into hexane as a function of the polyether concentration in the aqueous phase. Within the limits of experimental error the partition coefficient is independent of the aqueous concentration and has a value of $8.5 \pm 0.5 \times 10^3$ (SEM) in favor of hexane

negatively charged and mixed lipids. Furthermore, it does not seem to depend on the nature of the fatty acid tails, for the fatty acid residues of asolectin are largely unsaturated, those of PG and PE contain cyclopropyl groups and DC, of course, contains no long chain fatty acids at all.

### *E. The Partition Coefficient of XXXII into Hexane*

As the partition coefficient of the neutral polyether into the membrane phase is an important parameter in the theoretical interpretation of the results, the partition coefficient of XXXII between water and hexane was measured as a function of polyether concentration. Fig. 9 illustrates that the partition coefficient of XXXII is about $8.5 \times 10^3$ in favor of hexane and is independent of the polyether concentration within the limits of experimental error. We may therefore tentatively conclude that the cubic dependence of conductance on polyether concentration is not due to the partition coefficient being a function of the polyether concentration. (This conclusion is tentative because hexane is not a perfect model of the interior of a bilayer membrane and also because adsorption at the membrane solution interface might be important.)

For comparison with the above data, it may be noted that the isomers *A* and *B* of the analogous polyether XXXI have partition coefficients into hexane of 6 and 7, respectively (Frensdorff, 1971*b*). The partition coefficient of XXXII into hexane is thus more than three orders of magnitude greater than that of XXXI, and this is presumably a major factor in determining its greater effectiveness on membranes, as will be discussed in the next section.

### *F. Comparison of the Effects of XXXI and XXXII on Bilayer Membranes*

Fig. 10 compares the conductances produced by XXXI and XXXII under identical conditions. Note that identical conductance sequences (Cs > Rb > K > Na ~ Li) are observed in both cases but that a thousand times more XXXI than XXXII is required to produce an order of magnitude increase

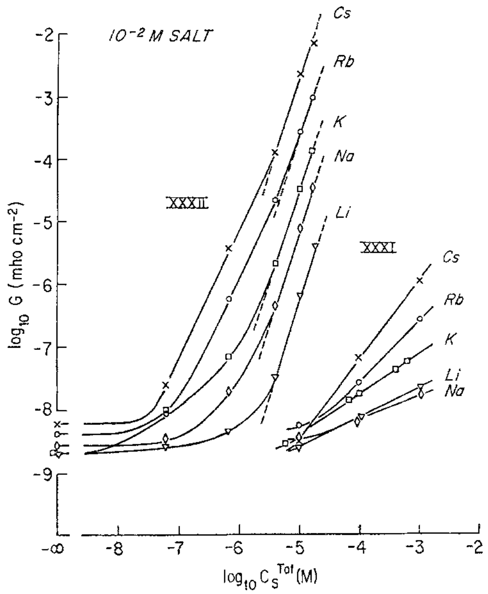

Fig. 10. Dependence of the conductance of an asolectin bilayer on polyether concentration in $10^{-2}$ M salt solutions. Note that the polyether XXXII is about a thousand times more effective in increasing the conductance than is the polyether XXXI

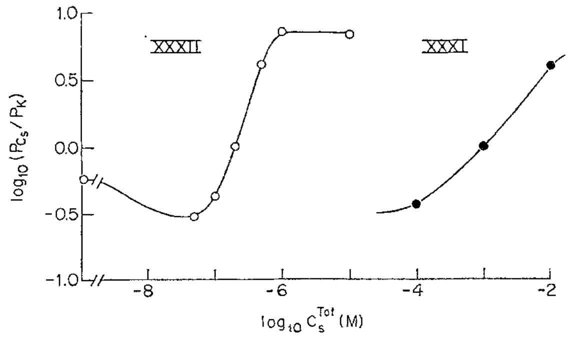

Fig. 11. Dependence of the permeability ratio on polyether concentration for XXXII and XXXI. The asolectin membrane is bathed in $10^{-2}$ M CsCl solution and the permeability ratio at a given polyether concentration is calculated from potential experiments similar to those illustrated in Fig. 3, as $KNO_3$ was added to one side of the membrane. Note that at high polyether concentrations the membrane is more permeable to Cs than to K while the reverse is true at low polyether concentrations

in conductance for a given ion. The greater potency of XXXII in increasing the membrane conductance agrees qualitatively with the greater partition coefficient of this molecule (*see* Sect. *E*).

In the presence of the polyethers XXXI or XXXII the relative permeability of the membrane to Cs and K, as measured by potential experiments similar to those illustrated in Fig. 3, depends on the polyether concentrations as illustrated in Fig. 11. At high polyether concentrations the membrane is more permeable to Cs whereas at low polyether concentrations the membrane becomes more permeable to K. The shift in the XXXI curve parallels that observed for XXXII, but occurs at a concentration about $10^4$ times higher. As with the relative conductance measurements (Fig. 10), this finding agrees qualitatively with the observation that XXXII has a partition coefficient into hexane of more than $10^3$ times that of XXXII (Sect. *E*). An analysis of why the $P_{\mathrm{Cs}}/P_{\mathrm{K}}$ permeability ratios depend on the polyether concentration and eventually invert and why this inversion occurs before the $G_{\mathrm{Cs}}/G_{\mathrm{K}}$ ratios invert will be postponed until the Discussion.

## Discussion

### *A. The Polyethers Probably Act as Neutral Carriers Rather than Pores*

All the data presented in Results will be shown to be compatible with a "carrier" model which postulates that two or three polyethers combine with a single cation to solubilize it in the liquid-like hydrocarbon interior of the membrane. The theoretical details of the model have been relegated to an Appendix, but it is probably apparent intuitively that the cubic dependence of conductance on polyether concentration for all cations in the region around $C_s^{\mathrm{Tot}} = 10^{-5}$ M (Figs. 2 and 5), the square dependence of conductance on polyether concentration for the ions, cesium and rubidium when $10^{-8} < C_s^{\mathrm{Tot}} < 5 \times 10^{-6}$ M (Fig. 5*a* and *b*), and the linear dependence of conductance on salt activity when the concentration is low (and ionic strength maintained a constant if the membrane bears a net charge) are all compatible with such a carrier model. The macrotetralide actin antibiotics (e.g. Eisenman *et al.*, 1972) and valinomycin (e.g. Stark & Benz, 1971) also act as carriers, but form 1:1 complexes with the alkali metal cations as indicated by the linear dependence of conductance on both the salt and antibiotic concentration, by the ability of these antibiotics to extract cations stoichiometrically into a bulk organic phase (Eisenman, Ciani & Szabo, 1969) and by X-ray (Kilbourn, Dunitz, Pioda & Simon, 1967), NMR

(Shemyakin, Ovchinnikov, Ivanov, Antonov, Vinogradova, Shkrob, Malenkov, Evstratov, Laine, Melnik & Ryabova, 1969) and other physical chemical evidence. Furthermore, nonactin and valinomycin have very low association constants with the alkali metal cations ($<0.1$ liters per mole, Stark & Benz, 1971). It is a combination of two factors, the cubic dependence of conductance on polyether concentration and the significant degree of association which occurs in the aqueous phase, which gives rise to the phenomena observed with the polyether but not with the antibiotics; namely, the maxima in the conductance *vs.* salt concentration curves and the discrepancies between the permeability and conductance ratios at high salt concentrations.

It is certainly possible that the cubic dependence of conductance on polyether concentration results from three polyether molecules stacking in some manner to form a "pore", but there are three additional reasons why we believe this is not the case. Molecular models of a bilayer and the polyether (Fig. 1) indicate that three polyether molecules could not stack up in any manner to form a "pore" unless the membrane was drastically thinned at that point. Furthermore, the selectivity induced by the polyethers is quite substantial (Fig. 2) and decreases in the lyotropic sequence, $Cs > Rb > K > Na > Li$. Cesium, however, is known to be too large to fit easily through the 18-membered polyether ring, as indicated by both measurements on molecular models (Pedersen, 1970) and direct X-ray analysis (Bright & Truter, 1970*a*, *b*). The experimental fact that the most permeable ion is unable to fit through the polyether ring indicates that three polyethers are not simply stacking up like life savers in a package to form a pore. Finally, freezing the bilayer membrane reduces the conductance produced by the polyether several orders of magnitude (S. Krasne, *personal communication*) in much the same manner as it does for the typical neutral carriers nonactin and valinomycin (Krasne, Eisenman & Szabo, 1971). If the polyethers act by forming a pore, as does gramicidin, one would expect the conductance to have been largely unaffected by freezing.

In the absence of any experimental evidence to the contrary, we will assume that the polyether acts as a "carrier" rather than a "pore". The more subtle question of whether the polyethers, or indeed any of the so-called carriers, transport via a Nernst-Planck diffusion process, as is assumed here or via an Eyring jump process (e.g. Läuger & Stark, 1970) must be regarded as unresolved. As equations of the same form are obtained in both cases for the conductance, measured in the limit of a small applied voltage (Läuger & Stark, 1970), the question is irrelevant for the purpose of this paper.

### *B. Comparison of Permeability and Conductance Ratios*

The basic conclusion from the theoretical Appendix [Eq. (A.29)] is reproduced here as Eq. (6):

$$\frac{G_j}{G_i} \cdot \frac{P_i}{P_j} = \left( \frac{1 + K_{is}^{+} a_i}{1 + K_{js}^{+} a_j} \right)^3 . \tag{6}$$

Eq. (6) predicts that the permeability and conductance ratios should be equal when the salt concentration is sufficiently low that aqueous phase association is negligible ($K_{is}^{+}\, a_i \ll 1$). The permeability and conductance ratios do in fact agree at a salt concentration of $10^{-3}$ M (Table 1). At higher salt concentrations, when aqueous phase association is expected to become important, the conductance and permeability ratios should disagree (and do). Before applying Eq. (6) to the experimental data to calculate the values of the association constants, we shall consider the system intuitively.

An examination of Eqs. (2) through (5) in the text [or Eqs. (A.10), (A.13) and (A.14) leading to Eq. (A.19) in the Appendix] should make clear why the conductance ratios depend on salt concentration when association in the aqueous phase is significant. The conductance is simply proportional to the cube of the *free* polyether concentration in the aqueous phase, and this is reduced by the formation of relatively impermeant 1:1 complexes in the aqueous phase. If association is significant, and different, for two ions the conductance ratios will therefore change with the ionic concentration according to Eq. (A.19).

Why then, do the permeability ratios not also change with an increase in salt concentration when association occurs more on one side of the membrane than the other, and the free concentration of polyether in the bulk aqueous phases must consequently differ on the two sides? The answer is that a difference in the free polyether concentration produces no potential across the membrane because the rate-limiting step for the movement of the neutral species is diffusion in the unstirred layer (McLaughlin, Eisenman & Szabo, 1972). This implies that at the two membrane solution interfaces the concentrations of the free polyether species are virtually identical.

A quantitative comparison of the permeability and conductance ratios will now be made to estimate the aqueous association constants required to produce the discrepancies in permeability and conductance ratios observed at high salt concentrations. In the left-hand portion of Fig. 12 the conductance ratios have been divided by the permeability ratios and plotted as a function of salt concentration on a logarithmic scale. These experimental data are referred to lithium and it may be noted that the empirical curves for all

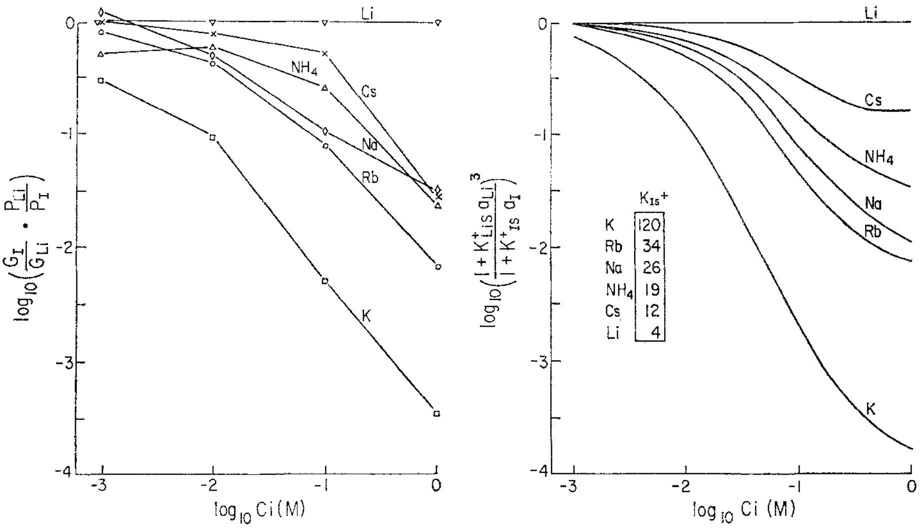

Fig. 12. *Left*: Experimentally observed dependence of the quotient of the conductance and permeability ratios on the salt concentration. The data points were obtained from combining the data in Figs. 4 and 6. *Right*: Theoretically predicted dependence of the quotient of the conductance and permeability ratios on salt concentration from Eq. (6). The curves have been drawn with the indicated values of the aqueous association constants for the formation of the 1:1 polyether cation complex. Note that there is a reasonably good agreement between the shapes of the theoretical and experimental curves, and that the association constants used to produce the theoretical curves agree very well with those measured directly for the analogous polyether XXXI (Table 2)

the other ions move in the downward direction, indicating that their conductances decrease with respect to lithium as a function of salt concentrations. The right-hand side of Fig. 12 plots the term $\left(\frac{1+K_{is}^{+}a_i}{1+K_{js}^{+}a_j}\right)^3$ as a function of salt activity $a_i$ for the indicated values of the association constants[5]. Note that with a few minor expections[6], Eq. (6) is capable of explaining most of the discrepancies between the permeability and conductance ratios; the shape of the theoretical curves resembles closely the experimental curves in the left-hand portion of the figure. Aqueous phase association is therefore a plausible explanation for the observed phenomenon. The indication that it is indeed the correct explanation comes from quantitative comparison of the values of the association constants derived from Fig. 12

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[5] A first estimate of the association constants was made at $10^{-2}$ M, where it was assumed that the association for Li was negligible. The value of 4 was chosen for lithium to give a best fit, by eye, to the data at higher concentrations.

[6] The discrepancy between the potassium permeability and conductance ratios at the lowest salt concentration, for example, is larger than predicted by the equation. This, however, is not too surprising as XXXII is a mixture of several isomers, which might have different association constants, as do the isomers of XXXI (Frensdorff, 1971*a*).

Table 2. Comparison of values of aqueous complex formation constants deduced from bilayer membrane measurements for XXXII with those directly measured for XXXI

| Ion | Membrane | | Direct | | | |
|---|---|---|---|---|---|---|
| | (Potential *vs.* conductance)[a] | (Conductance *vs.* salt concentration)[b] | (Glass electrode)[c] | | (Calorimetric)[d] | |
| | XXXII | XXXII | XXXIA | XXXIB | XXXIA | XXXIB |
| K | 120[e] | 100 | 150 | 60 | 102 | 40 |
| Rb | 34 | 21 | – | – | 31 | 4 |
| Na | 26 | | 31–70 | 16–40 | – | – |
| $NH_4$ | 19 | | 25 | 6.5 | 22 | 16 |
| Cs | 12 | 9 | 18 | 7 | 13 | – |
| Li | 4 | | 4 | – | – | – |

[a] From the comparison between permeability ratios and conductance ratios in Fig. 12.

[b] From the dependence of conductance on salt activity illustrated in Fig. 7.

[c] Measurements for the two different isomers A and B by Frensdorff (1971*a*) using cation selective glass electrodes.

[d] Calorimetric measurements by Izatt *et al.* (1971).

[e] All values are in liters per mole.

with the values obtained from direct measurements on the analogous polyether XXXI[7] and from the values obtained from the conductance data of Fig. 7. Such a comparison is made in Table 2, where the agreement is seen to be satisfactory, both as to the sequence and the magnitude.

### *C. Dependence of Conductance on Salt Concentration*

Fig. 7 illustrates that the conductance depends on the concentration of permeant cation in the aqueous phase in a complex manner; it increases linearly at low salt concentration, then passes through a maximum and finally decreases. The data were fitted with the empirical Eq. (5), which was derived from the minimal assumptions that the conductance depends on the cube of the free polyether concentration in the aqueous phase [Eq. (2)] and that a significant degree of association occurs in the aqueous phase, the 1:1 complexes being relatively impermeable. Eq. (A.17) was derived on the basis of a carrier model and is in fact identical in form to Eq. (5). It is apparent from the good fit of the theoretical curves to the experimental data (Fig. 7) that aqueous phase association is a reasonable interpretation

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[7] It is necessary to use data from the polyether XXXI because the solubility of XXXII in water was insufficient for Frensdorff to measure the association constants directly. H. K. Frensdorff (*personal communication*) has, however, measured the association constants of both XXXI and XXXII for potassium in methanol and these values are virtually identical, supporting the claim that these polyethers should have very similar aqueous phase association constants.

of the maxima observed[8]. That it is in fact the correct one comes from a comparison of the aqueous phase association constants required to fit the data in Fig. 7 with both the constants derived from a comparison of the permeability and conductance ratios, and those obtained by independent direct measurements on the analogous polyether XXXI. The agreement is excellent, as an examination of Table 2 indicates.

### *D. The Nature of the Polyether-Cation Complexes*

*Evidence for the Existence of 2:1 Complexes.* As Fig. 5 illustrates, there is a moderately wide range over which the conductance depends on the square of the polyether concentration in the presence of Cs and Rb, suggesting that the polyether is forming 2:1 complexes with these ions. Furthermore, the fact that the permeability and conductance sequences (Figs. 4 and 6) are very different from the sequence of the 1:1 aqueous association constants (Table 2), can be used to argue that more than one polyether is combining with a cation to solubilize it in the membrane. Finally, 2:1 complexes formed between the 18-crown-6 polyethers and the alkali metal cations Cs and Rb have been isolated in crystal form (Pedersen, 1970) and there is also evidence from potentiometric studies (Frensdorff, 1971*a*) that they exist in solution. These investigators note that the formation of 2:1 complexes appears to be governed by the relative size of the polyether ring and the complexed cation. With 18-crown-6 compounds, for example, Cs and Rb are larger than the ring size and form stable 2:1 compounds, possibly of the "sandwich" type with the cation as the filling; K and the smaller alkali metal cations, on the other hand, can fit into the plane of the 18-membered ring and might be expected to complex less readily with a second polyether. Such 2:1 complexes would certainly be much more lipid soluble, hence more permeable, than the more prevalent 1:1 complexes. The stray electric field emanating from these latter complexes will increase

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[8] The major apparent discrepancy occurs in the potassium point at the highest salt concentration. The conductance does not, however, depend exactly on the cube of the polyether concentration in $10^{-1}$ M KCl, because association has lowered the polyether concentration significantly and the relationship is nearer a square than a cube dependence. This is expected from the theory, and hence the deviation from the theoretical curve of Fig. 7 is also expected. It was not felt worthwhile to complicate the theoretical expressions further to incorporate this small deviation. It should be noted that there are other possible mechanisms, such as rate-limiting processes at the interfaces, which could give rise to maxima in the conductance *vs.* salt concentration curves. Such rate-limiting processes would not appear to be important for the polyether XXXII because the current-voltage curve is superlinear even in the presence of $10^{-1}$ M KCl (*unpublished observation*), a concentration where the conductance is decreasing markedly with an increase in salt activity (Fig. 7). Superlinearity implies the rate-limiting step for ion permeation is still the movement of the complex through the membrane (Läuger & Stark, 1970).

the free energy required to take the complex into a medium of low dielectric constant whereas the formation of a "sandwich" would serve the function of screening the ion well, a necessary requirement for solubilizing it in the membrane.

*Evidence for 3:1 Complexes.* Whereas there is moderately strong evidence for the formation of 2:1 complexes, the evidence for the existence of a lipid soluble 3:1 complex hinges entirely[9] on the cubic dependence of conductance on polyether concentration for all ions in the region around $C_s^{\mathrm{Tot}} = 10^{-5}$ M (*see* Fig. 5). This cubic dependence is presumably not caused by any specific interactions of the polyether with either the head or tail group of the lipids (*see* Results, Sect. *D*), and not caused by a variation in the partition coefficient with concentration (*see* Results, Sect. *E*). In the absence of any experimental evidence to the contrary we therefore tentatively conclude the permeant species is the 3:1 complex.

## *E. Comparison of the XXXII and XXXI Data*

We consider first the conductance data illustrated in Fig. 10 for the polyethers XXXII and XXXI. The selectivity sequences are identical for both polyethers, but XXXII is about a factor of $10^3$ more potent than XXXI, in agreement with John Rowell's original observation (*personal communication*). The partition coefficient of XXXII is also greater than that of XXXI by a factor of about $10^3$, and this explains to a large extent its greater effectiveness in enhancing the conductance of a bilayer membrane. To illustrate the point analytically, a combination of Eqs. (A.2), (A.4) and (A.13) yields Eq. (7) which indicates that the ratio of the polyether concentrations required to produce a given increase in the conductance is proportional to the ratio of the partition coefficients of the neutral carriers ($k_s$) all other factors being equal (i.e.,

$$G = \frac{F^2}{d} u_{i s_k}^{*} a_i(0) (k_s C_s)^k \overline{K}_{i s_k}^{+}; \qquad (7)$$

mobilities, $u_{i s_k}^{*}$; salt concentrations, $a_i(0)$; heterogeneous association constants, $\overline{K}_{i s_k}^{+}$, and stoichiometry, $k$). Qualitatively this is the case; the shift is in the correct direction and of the correct order of magnitude.

One puzzling feature of the results previously obtained for the polyether XXXI (Eisenman *et al.*, 1968) was the observation that the permeability and conductance did not agree with one another, even at low salt concentrations. In particular, the cesium and potassium ratios were inverted, the permeability sequence in a $10^{-2}$ M salt solution being K > Rb > Cs > Na > Li. Such an inversion of the permeability ratios can also be produced for the polyether XXXII by lowering the polyether concentration, as illustrated in Fig. 11. Conversely, if measurements are carried out with XXXI at higher concentrations than those used initially by Eisenman *et al.* (1968), this polyether produces a

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[9] No 3:1 complexes have been isolated in crystals, possibly for energetic reasons, and there is no evidence from binding studies for such a complex existing in solution, possibly because insufficiently high concentrations of the polyether have been used in such studies. (Recall that the aqueous concentration of $10^{-5}$ M corresponds to a membrane concentration of about $10^{-1}$ M if the partition coefficient of the polyether into hexane and the membrane are similar.)

cesium selective membrane (Fig. 11). This reversal of the Cs-K permeability is similar for the two polyethers, but occurs at about a concentration of $10^{-3}$ for XXXI and $10^{-7}$ for XXXII (Fig. 11). It is clear that the difference between the two polyethers may be explained qualitatively in terms of the higher partition coefficient of XXXII.

The different effects of the polyethers XXXI and XXXII on bilayer membranes can thus be largely explained in terms of their different partition coefficients; but, the reasons why the permeability coefficients depend on the polyether concentration and why the permeability and conductance ratios for Cs and K disagree in the transition region remain to be explained. The behavior seen in Fig. 11 is predicted by the carrier model, as can be seen from an examination of Eq. (A.27). At high polyether concentrations (above a value of about $10^{-6}$ M for XXXII and $10^{-2}$ M for XXXI, where Cs is more permeable than K) the permeability ratios should be independent of polyether concentration because the stoichiometries are identical. The same parameters occur in the permeability and conductance ratios and these two ratios will be identical when association in the aqueous phase can be ignored and the stoichiometries are identical [compare Eqs. (A.27) and (A.19)]. As the polyether concentration is lowered, the most permeant species will eventually be the 1:1 complexes. The association constants of the 1:1 complex in water for potassium are greater than those for cesium (*see* Table 2) for both XXXI and XXXII, hence it is reasonable that the membrane is more permeable to K than to Cs at the lowest polyether concentrations. [As can be seen from Eq. (A.27), the permeability ratios will equal the ratio of the association constants if the mobilities and partition coefficients of the Cs and K complexes into the membrane are identical.] The carrier model is therefore capable of explaining why the permeability ratios for Cs and K invert.

It is also capable of explaining why the permeability and conductance ratios are different in this region; that is, why the permeability ratio inverts and the membrane becomes potassium permselective before the conductance ratio changes. The explanation hinges on a combination of two effects, the differing stoichiometry and the significant degree of association in the aqueous phase, as may be seen by examining Eq. (A.27). Permeability and conductance measurements are compared at the same total concentration of polyether $C_s^{\mathrm{Tot}}$ but the effective concentration in the membrane for the permeability measurement will be lower than that for the Cs conductance measurement and higher than that for the potassium conductance measurement because K forms stronger complexes than Cs. To express Eq. (A.27) verbally, aqueous phase association is of little importance in permeability measurements if the two ions form complexes of the same stoichiometries with the polyether. The permeability ratios will be independent of salt concentration in this case. Consider, however, the case where the conductance measurements are made at a total polyether concentration where the cesium conductance is slightly greater than the potassium conductance and the permeant species is the 2:1 complex for cesium but the 1:1 complex for potassium. For the permeability measurement, made at the same total concentration of polyether, the free concentration at the membrane solution interfaces, and therefore within the membrane, will lie between the free concentrations for the two conductance measurements, lower than in the case of cesium, higher than in the case of potassium. The number of permeant cesium complexes, however decreases with the square of the polyether concentration, whereas those of potassium change with only the first power. Thus, at this total polyether concentration, the permeability of the cesium ion is less than that of the potassium ion, or the crossover occurs in the permeability ratios at a higher concentration of polyether than the conductance ratios. If the formation of 1:1 complexes in the aqueous phase did not reduce the free polyether concentration to a significant degree, crossovers would still occur but at the same concentration for both the permeability and conductance measurements and the ratios in both cases would always be equal. The carrier model is thus capable of accounting for all the phenomena observed with the polyethers.

### *F. Biological Implications*

If, on a squid axon or other convenient preparation one could observe the conductance due to a carrier not normally present in the nerve rather than the endogenous Na and K "channels" a number of novel experiments would be possible. For example, carriers and channels in a bilayer behave in a markedly different manner as the membrane is frozen (Krasne *et al.*, 1971) and the nerve preparation could be cooled in an attempt to ascertain whether the sodium and potassium channels are really channels or carriers. The binding of any charged species to the nerve membrane should be readily detectable because the conductance produced by a neutral carrier depends on the surface potential of the membrane. The effect of pH (McLaughlin *et al.*, 1970; Szabo, Eisenman, McLaughlin & Krasne, 1972), divalent ions (McLaughlin, Szabo & Eisenman, 1971) and certain charged drugs such as the salicylates and local anaesthetics (McLaughlin, 1972) on the surface potential of artificial bilayer membranes has already been examined using this technique.

Unfortunately, the addition of monactin, a typical potassium selective neutral carrier, to a squid axon did not produce any appreciable increase in the conductance (Stillman, Gilbert & Robbins, 1969), possibly because the innate potassium conductance of the axon is so high. When the axon is perfused with a solution where part of the potassium is replaced by cesium, however, the conductance drops to an anomalously low value (Chandler & Meves, 1965), lower than one would predict from the dilution of the potassium concentration. Thus, the addition of a cesium selective neutral carrier such as the polyether XXXII to a squid axon perfused with cesium may produce a detectable increment in the conductance and the polyether prove to be a suitable "probe".

## Appendix

### *Theoretical Description of the Effects of the Polyethers on Membrane Conductance and Permeability in Terms of a Carrier Model*

*Description of the System.* This analysis extends previous theoretical treatments of a carrier model (e.g. Ciani, Eisenman & Szabo, 1969; Markin, Krishtalik, Liberman & Topaly, 1969; Läuger & Stark, 1970) to include the case where more than one carrier is required to solubilize an ion in the interior of the membrane. The expression for the conductance is derived only for the limiting case of a vanishingly small applied voltage ($<25$ mV where the current-voltage curve is linear) and the expression for the potential is derived only for the steady-state or zero current case. The use of the

Nernst-Planck formulation assumes the validity of the diffusion equation, but expressions identical in form to those derived here can also be obtained from an Eyring model (e.g. Läuger & Stark, 1970). The basic assumption of this treatment is that the rate-limiting step for ion permeation is the diffusion of the carrier-ion complex through the membrane, which implies that equilibrium considerations are sufficient to predict the relative conductance and permeability properties of the system. The assumption was tested experimentally by studying the form of the current-voltage curves, which we found to be superlinear over the entire range of salt concentrations examined. This implies that no other steps are rate limiting (Läuger & Stark, 1970).

The neutral carrier will be denoted by the symbol $S$, which in this case stands for a polyether molecule, and the cations by the symbol $I$ ($s$ and $i$ when used as subscripts). The following reactions occur in both the aqueous and membrane phases:

$$I^+ + kS \underset{K^+_{is_k}}{\rightleftharpoons} IS_k^+ \quad (k=1,2,\dots n), \qquad K^+_{is_k} = \frac{a_{is_k}}{a_i a_s^k} \tag{A.1}$$

where $a$ denotes the activity of the species in moles per liter and $K^+_{is_k}$ ($k = 1, 2, \dots n$) is the equilibrium association constant for the $k^{\text{th}}$ homogeneous reaction. It is assumed that a negligible quantity of complexes of the type $I_m S_n^{m+}$, where $m > 1$ are formed. The experimental justification for this assumption is that such complexes are not observed for the polyethers in bulk aqueous solutions (Frensdorff, 1971*a*).

Eq. (A.1) describes the homogeneous reactions which can occur in either the aqueous or organic phases. Eq. (A.2) describes the heterogeneous reaction by which the neutral species $S$ crosses the membrane-solution interface in terms of a partition coefficient $k_s$:

$$S \underset{k_s}{\rightleftharpoons} S^*, \qquad k_s = \frac{C_s^*}{C_s} \tag{A.2}$$

where the asterisk ($*$) denotes a quantity characteristic of the membrane phase. Similarly, Eq. (A.3) describes the partition equilibrium of the univalent charged species $IS_k^+$

$$IS_k^+ \underset{k^+_{is_k}}{\rightleftharpoons} IS_k^{+*}, \qquad k_{is_k} = \frac{a^*_{is_k}}{a_{is_k}} \exp(+F\psi^*/RT) \tag{A.3}$$

where $\psi^*$ is the electrostatic potential in the interior of the membrane. (A potential difference between the membrane and the bulk aqueous solution will arise if there is a surface charge on the membrane due to a net charge on the polar head group.) The concentrations of the charged carrier-cation complexes within the membrane are assumed to be sufficiently high that they determine its electrical properties, but not so high that they produce an appreciable perturbation of the constant potential within the membrane by the formation of a space charge. That is, their concentration is assumed to be less than $5 \times 10^{-5}$ M (Neumcke & Läuger, 1970), the experimental justification for this assumption being the observation that the graph of the conductance *vs.* the concentration of neutral carrier does not show any tendency to saturate (*see* Fig. 2).

Ideal behavior will be assumed for all species in the membrane phase as well as for the $S$ and $IS_k^+$ species in the aqueous phase. Activities $a$ will therefore be replaced by concentrations $C$ in all further equations. Activity coefficient corrections will, however, be made for the concentrations of $I^+$ in the aqueous phase.

In this treatment it is immaterial whether the ion combines with the carrier(s) in the aqueous phase and crosses the membrane solution interface as a complex or combines with the carrier(s) at the interface of the membrane phase as illustrated in Eq. (A.4), which is obtained by combining Eqs. (A.1), (A.2) and (A.3):

$$I_{x=0}^{+} + kS^{*} \underset{\overline{K}_{is_k}^{+}}{\rightleftharpoons} IS_k^{+*}, \qquad \overline{K}_{is_k}^{+} \equiv \frac{C_{is_k}^{*}}{a_i \exp\left(-\dfrac{F\psi^{*}}{RT}\right)(C_s^{*})^{k}} = \frac{k_{is_k} K_{is_k}^{+}}{(k_s)^{k}}. \tag{A.4}$$

Stark and Benz (1971) have distinguished between these two possibilities for the carrier valinomycin on the basis of kinetic measurements and have shown that most of the ions enter the membrane via the pathway described by Eq. (A.4) for this carrier.

*The Chemical Composition of an Ionic Solution in the Presence of a Neutral Carrier.* We desire an expression for the equilibrium aqueous concentrations of the carrier $C_s$ and its complexes $C_{is_k}$ in terms of the two experimentally accessable variables, the ionic activity $a_i$ and the total concentration of carrier added to the aqueous phase $C_s^{\mathrm{Tot}}$. For $m$ species of cations in the aqueous solution, Eq. (A.1) and the assumption of ideal behavior yields:

$$C_s = \frac{C_{is}}{K_{is}^{+} a_i} = \frac{C_{is_2}}{K_{is_2}^{+} a_i C_s} = \cdots \frac{C_{is_n}}{K_{is_n}^{+} a_i C_s^{n-1}} \tag{A.5}$$

where $i$ refers to any of the $m$ cation species present in the aqueous solution. By adding the numerators and denominators the following equation is obtained:

$$C_s = \sum_{\substack{i=1\\k=1}}^{m,n} C_{is_k} \Big/ \sum_{\substack{i=1\\k=1}}^{m,n} K_{is_k}^{+} a_i C_s^{k-1}. \tag{A.6}$$

Eq. (A.6) may be simplified for the case under consideration by assuming that

$$C_s V + \sum_{\substack{i=1\\k=1}}^{m,n} C_{is_k} V \gg C_s^* V^* + \sum_{\substack{i=1\\k=1}}^{m,n} C_{is_k}^* V^*. \tag{A.7}$$

The left-hand side of Eq. (A.7) is the total amount of carrier in the aqueous phase, which is assumed to be much greater than the total amount of carrier which has partitioned into the membrane phase. [The ratio of the volume of the aqueous solution to the volume of lipid added to the chamber $V/V^*$ is greater than $30\,\text{ml}/10^{-3}\,\text{ml} = 3 \times 10^4$ whereas the partition coefficient of XXXII has been measured to be less than $10^4$ in favor of an organic phase similar to the interior of the membrane (Fig. 9). At most then, 30% of the total amount of polyether added to the aqueous phase could be in the lipid phase. The actual loss of polyether to the excess lipid is probably much less than this because measurements were made a few minutes after the addition of the polyether, a sufficient time for the carrier to equilibrate with the bilayer but not with the lipid in the torus.] The conservation equation for the carrier is therefore

$$C_s^{\text{Tot}} = C_s + \sum_{\substack{i=1\\k=1}}^{m,n} C_{is_k} \tag{A.8}$$

where $C_s^{\text{Tot}}$ is the total concentration of $S$ added to the aqueous phase and $C_s$ is the free concentration present.

$$C_s = \frac{C_s^{\text{Tot}}}{\left(1 + \sum\limits_{\substack{i=1\\k=1}}^{m,n} K_{is_k}^+ C_s^{k-1} a_i\right)}. \tag{A.9}$$

For the experimental conditions encountered in this investigation, the concentration of polyether $C_s$ was always sufficiently low that the activity of the ions $a_i$ was not significantly affected by association. Since only the 1:1 $IS^+$ complex exists in significant quantities in a solution of high dielectric constant such as water (Frensdorff, 1971*a*), it is unnecessary to sum over the index $k$ in Eq. (A.9) and this equation simplifies to:

$$C_s = \frac{C_s^{\text{Tot}}}{\left(1 + \sum\limits_{i=1}^{m} K_{is}^+ a_i\right)}. \tag{A.10}$$

Combining Eq. (A.10) with Eq. (A.1) the expression for the aqueous concentration of the complex formed from the $i^{\text{th}}$ ion and $k$ polyether molecules $C_{is_k}$ is obtained:

$$C_{is_k} = K_{is_k}^+ a_i \left(C_s^{\text{Tot}} \Big/ 1 + \sum_{i=1}^{m} K_{is}^+ a_i\right)^k. \tag{A.11}$$

Eq. (A.11) expresses the concentration of the permeant $IS_k$ complex in terms of the known concentrations of polyether and salt in the aqueous phase.

*The conductance in the Limit of Zero Applied Voltage.* If the Nernst-Planck equations are assumed to be valid, the conductance measured in the limit of zero applied voltage $G_0$ of a membrane separating identical solutions is given by (Eisenman *et al.*, 1968; Ciani *et al.*, 1969):

$$G = F^2 \Big/ \int_0^d \left[ 1 \Big/ \sum_{\substack{i=1\\k=1}}^{m,n} u^*_{is_k} C^*_{is_k}(x) \right] dx \qquad \text{(A.12)}$$

where $F$ is the Faraday, $d$ the membrane thickness, $C^+_{is_k}(x)$ the concentration of the $IS_k$ complex within the membrane and $u^*_{is_k}$ its mobility, which is assumed to be a constant. The essential assumption required to derive this equation is that the rate-limiting step for ion movement is diffusion of the complex through the interior of the membrane. When the total concentration of charged $IS_k^+$ complexes within the membrane is sufficiently low ($< 5 \times 10^{-5}$ M; Neumcke & Läuger, 1970), as may be shown to be the case here, we may assume that the concentration profile is constant and Eq. (A.1) reduces to:

$$G = \frac{F^2}{d} \sum_{\substack{i=1\\k=1}}^{m,n} u^*_{is_k} C^*_{is_k}. \qquad \text{(A.13)}$$

At equilibrium, the concentration of the permeant complex within the membrane $C^*_{is_k}$ is related to its bulk concentration in the aqueous phase $C_{is_k}$ by

$$C^*_{is_k} = k_{is_k} C_{is_k} \exp(-F\psi^*/RT) \qquad \text{(A.14)}$$

where $\psi^*$ is the potential difference between the membrane and the bulk aqueous phase. Eq. (A.14) is merely the expression of electrochemical equilibrium, but Neumcke and Läuger (1970) have argued that the Boltzmann expression for the ion concentration in the aqueous phase will be valid even when a potential is applied to the membrane. We have shown (McLaughlin *et al.*, 1970, 1971) that the surface potential $\psi^*$ may be described to a rather good approximation by the Gouy-Chapman equation for the diffuse double layer

$$\exp\left(\frac{+F\psi^*}{RT}\right) = \left(\alpha + \sqrt{\alpha^2+1}\right)^{\frac{2\sigma}{|\sigma|}}, \qquad \alpha = 136\,|\sigma| \Big/ \sqrt{\sum_{i=1}^{m} C_i} \qquad \text{(A.15)}$$

where $\sigma$ is the charge density in units of electronic charge per Å$^2$ and $\Sigma_i C_i$ is the total electrolyte concentration in moles/liter. This form of the equation is applicable when only monovalent ions are present and the temperature is 22 °C. Combining Eqs. (A.11), (A.13), (A.14) and (A.15) yields the general expression for the conductance

$$G=\frac{F^2}{d}\sum_{\substack{i=1\\k=1}}^{m,n} u^*_{i s_k} k_{i s_k} K^+_{i s_k} a_i \left(\frac{C_s^{\mathrm{Tot}}}{1+K_{is} a_i}\right)^k \frac{1}{(\alpha+\sqrt{\alpha^2+1})}^{\frac{2\sigma}{|\sigma|}}. \tag{A.16}$$

Note that whenever the surface charge is negligible ($\alpha \ll 1$) the last term reduces to unity and that when only a single cation species is present the summation over $i$ may be ignored.

Consider now the dependence of the conductance on the polyether and salt concentrations. The experimental observation (Figs. 2 and 5) that the conductance depends on the cube of the polyether concentration in the region around $C_s^{\mathrm{Tot}}=10^{-5}$ M implies that only the cubic term of the polynomial in Eq. (A.16) is important in this region (i.e., the permeant species are essentially all the 3:1 complexes). The conductances in the presence of a single salt may therefore be described by the following expression in the region of polyether concentration around $10^{-5}$ M:

$$G=\frac{F^2}{d} u^*_{i s_3} k_{i s_3} K^+_{i s_3} a_i \left(\frac{C_s^{\mathrm{Tot}}}{1+K^+_{is} a_i}\right)^3 \frac{1}{(\alpha+\sqrt{\alpha^2+1})}^{\frac{2\sigma}{|\sigma|}}. \tag{A.17}$$

When the surface charge produces a significant potential, as is the case for asolectin membranes (McLaughlin *et al.*, 1970, *see* Fig. 2), the dependence of conductance on salt concentration is extremely complex. If, however, only monovalent ions are present and the ionic strength is maintained a constant with a relatively impermeant or "indifferent" electrolyte like lithium chloride, the term in $\sigma$ will remain a constant. This is why the ionic strength was held constant in the experiments illustrated in Fig. 7. At a constant ionic strength and a polyether concentration of $10^{-5}$ M, the conductance will depend on the concentration of permeant ions in the aqueous phase in the following manner

$$G=(\gamma+\delta_i a_i)\left(\frac{C_s^{\mathrm{Tot}}}{1+K^+_{is} a_i}\right)^3 \tag{A.18}$$

where the values of the constants $\gamma$ and $\delta$ are apparent from a comparison of Eqs. (A.17) and (A.18).

The ratios of the conductance measured for two different ions $I$ and $J$ at the same concentrations in the presence of $10^{-5}$ M XXXII [where the cubic term dominates Eq. (A.16) and Eq. (A.17) is valid] are given by:

$$\frac{G_j}{G_i}=\frac{u^*_{j s_3} k_{j s_3}}{u^*_{i s_3} k_{i s_3}} \frac{K^+_{j s_3}}{K^+_{i s_3}} \cdot \frac{(1+K^+_{is} a_i)^3}{(1+K^+_{js} a_j)^3}. \tag{A.19}$$

Note that the polyether concentration cancels, as does the term in $\alpha$.

*The Membrane Potential and the Permeability Ratios.* When the Nernst-Planck flux equations and the Einstein relation ($D = RTu$) are assumed to be valid the condition for zero current flow indicates that:

$$0 = \sum_{\substack{i=1\\k=1}}^{m,n} u^*_{i s_k} \left( \frac{dC^*_{i s_k}}{dx} + \frac{F}{RT} C_{i s_k} \frac{d\psi^*}{dx} \right). \tag{A.20}$$

If the mobilities of all the complexes are assumed to be constant, dividing Eq. (A.20) by $\sum_{i,k}^{m,n} u^*_{i s_k} C^*_{i s_k}$ and integrating directly across the membrane from $x = 0$ to $x = d$ yields:

$$\psi^*(d) - \psi^*(0) = -\frac{RT}{F} \ln \frac{\sum_{i,k=1}^{m,n} u^*_{i s_k} C^*_{i s_k}(d)}{\sum_{i,k=1}^{m,n} u^*_{i s_k} C^*_{i s_k}(0)} \tag{A.21}$$

which is the expression for the diffusion potential within the membrane in terms of $C^*_{i s_k}(d)$ and $C^*_{i s_k}(0)$, the concentration of the permeant $IS_k$ complexes within the membrane at the interfaces.

By assuming that the heterogeneous reactions described by Eq. (A.4) are in equilibrium at the membrane-solution interfaces, expressions for $C^*_{i s_k}(d)$ and $C^*_{i s_k}(0)$ may be inserted into Eq. (A.21) to yield:

$$V_0 = \frac{RT}{F} \ln \frac{\sum_{i,k=1}^{m,n} u^*_{i s_k} k_{i s_k} K^+_{i s_k} \left( \frac{C^*_s(d)}{ks} \right)^k a''_i}{\sum_{i,k=1}^{m,n} u^*_{i s_k} k_{i s_k} K^+_{i s_k} \left( \frac{C^*_s(0)}{ks} \right)^k a'_i} \tag{A.22}$$

which is the desired expression for the total potential difference between the two solutions $V_0$. The concentrations of the polyether within the membrane at the interfaces $C^*_s(0)$ and $C^*_s(d)$ must, however, be expressed in terms of the known concentrations of polyether in the bulk aqueous solutions. Different expressions are obtained for the potential depending on whether the rate-limiting step for the permeation of the neutral species is diffusion in the membrane, the rate of crossing the membrane solution interface, or diffusion in the aqueous unstirred layers. It is known, however, that the rate-limiting step for the neutral species is diffusion through the unstirred layers (McLaughlin *et al.*, 1972). In this case, the concentrations of the neutral species are the same at both interfaces and to a good approximation for the polyether in an alkali metal chloride solution:

$$C^*_s(0) = C^*_s(d) = \frac{k_s}{2}(C'_s + C''_s) \equiv k_s C^{\text{eff}}_s. \tag{A.23}$$

Inserting the value of $C_s$ from Eq. (A.10) into Eq. (A.23) yields:

$$C_s^{\mathrm{eff}}=\frac{1}{2}\left(\frac{C_s^{\mathrm{Tot}'}}{1+\sum\limits_{i=1}^{m} K_{is}^{+}a_i'}+\frac{C_s^{\mathrm{Tot}''}}{1+\sum\limits_{i=1}^{m} K_{is}^{+}a_i''}\right). \tag{A.24}$$

If Eq. (A.24) is inserted into Eq. (A.21) the following expression for the membrane potential is obtained:

$$V_0=\frac{RT}{F}\ln\frac{\sum\limits_{i,k=1}^{m,n} u_{is_k}^{*}k_{is_k}K_{is_k}^{+}(C_s^{\mathrm{eff}})^k a_i''}{\sum\limits_{i,k=1}^{m,n} u_{is_k}^{*}k_{is_k}K_{is_k}^{+}(C_s^{\mathrm{eff}})^k a_i'}. \tag{A.25}$$

When one aqueous solution (′) contains the cations $I$ and $J$ at activities $a_i'$ and $a_j'$ and the other side (″) contains the same cations at activities $a_i''$ and $a_j''$ and both sides contain the same total concentration of polyether $C_s^{\mathrm{Tot}}$, Eq. (A.25) may be written as:

$$V_0=\frac{RT}{F}\ln\left(\frac{a_i'+\beta a_j'}{a_i''+\beta a_j''}\right). \tag{A.26}$$

The parameter $\beta$ is formally equivalent to the permeability ratio $P_j/P_i$ of the Goldman-Hodgkin-Katz equation and is equal to:

$$\beta=\frac{P_j}{P_i}=\frac{\sum\limits_{k=1}^{m} u_{js_k}^{*}k_{js_k}K_{js_k}^{+}(C_s^{\mathrm{eff}})^k}{\sum\limits_{k=1}^{m} u_{is_k}^{*}k_{is_k}K_{is_k}^{+}(C_s^{\mathrm{eff}})^k}. \tag{A.27}$$

In general, therefore, the permeability ratios will depend on both the polyether and salt concentrations. Note, however, that if the same term (i.e., the $k^{\mathrm{th}}$) of the polynomials in the numerator and denominator of Eq. (A.27) dominates all the other terms, $(C_s^{\mathrm{eff}})^k$ cancels and the permeability ratios become independent of both carrier and salt concentration. The polynomials in Eq. (A.27) are similar to those in the expression for the conductance [Eq. (A.16)] in that both contain the product of the mobility, partition coefficient and association constants. It can be shown that the $k^{\mathrm{th}}$ term in the numerator and denominator of Eq. (A.27) will dominate all the other terms if the slopes of the $G_0$ *vs.* $C_s^{\mathrm{Tot}}$ curves are $k$ over a significant range for the ions $I$ and $J$ on a log-log plot. The slopes are in fact 3 (*see* Fig. 5) for all the alkali metal cations at a polyether concentration of $10^{-5}$ M[10]. The permeability ratios, measured at this polyether concentration, will therefore be equal to:

$$\frac{P_j}{P_i}=\frac{u_{js_3}^{*}k_{js_3}K_{js_3}^{+}}{u_{is_3}^{*}k_{is_3}K_{is_3}^{+}}. \tag{A.28}$$

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[10] The one exception is the potassium curve at high salt concentrations.

Combining Eq. (A.28) with Eq. (A.19), the following relationship between the permeability and conductance ratios, measured at a polyether concentration of $10^{-5}$ M, is obtained:

$$\frac{G_j}{G_i} \cdot \frac{P_i}{P_j} = \left( \frac{1 + K_{is}^{+} a_i}{1 + K_{js}^{+} a_j} \right)^3 . \tag{A.29}$$

The permeability and conductance ratios should therefore be equal at low salt concentrations where association is negligible, and should differ at higher salt concentrations if the association constants for the formation of the 1:1 aqueous complexes differ for the two ions.

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## References

Bright, D., Truter, M. R. 1970*a*. Crystal structures of complexes between alkali metal salts and cyclic polyethers. *J. Chem. Soc.*, (*B*). 1970:1544.

Bright, D., Truter, M. R. 1970*b*. Crystal structure of a cyclic polyether complex of alkali metal thiocyanate. *Nature* **225**:176.

Cass, A., Finkelstein, A., Krespi, V. 1970. The ion permeability induced in thin lipid membranes by the polyene antibiotics nystatin and amphotericin B. *J. Gen. Physiol.* **56**:100.

Chandler, W. K., Meves, H. 1965. Voltage-clamp experiments on internally perfused giant axons. *J. Physiol.* **180**:788.

Christensen, J. J., Hill, J. O., Izatt, R. M. 1971. Ion binding by synthetic macrocyclic compounds. *Science* **174**:459.

Ciani, S., Eisenman, G., Szabo, G. 1969. A theory for the effects of neutral carriers such as the macrotetralide actin antibiotics on the electrical properties of bilayer membranes. *J. Membrane Biol.* **1**:1.

Eisenman, G., Ciani, S. M., Szabo, G. 1968. Some theoretically expected and experimentally observed properties of lipid bilayer membranes containing neutral molecular carriers of ions. *Fed. Proc.* **27**:1289.

Eisenman, G., Ciani, S. M., Szabo, G. 1969. The effects of the macrotetralide actin antibiotics on the equilibrium extraction of alkali metal salts into organic solvents. *J. Membrane Biol.* **1**:294.

Eisenman, G., Szabo, G., McLaughlin, S., Ciani, S. 1972. Molecular basis for the action of macrocyclic carriers on passive ion translocation across lipid bilayer membranes. *J. Bioenergetics*. (*In Press*.)

Frensdorff, H. K. 1971*a*. The stability constants of cyclic polyether complexes with univalent cations. *J. Amer. Chem. Soc.* **93**:600.

Frensdorff, H. K. 1971*b*. Salt complexes of cyclic polyethers. Distribution equilibria. *J. Amer. Chem. Soc.* **93**:4684.

Goldman, D. E. 1943. Potential, impedance, and rectification in membranes. *J. Gen. Physiol.* **27**:37.

Hodgkin, A. L., Katz, B. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol.* **116**:473.

Hopfer, U., Lehninger, A. L., Lennarz, W. J. 1970. The effect of the polar moiety of lipids on the ion permeability of bilayer membranes. *J. Membrane Biol.* **2**:41.

Izatt, R. M., Nelson, D. P., Rytting, J. H., Haymore, B. L., Christensen, J. J. 1971. A calorimetric study of the interaction in aqueous solution of several uni- and bivalent metal ions with the cyclic polyether dicyclohexyl-18-crown-6 at 10, 25 and 40°. *J. Amer. Chem. Soc.* **93**:1619.

Kilbourn, B. T., Dunitz, J. D., Pioda, L. A. R., Simon, W. 1967. Structure of the $K^+$ complex with nonactin, a macrotetralide possessing highly specific $K^+$ transport properties. *J. Mol. Biol.* **30**:559.

Krasne, S., Eisenman, G., Szabo, G. 1971. Freezing and melting of lipid bilayers and the mode of action of nonactin, valinomycin and gramicidin. *Science* **174**:412.

Läuger, P., Stark, G. 1970. Kinetics of carrier-mediated ion transport across lipid bilayer membranes. *Biochim. Biophys. Acta* **211**:458.

Markin, V. S., Krishtalik, L. I., Liberman, E. A., Topaly, V. P. 1969. Mechanism of conductivity of artificial phospholipid membranes in the presence of ion carriers. *Biofizika* **14**:256.

McLaughlin, S. G. A. 1972. Local anaesthetics, salicylate and the surface potential of bilayer membranes. *Biophys. Soc. Abstr.* **12**:181a.

McLaughlin, S. G. A., Eisenman, G., Szabo, G. 1972. The rate limiting step for the permeation of a cyclic polyether through bilayer membranes. (*In Preparation.*)

McLaughlin, S. G. A., Szabo, G., Eisenman, G. 1971. Divalent ions and the surface potential of charged phospholipid membranes. *J. Gen. Physiol.* **58**:667.

McLaughlin, S. G. A., Szabo, G., Eisenman, G., Ciani, S. M. 1970. Surface charge and the conductance of phospholipid membranes. *Proc. Nat. Acad. Sci.* **67**:1268.

Neumcke, B., Läuger, P. 1970. Space charge-limited conductance in lipid bilayer membranes. *J. Membrane Biol.* **3**:54.

Parsegian, A. 1969. Energy of an ion crossing a low dielectric membrane: solutions to four relevant electrostatic problems. *Nature* **221**:844.

Pedersen, C. J. 1967. Cyclic polyethers and their complexes with metal salts. *J. Amer. Chem. Soc.* **89**:7017.

Pedersen, C. J. 1970. Crystalline salt complexes of macrocyclic polyethers. *J. Amer. Chem. Soc.* **92**:386.

Shemyakin, M. M., Ovchinnikov, Yu. A., Ivanov, V. T., Antonov, V. K., Vinogradova, E. I., Shkrob, A. M., Malenkov, G. G., Evstratov, A. V., Laine, I. A., Melnik, E. I., Ryabova, I. D. 1969. Cyclodepsipeptides as chemical tools for studying ionic transport through membranes. *J. Membrane Biol.* **1**:402.

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

Stillman, I., Gilbert, D., Robbins, M. 1969. Effect of monactin on potassium currents of squid giant axons. *Biophys. Soc. Abstr.* **9**:A-250.

Szabo, G. 1969. The effect of neutral molecular complexes of cations on the electrical properties of lipid bilayer membranes. Ph. D. Thesis. University of Chicago, Chicago, Ill.

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

Szabo, G., Eisenman, G., McLaughlin, S. G. A., Krasne, S. 1972. Ionic probes of membranes structures. *Proc. N.Y. Acad. Sci.* (*In Press.*)

Tosteson, D. C. 1968. Effect of macrocyclic compounds on the ionic permeability of natural and artificial membranes. *Proc. Fed. Amer. Soc. Exp. Biol.* **27**:1269.