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Influence of Molecular Variations of Ionophore and Lipid on the Selective Ion Permeability of Membranes: I. Tetranactin and the Methylation of Nonactin-type Carriers

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Summary. The manner in which the molecular structure of the carrier and the lipid composition of the membrane modulate the membrane selectivity among monovalent cations has been investigated for nonactin, trinactin, and tetranactin, which differ only in their degrees of methylation, and for membranes made of two lipids, phosphatidyl ethanolamine and glyceryl dioleate, in which "equilibrium" and "kinetic" aspects of permeation, respectively, are emphasized. Bilayer permeability ratios for Li, Na, K, Rb, Cs, Tl, and NH_4 have been characterized and resolved into "equilibrium" and "kinetic" components using a model for carrier-mediated membrane transport which includes both a trapezoidal energy barrier for translocation of the complex across the membrane interior and a potential-dependence of the loading and unloading of ions at the membrane-solution interfaces. The bilayer permeability properties due to tetranactin have been characterized in each of these lipids and found not only to be regular but to be systematically related to those of the less methylated homologues, trinactin and nonactin. This analysis has led to the following conclusions: (1) The change in lipid composition alters the relative contributions of "kinetic" *vs.* "equilibrium" components to the observed carrier-mediated selectivity. (2) Increased methylation of the carrier increases the contribution of the "kinetic" component to the selectivity relative to that of the "equilibrium" component and additionally alters the "equilibrium" component sufficiently that an inversion in Cs—Na selectivity occurs between trinactin and tetranactin. (3) For all ions and carriers examined, the "reaction plane" for ion-carrier complexation and the width for the "diffusion barrier" can be represented by the same two parameters, independent of the ion or carrier, so that in all cases the complexation reaction senses 10% of the applied potential and the plateau of the "diffusion barrier" extends across 70% of the membrane interior.

The present paper analyzes the effects on selective cation permeation of varying the degree of methylation of nonactin-type molecules and of varying the lipid composition of the membrane, a subject reported upon more briefly elsewhere (Eisenman, Krasne & Ciani, 1975). This paper is part of a series of papers examining the selective ion permeation induced in bilayer membranes of varied composition by a number of structurally-

related synthetic and naturally occurring cation carriers. Our goal is to determine the influence of the molecular structure of the carrier both on its equilibrium binding of cations and on the selective permeability to cations which it confers on a bilayer membrane. We believe that the understanding of structure-selectivity relationships which studies such as these provide will ultimately be of use in inferring the molecular structures of ion-binding sites in biological systems from their selectivity properties.

The selective cation permeabilities (seen both in membrane potentials and membrane conductances) conferred upon a bilayer by a carrier molecule may reflect not only the equilibrium selectivity intrinsic to the carrier molecule, as initially suggested (Pressman, Harris, Jagger & Johnson, 1967; Eisenman, Ciani & Szabo, 1968; Ciani, Eisenman & Szabo, 1969), but have also been shown to depend on the rates of ion-carrier complexation at the membrane-solution interface (Markin, Kristalik, Liberman & Topaly, 1969; Läuger & Stark, 1970; Stark & Benz, 1971; Ciani, Eisenman, Laprade & Szabo, 1973*a*; Laprade, Ciani, Eisenman & Szabo, 1975). If the chemical reactions between the carriers and the carried ions occur sufficiently rapidly (both in the aqueous solutions and at the membrane solution interfaces) that they can be considered to be at equilibrium relative to the rate of movement of the ion-carrier complexes across the membrane interior, then the ionic permeabilities deduced from zero-current transmembrane potential measurements reflect simply the product of the equilibrium selectivity intrinsic to the carrier molecule in the membrane and the mobility ratios of the ion-carrier complexes, the latter term being neglected in the case of "isosteric" [1] complexes such as those formed by the present carriers. This realm of behaviors has been termed the "equilibrium domain" (Ciani *et al.*, 1973*a*, p. 80; Eisenman, Szabo, Ciani, McLaughlin & Krasne, 1973, p. 144). If, however, the chemical reactions between the carrier and carried ions occur at a rate which is comparable to or slower than the rate of movement of the ion-carrier complexes across the membrane interior, then the ionic permeability properties conferred upon the membrane also reflect the rates of ion-carrier complexation at the membrane-solution interface

1 "Isosteric" complexes refer to those ion-carrier complexes for which the overall size and shape as well as the externally viewed charge distribution of the complex are approximately the same for all cations. A discussion of "isostericity" as well as the justification for considering the complexes with different ions of nonactin- and valinomycin-type carriers to be "isosteric" can be found in Eisenman *et al.* (1969, p. 323), Eisenman *et al.* (1973, p. 156ff), or Szabo *et al.* (1973, p. 226ff.).

(Markin *et al.*, 1969; Läuger & Stark, 1970; Stark & Benz, 1971; Ciani *et al.*, 1973*a*, Laprade *et al.*, 1975), this realm of behaviors having been termed the "kinetic domain" (Ciani *et al.*, 1973*a*, p. 95; Eisenman *et al.*, 1973, p. 144). In this latter case, however, it has been shown (Ciani *et al.*, 1973*a*) that the selectivity in ionic permeability inferred from the steady-state membrane potential or conductance can be analytically decomposed into a voltage-independent "equilibrium" component of selectivity intrinsic to the carrier molecule and independent of the lipid together with a voltage-dependent "kinetic" component whose magnitude depends not only on the carrier molecule but also upon the lipid composition of the membrane.

In this paper the analysis of the separate "equilibrium" and "kinetic" components contributing to the observed ionic permeabilities is based upon steady-state electrical measurements of bilayer membrane potentials and conductances which are interpreted according to a model, presented by Ciani (1976) in the following article, which has evolved from the work of a number of investigators (Ciani *et al.*, 1969, 1973*a*; Ciani, Laprade, Eisenman & Szabo, 1973*b*; Markin *et al.*, 1969; Läuger & Stark, 1970; Hall, Mead & Szabo, 1973; Hladky, 1973, 1974; Feldberg & Kissel, 1975; Laprade *et al.*, 1975) and which is capable of rationalizing the electrical properties observed for carrier-mediated ion transport through bilayers formed from a wide variety of lipid compositions. In particular, the present data are most consistently fit by a model (*see* Fig. 1) which incorporates both a trapezoidal energy barrier (Hall, Mead & Szabo, 1973; Hladky, 1974) for the transfer of the ion-carrier complex across the membrane interior and a plane for ion-carrier complexation located at a point near the membrane surface, but within the membrane, which senses some fraction of the voltage drop across the membrane (Hladky, 1974; Feldberg & Kissel, 1975; Andersen & Fuchs, 1975). The potential energy profile corresponding to this model is illustrated in Fig. 1 in which α is the width of the plateau of the "diffusion barrier" (in units of membrane thickness) and β is the position of the ion-carrier "reaction plane" in the membrane (also in units of membrane thickness).[2] In addition, it is assumed that the rate at which the ion-carrier complex is supplied to the membrane from pre-formed complexes in the aqueous phase is much slower (due to diffusion through the unstirred layer and the lifetime of

2 When defining these parameters in terms of membrane thickness, one assumes that the applied potential falls as a constant gradient across the entire core of the membrane. Alternatively, these symbols could be defined as fractions of the voltage drop across the membrane with no restriction on the profile of the applied potential within the membrane.

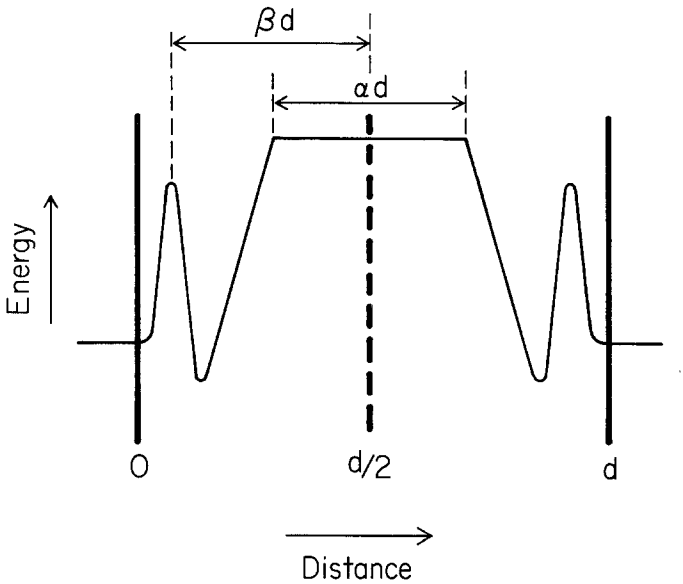


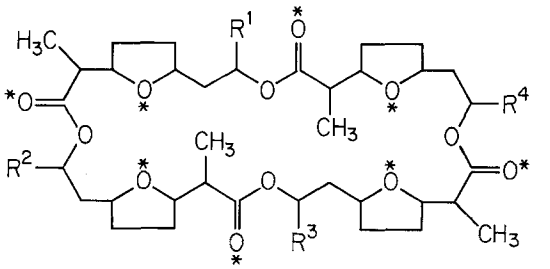
Fig. 1. Schematic diagram of the "extended" model for the potential energy barriers surmounted by an ion and carrier in the course of ion transport across a bilayer membrane. The ordinate is free energy. The abscissa is distance, with the heavy vertical lines at 0 and d representing the left- and right-hand membrane-solution interfaces, respectively. The energy level in the aqueous phase represents the combined free energies of ion hydration and carrier solubilization in the membrane. The outermost peak represents the energy of the "transition state" for formation of the ion-carrier complex in the membrane, the locus of this peak being termed the "reaction plane" and being located a distance βd from the midpoint of the membrane. The "wells" in the membrane represent the energy of the "equilibrium state" for the ion-carrier complex inside the membrane. The trapezoid in the middle of the membrane, termed the "diffusion barrier", represents the energy barrier which must be surmounted by an ion-carrier complex in crossing the membrane interior; the plateau of this barrier has a width of αd

the charged species in the aqueous phase) than the rate at which the complex is supplied via complexation between the ion and carrier at the reaction plane in the membrane.[3]

The present paper extends previously reported observations (Szabo, Eisenman & Ciani, 1969; Szabo, Eisenman, Ciani, Laprade & Krasne, 1973; Ciani *et al.*, 1973*a*; Eisenman *et al.*, 1973; Hladky, 1974; Laprade *et al.*, 1975; Benz & Stark, 1975; Feldberg & Kissel, 1975) on the membrane permeability properties of the macrotetralide actin molecules in two

3 This assumption corresponds to the "R_{is} mechanism" of Ciani *et al.* (1973*a*) and has been previously shown to be the predominant mechanism by which charged species are supplied to the membrane for the nactins and valinomycin (Stark & Benz, 1971; Ciani, Gambale, Gliozzi & Rolandi, 1975).

MACROTETRALIDES



$R^1 = R^2 = R^3 = R^4 = CH_3$ NONACTIN

$R^1 = CH_3$ $R^2 = R^3 = R^4 = C_2H_5$ TRINACTIN

$R^1 = R^2 = R^3 = R^4 = C_2H_5$ TETRANACTIN

Fig. 2. Chemical formulae of the macrotetralide actin antibiotics nonactin, trinactin, and tetranactin

ways. First, we have characterized tetranactin, which has not been previously studied and which has one more methyl group than the most highly methylated nactin (trinactin) studied so far (*see* Fig. 2). Second, using the extended model for membrane permeation (Ciani, 1976) described by Fig. 1, we have made a quantitative analysis and comparison of the "equilibrium" and "kinetic" contributions to the observed ion selectivities of nonactin, trinactin, and tetranactin in membranes having two different lipid compositions. One lipid, phosphatidyl ethanolamine/decane (PE/dec), was chosen because previous observations (Ciani *et al.*, 1973*a*; Szabo *et al.*, 1973) suggested that the observed permeability ratios should reflect chiefly the "equilibrium" component of the selectivity while the other lipid, glyceryl dioleate/decane (GDO/dec), was chosen because in this lipid marked "kinetic" components are manifested (Ciani *et al.*, 1973*a*; Hladky, 1974; Laprade *et al.*, 1975; Feldberg & Kissel, 1975). The results presented below are in complete accord with these expectations and verify the practical usefulness of PE/dec membranes as a bilayer system in which to study the equilibrium aspects of permeability with almost no complications due to kinetic effects.

Materials and Methods

Materials

Glyceryl dioleate-decane (GDO/dec) membranes were formed from a 5% (v/v) solution in *n*-decane of glyceryl dioleate (Pfaltz and Bauer, 90% purity mixture of the 1–2 and 1–3 esters). Phosphatidyl ethanolamine-decane (PE/dec) membranes were formed from a 25 mg/ml solution of bacterial phosphatidyl ethanolamine (Supelco, 99% purity) in *n*-decane. *n*-decane (Aldrich, 99.9+% grade) was used without further purification. Nonactin was a gift from Barbara Sterns of Squibb, trinactin was a gift from Hans Bickel, and tetranactin was a gift from W. Simon and from K. Ando. Distilled water, subsequently deionized on a Super Q column to >18 megohm-cm^2 resistance, was used for preparation of solutions from analytical grade reagents (ultrapure, where available).

Methods

Electrical Methods

All experiments were performed at 23 °C ± 1 °C. The electrical methods were the same as those used in previous studies (Szabo *et al.*, 1969; Laprade *et al.*, 1975) with the following particulars to be noted. Ag—AgCl electrodes were routinely used, two for the zero-current potential (V_0) measurements and four for the current-voltage (I–V) measurements. An electrometer (Keithley 602) was used to measure the transmembrane potential or current. A function generator (Interstate Electronics Corp., F 5 3 A) supplied the electrical signals for the zero-current conductance (±10 mV amplitude square wave symmetric with respect to zero, frequency 0.01 to 0.04 Hz) and current-voltage (triangular wave symmetric with respect to zero, sweep rate ±60 mV/sec) measurements. Concentration polarization in the aqueous phase was determined to be negligible over the experimental range of sweep rates from 6 to 120 mV/sec as judged by the frequency independence of the $I-V$ characteristic. Since electrode and solution polarization were found negligible for all of the experiments reported here, electronic voltage clamping of the membrane potential was unnecessary and was not used.

Methods for Membrane Formation and the Addition of Carrier

Membranes were formed on a teflon partition separating two aqueous compartments of 20 ml volume each according to previously published methods (Szabo *et al.*, 1969) with the slight modification that the membrane was formed from a total of 4 µl of lipid solution applied to the hole in the teflon partition by adding 2 µl to each side of the partition using a micropipette. This method of forming membranes symmetrically was important for obtaining current-voltage curves which were symmetrical about zero voltage.

Aliquots of stock ethanol solutions of the carrier were added either to the aqueous or lipid solutions. The final concentration of ethanol in the aqueous phase never exceeded 1%. When the carrier was added to the lipid solution, the ethanol was first evaporated from an aliquot of the stock solution of the carrier, and then the lipid-decane solution was added to give the final concentration stated. The decision as to whether to add the carrier via the lipid or via the aqueous phase depended on which was optimal for the particular measurement and the particular lipid according to the following considerations:

a. Zero-current conductance measurements. In order to circumvent the time dependence[4] of conductance when adding tetranactin to the aqueous phase, we have added this carrier routinely to the lipid phase when making zero-current conductance measurements, so as to be "torus-buffered" (Hladky, 1972, 1973). An additional benefit of performing the experiment in this way is that the membrane conductance is then expected to be independent of any complexation between ion and carrier which may take place in the aqueous solutions (*see* Hladky, 1972; Benz *et al.*, 1973). To optimize further the ability of the torus to buffer the carrier concentration in the membrane, we have in these circumstances also used small membrane areas (0.15–0.2 mm^2) (*see* Stark and Benz, 1971).

b. Zero-current membrane potential measurements in ionic mixtures. We have determined, for all of the carrier molecules reported here, that the membrane potential behavior in ionic mixtures is independent of whether the carrier is added to the lipid phase or symmetrically to the aqueous solutions on both sides of the membrane, provided only that the membrane conductance is significantly (at least 50 times) higher in the presence of the carrier than in its absence. We have therefore added the carrier in the way that was most convenient for each lipid. For membranes made from PE/dec it was more convenient to add the carrier to the aqueous phase because the amount of carrier which it was necessary to add to the lipid to produce high enough conductances frequently interferred with the thinning of the bilayer. For the more highly conducting membranes made from glyceryl dioleate, it was more convenient to add the carrier to the lipid phase because these membranes tend to be more breakable, and the steady-state values of potential are more rapidly attained upon reforming bilayers when the carrier is already present in the lipid phase.

c. Current-voltage measurements. When making the current-voltage ($I-V$) measurements at the routine sweep rate of 60 mV per sec, we have found that the "normalized conductance" (i.e., the conductance at a given voltage divided by that in the limit of zero voltage) was about 10% higher if the carrier had been introduced via the lipid phase than if it had been added to the aqueous phase. This discrepancy is anticipated because of the fact that the membrane area increases as a function of the applied voltage (White, 1970; Hladky, 1973), and the conductance level of the membrane reflects these area changes almost instantaneously when the carrier is in the lipid phase, whereas there is a time lag if the carrier is added via the aqueous phase (probably because of a limitation in the rate of crossing the aqueous unstirred layer, *cf.* Stark & Benz, 1971; Hladky, 1973). Therefore, to minimize the effect of membrane area changes, we have added the carrier to the aqueous phase when performing current-voltage measurements.

Numerical Methods

Analysis of the kinetic parameters from current-voltage and zero-current membrane-potential experiments required curve-fitting the experimental points to the theoretically

4 If monactin or valinomycin is introduced via the aqueous phase, long time dependences have been reported in the level of membrane conductance (Stark & Benz, 1971; Benz, Stark, Janko & Läuger, 1973; Hladky, 1973), these time dependences being due to the slow equilibration of the carrier molecule between the aqueous phase and both the membrane and bulk lipid solution surrounding the membrane. By contrast, when these carriers are added via the lipid phase, the level of membrane conductance is reported to be independent of time over at least a one-hr period from the time at which the membrane goes black (Stark & Benz, 1971) implying that the carrier concentration in the membrane remains constant in this case and indicating that as carrier molecules leave the membrane to enter the aqueous solution, they are replaced by molecules from the surrounding membrane torus. For tetranactin and trinactin, we observed both of these phenomena.

predicted ones. In order to obtain the parameters which would give an optimum fit of the measured quantities to the predicted ones, we have used a least squares fit program on a digital computer. This program, starting with hand-calculated initial guesses of the parameters, varies them until the sum of the squares of the differences between all the given and predicted points is minimized. Because of the lack of uniqueness in the ability of any particular combination of parameters to fit the analytical functions, a particular rationale and set of assumptions was followed in carrying out the curve fitting. The procedure which we have used, its justification, and the sensitivity of the results to the set of assumptions we have made are discussed in detail in the Appendix.

Results

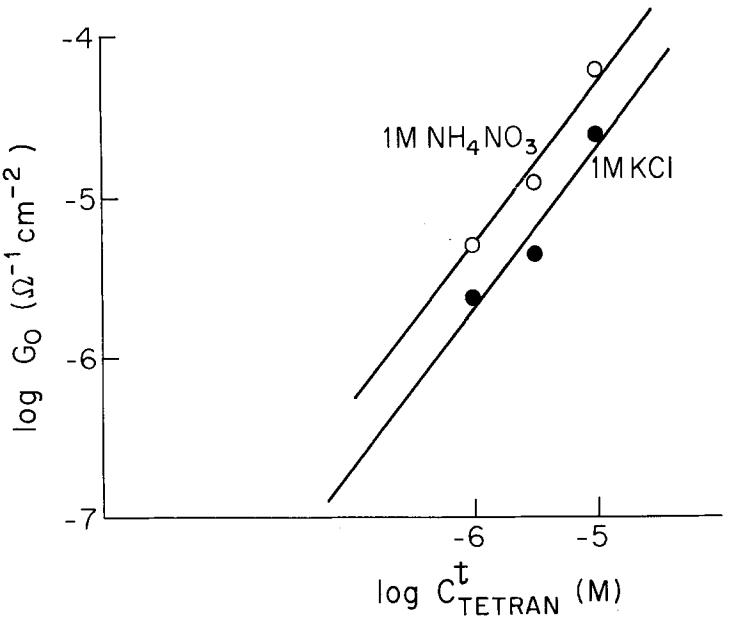
To interpret and analyze the experimental data presented in this section, we make use of a number of theoretical expectations for the model of Fig. 1, derived by Sergio Ciani (1976) in the article which follows, where the assumptions underlying them are presented.

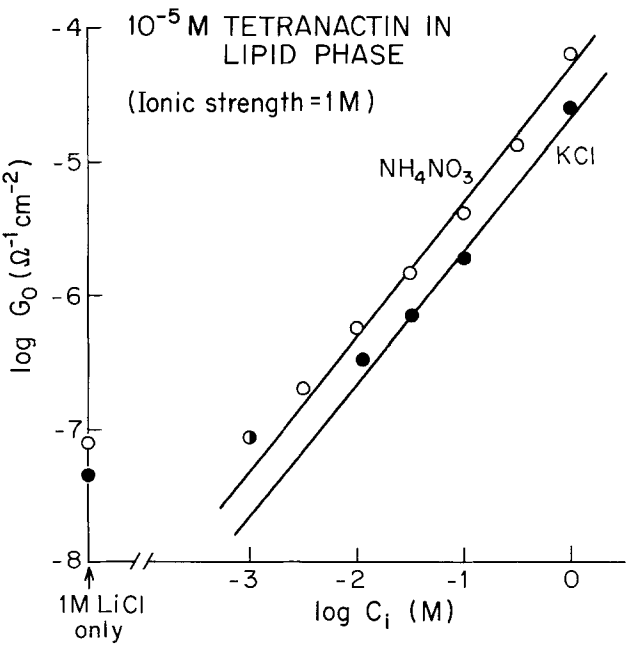
Characterization of the Steady-State Electrical Properties of Membranes in the Presence of Tetranactin

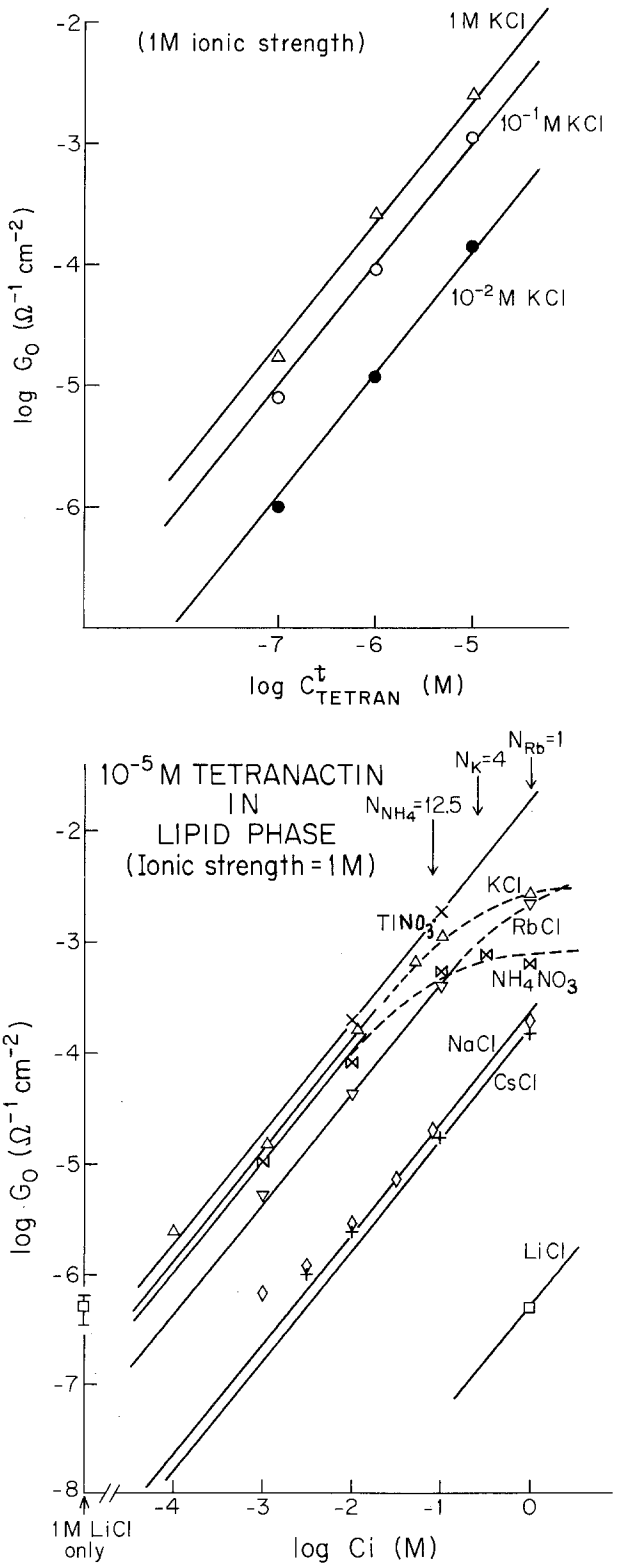
The Zero-Current (Zero-Potential) Conductance Behavior

The membrane conductances observed in the limit of zero current (and zero transmembrane potential) at different (symmetrical) concentrations of salt and carrier are plotted as points for PE/dec membranes in Fig. 3*a* and *b* and for GDO/dec membranes in Fig. 4*a* and *b*. For comparison, the solid curves in these figures have been drawn according

Fig. 3. (*a*) The dependence of the conductance of PE/dec membranes on the tetranactin concentration. The ordinate is the logarithm of the steady-state conductance of tetranactin-containing, PE/dec membranes in 1 M KCl or 1 M NH_4NO_3. The abscissa is the concentration of tetranactin in the bulk lipid solution from which the membranes were formed. The solid lines have been drawn to the unit slope expected from Eq. (1) for the proportionality between the membrane conductance and the concentration of carrier in the bulk lipid phase. (*b*) The dependence of the conductance of PE/dec membranes on the aqueous permeant ion concentration. The ordinate is the logarithm of the steady-state conductance of tetranactin-containing, PE/dec membranes in varied concentrations of either KCl or NH_4NO_3. The abscissa is the concentration of permeant ion in the aqueous phase. The ionic strength was maintained constant at 1 M with the relatively impermeant electrolyte LiCl. The solid lines have been drawn to the unit slope expected for the "equilibrium domain" limit of Eq. (1) for the proportionality between the membrane conductance and the concentration of permeant ion


-4
-5
-6
-7
log G_O ($\Omega^{-1}cm^{-2}$)
1M NH_4NO_3
1M KCl
-6
-5
log C^t_{TETRAN} (M)


10^{-5} M TETRANACTIN IN LIPID PHASE
(Ionic strength = 1 M)
-4
-5
-6
-7
-8
log G_O ($\Omega^{-1}cm^{-2}$)
NH_4NO_3
KCl
-3
-2
-1
0
log C_i (M)
1M LiCl only


(1M ionic strength)
1M KCl
10^{-1}M KCl
10^{-2}M KCl
log G_o (Ω^{-1} cm^{-2})
log C^t_{TETRAN} (M)
10^{-5}M TETRANACTIN
IN
LIPID PHASE
(Ionic strength = 1M)
N_K=4
N_{Rb}=1
N_{NH_4}=12.5
KCl
TlNO$_3$
RbCl
NH_4NO_3
NaCl
CsCl
LiCl
1M LiCl only
log Ci (M)

to the theoretical expectation for the zero-current conductance, G_0, derived by Ciani (1976) for the model illustrated in Fig. 1,

$$G_0 = \frac{z^2 F^2}{RT} \Omega_i \frac{c_s^t c_i}{1 + N_i c_i}. \quad (1)$$

Here c_i is the aqueous concentration of ion I^+, c_s^t is the concentration of tetranactin in the bulk lipid phase, and Ω_i is a concentration-independent parameter given by Eq. (44) of Ciani (1976). R, T, F, and z have their usual meanings. In addition, Eq. (1) contains the "kinetic" parameter N_i defined in Eq. (45) of Ciani (1976). This kinetic term defines the ion concentration-dependent deviations from the "equilibrium domain", which are, of course, insignificant in the linear region of $\log G_0$ *vs.* $\log c_i$ where the term $N_i c_i$ is negligible (*cf.* Figs. 3*b* and 4*b*). We present this kinetic term for completeness in describing these zero-current conductance properties, but for the conductance-voltage behaviors and for selectivity considerations, we confine ourselves to the linear region.

Eq. (1) predicts that the conductance should be proportional to the carrier concentration; and this behavior is seen for both PE/dec and GDO/dec membranes, as inferred from the slopes of unity in Figs. 3*a* and 4*a*, respectively. By contrast, when the permeant ion concentration is varied, such a linearity is expected only if the kinetic limitations due to the N_i term in Eq. (1) are small; and it can be seen that such a linearity is observed for all ions only for PE/dec membranes (*see* Fig. 3*b*), whereas for GDO/dec membranes (*see* Fig. 4*b*), the conductance approaches a

Fig. 4. (*a*) The dependence of the conductance of GDO/dec membranes on the tetranactin concentration. The ordinate is the logarithm of the steady-state conductance of tetranactin-containing GDO/dec membranes in 10^{-2} M, 10^{-1} M, and 1 M KCl. The abscissa is the concentration of tetranactin in the bulk lipid solution from which the membranes were formed. The ionic strength was maintained constant at 1 M with LiCl. The solid lines have been drawn to the unit slope expected from Eq. (1) for the proportionality between the membrane conductance and concentration of carrier in the bulk lipid phase. (*b*) The dependence of the conductance of GDO/dec membranes on the aqueous permeant-ion concentration. The ordinate is the logarithm of the steady-state conductance of tetranactin-containing, GDO/dec membranes in varied concentrations of each of the alkali halide salts, $TlNO_3$, or NH_4NO_3. The abscissa is the concentration of permeant ion in the aqueous phase. The experimental data are plotted as points for the indicated cations Li (□), Na (◇), K (△), Rb (▽), Cs (+), Tl (×), NH_4 (⋈). (The same symbols will be used throughout.) The ionic strength was maintained constant at 1 M with LiCl except in the case of $TlNO_3$, for which the ionic strength was held at 0.2 M with $LiNO_3$ for solubility reasons. The solid lines have been drawn according to the unit slopes expected from Eq. (1) for the low ion-concentration limit in which $N_i c_i \ll 1$. The dashed lines have been drawn according to Eq. (1) for the best-fit values of N_i which are $N_{Rb} = 1$, $N_K = 4$, $N_{NH_4} = 12.5$

limiting value at high ion concentrations for the most permeant ions (NH_4, K, and Rb), as is expected if the kinetic limitations intrinsic to the N_i term in Eq. (1) are significant for this lipid.[5]

In its effects on zero-current conductance, tetranactin, therefore, shows the same types of regular behavior as has been described previously for the less methylated nonactin homologues (Szabo *et al.*, 1969; Ciani *et al.*, 1973*a*; Laprade *et al.*, 1975; Feldberg & Kissel, 1975; Benz & Stark, 1975) and as are theoretically expected for the model illustrated in Fig. 1. It can also be shown to be more effective, on a mole for mole basis, than trinactin, bearing out the regular increasing effectiveness with increasing methylation previously reported (Szabo *et al.*, 1969), but this demonstration requires careful comparison in the same lipids as well as control of torus buffering (as, for example, by adding the carrier to the lipid phase) and recognition of kinetic effects.

The conductance-voltage behavior

The normalized conductances, G/G_0, calculated from current-voltage measurements are plotted, for a variety of applied voltages, as points in Fig. 5 for PE/dec (left) and GDO/dec (right) membranes, in the presence of tetranactin and ion concentrations sufficiently low that the normalized conductance is independent of ion concentration [*see* Ciani, 1976; Eq. (33)]. Comparing the left- and right-hand figures, it can be seen that the tetranactin-mediated conductance voltage behaviors are strikingly different for the two different lipid compositions. In PE/dec membranes the normalized conductance always increases as the applied voltage is increased, and the data points are closely similar for the different ions; whereas in GDO/dec the normalized conductance may either increase (*cf.* Li, Cs, Na), decrease (*cf.* NH_4, K, Tl), or stay nearly constant (*cf.* Rb) as the voltage is increased.

These different behaviors can be readily rationalized in terms of the theoretical expectations derived by Ciani (1976) for the present model and

5 Another possible source of such "saturation" in the behavior of $\log G_0$ *vs.* $\log c_i$ is that expected for a space charge limitation in the membrane. Such a saturation should be independent of the concentration of carrier, whereas the conductance levels at which a saturation occurs due to $N_i c_i$ should be proportional to the carrier concentration (Ciani *et al.*, 1973*a*). These authors have used this criterion for ruling out a space charge limitation for trinactin, and because the proportionality between $\log G_0(I)$ and $\log c_s'$ in Fig. 4*a* is the same at each concentration of KCl, we can rule out this effect as the source of the saturation in Fig. 4*b* for tetranactin as well.

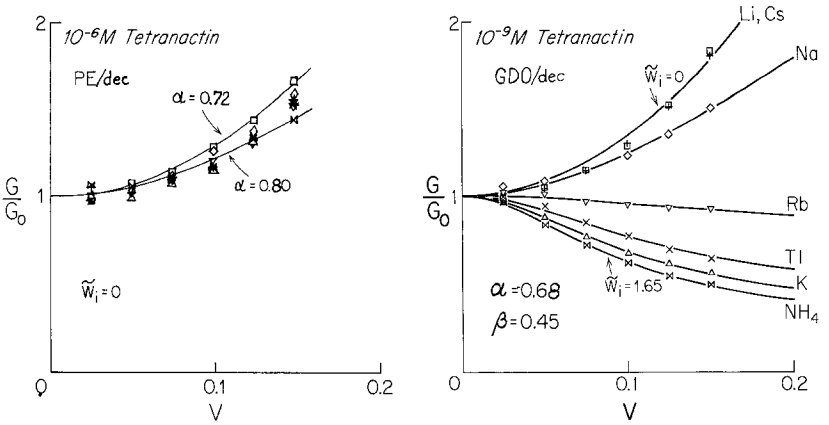


Fig. 5. Conductance-voltage behavior of tetranactin in PE/dec bilayers (left) and GDO/dec bilayers (right). The ordinate is the normalized conductance calculated by dividing the conductance measured at each voltage by the conductance measured in the limit of zero voltage. The abscissa is the applied transmembrane voltage. The curves were drawn according to Eq. (2) for GDO/dec and according to the "equilibrium domain" limit given by Eq. (3) for PE/dec. For the PE/dec data, note that the entire range of data can be fitted by a parameter α whose extreme values range from 0.72 (upper curve) to 0.80 (lower curve). Actually, these data are best described by a single α parameter ($\alpha = 0.72$) and very small values of $\tilde{w}_i$ (always less than 0.025) which are entirely negligible from the point of view of selectivity corrections. For GDO/dec bilayers the data are best-fitted assuming a single value for $\alpha = 0.68$, a single value for $\beta = 0.45$ and values of $\tilde{w}_i$ which range from the "equilibrium domain" for Li and Cs ($\tilde{w}_i = 0$) through the various values of $\tilde{w}_i$ given in the second column of Table 1 and corresponding to the "kinetic domain" for the remaining ions

given for the general case by

$$\frac{G}{G_0} = \frac{(\alpha + 4\tilde{w}_i)\sinh\frac{\phi}{2}}{\sinh\frac{\alpha\phi}{2} + 2\tilde{w}_i\phi\cosh\beta\phi}, \qquad 0 \leqq \frac{\alpha}{2}\beta \leqq 0.5^{\star} \tag{2}$$

where β designates the position of the "reaction plane" and ϕ is the transmembrane potential $\left(\text{in units of } \frac{zF}{RT}\right)$, and for the limiting case of the "equilibrium domain" (for which $\tilde{w}_i \ll \alpha$) by the simpler expression

$$\left(\frac{G}{G_0}\right)_{\text{Eq.}} = \frac{\alpha\sinh\frac{\phi}{2}}{\sinh\frac{\alpha\phi}{2}}. \tag{3}$$

⋆ As will be shown below (*see* particularly the Appendix), α_i and β_i appear to be independent of the ion for the present carriers so we have dropped the subscripts to these parameters for the present analysis.

The data for PE/dec membranes in Fig. 5 (left) are consistent with the expected "equilibrium domain" behavior of Eq. (3) and can be well fitted by choosing values of α within the ranges of those used to draw the solid curves in this figure. Clearly, a single value of $\alpha = 0.76 \pm 0.04$ can provide a reasonable fit for all of the data supporting the notion that the width of the "diffusion barrier" is approximately the same for all of the ions complexed with tetranactin.[6]

For GDO/dec membranes, the conductance-voltage behaviors are much more complex, as illustrated in Fig. 5 (right), in that they differ from ion to ion, ranging from the limiting "equilibrium domain" curves for Li and Cs to the curves for Rb, Tl, K, and NH_4 which show increasing degrees of "kinetic" limitations.

In order to fit this variety of conductance-voltage behaviors, the general expression given by Eq. (2) is required. Since a variety of combinations of α, β, and $\tilde{w}_i$ will produce a satisfactory fit of this experimental data, however, certain assumptions have been necessary for determining the values of these parameters. The Appendix is devoted to a detailed discussion of the procedure and rationale involved in fitting the conductance-voltage data for GDO/dec bilayers as well as the justification for our belief that the results obtained are physically meaningful. The set of assumptions which produces a unique fit of the data are as follows:

1. The value of α is independent of the complexed ion.

2. If the curve for G/G_0 *vs.* voltage which is the most increasing function of voltage is seen for complexes with two or more of the least strongly complexing cations, these ions may be taken to be in the "equilibrium domain" for their complexes with the carriers in this lipid.

3. The value of β is independent of the complexed ion.

4. The "equilibrium" component of the carrier-induced NH_4/Cs selectivity in GDO/dec bilayers is approximately the same as it is in PE/dec bilayers.

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6 A more reasonable interpretation than attributing the small differences seen from ion to ion to scatter is that α has the same value, 0.72, for all species and that very small kinetic effects exist even in this lipid for the most permeant ion-carrier complexes, these effects leading to the observed deviations from the ideal "equilibrium domain" curves. Assuming that these deviations are due to kinetic effects, one can calculate the largest possible values of $\tilde{w}_i$ which could account for these data, with $\alpha = 0.72$. From such calculations one finds that, for all of the ion-carrier complexes, $\tilde{w}_i < 0.025$ (*also see* Fig. 7 for nonactin and trinactin). These small kinetic effects would have a negligible effect on the selectivities calculated for PE/dec membranes, supporting the conclusion of Ciani *et al.* (1973*a*), and extending it to tetranactin, that measurements in PE/dec membranes can be considered to represent the "equilibrium domain" for all the nactins yet studied.

Table 1. Values of $\tilde{w}_i$ for tetranactin in GDO/dec bilayers[a]

Ion	$\tilde{w}_i$
Li	0
Na	0.012
K	0.54
Rb	0.08
Cs	0
Tl	0.24
NH_4	1.65

[a] $\alpha = 0.68$; $\beta = 0.45$.

Using these assumptions, and the analytical procedures described in detail in the Appendix, we have been able to deduce values for α and β and thence to determine the values of $\tilde{w}_i$, listed in column 2 of Table 1, which give the best fit of the GDO/dec conductance-voltage data by Eq. (2) as illustrated by the solid curves in Fig. 5 (right).[7]

From the close agreement between the observed data points and the theoretical curves, it is clear that the model satisfactorily describes the experimental data using a single value for α and for β. More importantly, the agreement of the α's and of the β's and the systematic trends in $\tilde{w}_i$'s for nonactin, trinactin, and tetranactin as well as the internal consistency with the zero-current potential data, all of which will be shown below, suggest that the parameters in the model have some correspondence to physical variables rather than simply being "fudge-factors" which increase the precision of the curve-fitting.

The Zero-Current Potential Behavior of Tetranactin

The data points in Fig. 6 illustrate the zero-current potentials observed upon adding NH_4NO_3 to one side of PE/dec (left) or GDO/dec (right) bilayers in the presence of symmetrical concentrations of tetranactin and of the chloride salts of the indicated cations. The theoretical expression

7 Specifically, the data for Li and Cs have been taken as representing the "equilibrium domain" (according to assumption 2) and were used to assign the value $\alpha = 0.68$. The NH_4 data were used (along with assumption 4 and the NH_4/Cs permeability ratio; *see* below) to assign the value $\beta = 0.45$. The curves in Fig. 5 (right) have been drawn according to Eq. (2) using these values for α and β and the values of $\tilde{w}_i$ listed for tetranactin in column 2 of Table 1.

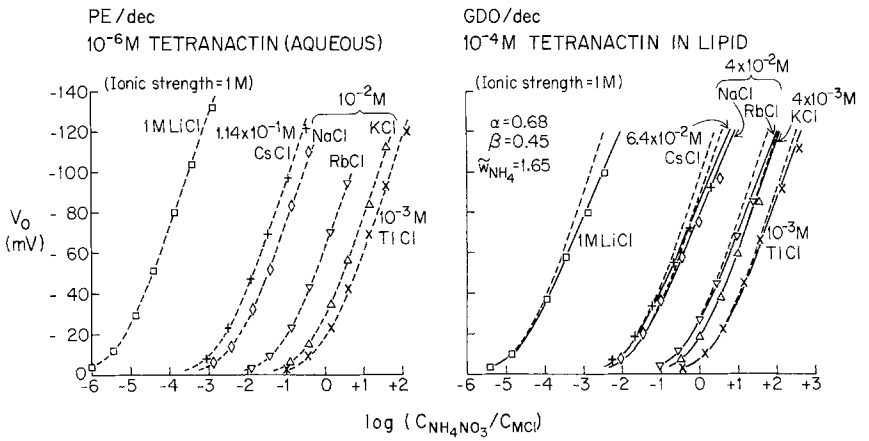

Fig. 6. Membrane potentials mediated by tetranactin in PE/dec bilayers (left) and GDO/dec bilayers (right) in ionic mixtures. The ordinate is the steady-state potential between the solutions on the two sides of the membrane. The abscissa is the logarithm of the ratio of the concentration of NH_4NO_3 to the concentration of the indicated cation. The experiments were carried out by adding small volumes of NH_4NO_3 to one side of a membrane bathed initially on both sides in the indicated salt solutions. The particular data presented were obtained in the presence of 10^{-6} M tetranactin in the aqueous phase on both sides of the membranes in the case of PE/dec and 10^{-4} M tetranactin in the lipid for GDO/dec; but entirely comparable data were obtained at different tetranactin concentrations and in controls where carrier was added via the lipid route for PE/dec and via the aqueous route for GDO/dec. The ionic strength was held constant at 1 M (usually using LiCl as a relatively impermeant electrolyte) except in the case of Tl^+ where, for solubility reasons, the ionic strength could only be kept at 0.2 M

expected of the present model is shown by Ciani (1976) to be the same as that for the previously used Eyring single barrier model with voltage independent interfacial reactions (Ciani *et al.*, 1973*a*),

$$V_0 = \frac{RT}{zF} \ln \frac{c_i'' + \left(\frac{P_j}{P_i}\right)_{app} c_j''}{c_i' + \left(\frac{P_j}{P_i}\right)_{app} c_j'}, \tag{4}$$

although the dependence of the "apparent permeability ratio" $(P_j/P_i)_{app}$ on voltage is different for the present model as will be discussed below. Here (′) and (″) refer to each side of the membrane and the subscripts i and j refer to ions I^+ and J^+.

In the case of the "equilibrium domain" (i.e., $\tilde{w}_i$, $\tilde{w}_j \approx 0$), the "apparent permeability ratios" are independent of the transmembrane potential and are equal to the "equilibrium permeability ratios" of the membrane,

given by

$$\left(\frac{P_j}{P_i}\right)_{\text{Eq.}} = \frac{\bar{K}_j \bar{A}^*_{js}}{\bar{K}_i A^*_{is}} \tag{5}$$

where $\bar{K}_i$ and $\bar{K}_j$ are the equilibrium constants for the heterogeneous interfacial association reactions of the carrier with ions I^+ and J^+, respectively (*cf.* Ciani *et al.*, 1973*a*, Eq. (17, 17*a*)), and $\tilde{A}^*_{is}$ and $\tilde{A}^*_{js}$ are the rate constants for translocation of the ion-carrier complex across the "diffusion barrier" (as defined in Eq. (24) of Ciani, 1976). That the zero-current potential behavior of PE/dec bilayers in the presence of tetranactin is consistent with the expectations for the "equilibrium domain" is illustrated by the excellent agreement between the data points for PE/dec in Fig. 6 and the solid curves in this figure, which have been drawn according to Eq. (4) using the (constant) values for the "equilibrium permeability ratios" listed in column 2 of Table 2.

In the case of the "kinetic domain," the "apparent permeability ratios" are not constants but are functions of the membrane potential which are given for the present model [*see* Ciani, 1976, Eq. (50)] by

$$\left(\frac{P_j}{P_i}\right)_{\text{app.}} = \left(\frac{P_j}{P_i}\right)_{\text{Eq.}} \frac{1+2\tilde{w}_i[f(\phi)]}{1+2\tilde{w}_j[f(\phi)]} \tag{6}$$

$$f(\phi) = \phi \frac{\cosh \beta\phi}{\sinh \frac{\alpha\phi}{2}}. \tag{7}$$

However, in the limit of small transmembrane potentials, the permeability ratios are again expected to be constants, these being given by

$$\left(\frac{P_j}{P_i}\right)_{V\to 0} = \left(\frac{P_j}{P_i}\right)_{\text{Eq.}} \frac{1+\frac{4\tilde{w}_i}{\alpha}}{1+\frac{4\tilde{w}_j}{\alpha}}. \tag{8}$$

Table 2. Permeability ratios for tetranactin in PE/dec and GDO/dec bilayers

Ion	PE/dec $\left(\frac{P_{NH_4}}{P_i}\right)$	GDO/dec $\left(\frac{P_{NH_4}}{P_i}\right)_{V\to 0}$	GDO/dec $\left(\frac{P_{NH_4}}{P_i}\right)_{\text{Eq.}}$
Li	1.79×10^5	3.45×10^4	3.70×10^5
Na	2.13×10^2	32.5	3.23×10^2
K	2.08	1.05	2.68
Rb	11.1	1.93	14.3
Cs	4.44×10^2	47.6	5.13×10^2
Tl	1.14	0.34	1.52
NH_4	[1]	[1]	[1]

We have termed the values of the "apparent permeability ratios" for this limiting case the "low-voltage permeability ratios." The dashed curves for GDO/dec bilayers in Fig. 6 (right) have been drawn according to Eq. (4) with these constant values defined by Eq. (8) to give the best fit to the data points observed at small potentials. These "low-voltage" permeability ratios are listed in column 3 of Table 2.

Two facts should be noted at this point. First, the permeability ratios induced by tetranactin are different in PE/dec and GDO/dec bilayers (*cf.* columns 2 and 3 of Table 2), consistent with PE/dec bilayers being in the "equilibrium domain" and GDO/dec bilayers reflecting the "kinetic domain" properties of tetranactin (as was also seen for their conductance-voltage behaviors). Second, the data points at higher transmembrane potentials for GDO/dec bilayers in Fig. 6 do not fall on the dashed curves expected if $(P_j/P_i)_{\text{app.}}$ were independent of voltage. This voltage dependence of the permeability ratios, which is in contrast to the behavior in PE/dec bilayers, is presumptive evidence for the "kinetic domain" behavior described by Eq. (6). The solid curves for the GDO/dec data of Fig. 6 have been drawn according to Eqs. (6) and (7) using the values for α, β, $\tilde{w}_{NH_4}$ and $\tilde{w}_j$ obtained from the conductance-voltage data in GDO/dec bilayers (*cf.* column 2 of Table 1). Note that these theoretical curves fit the data well, again lending support to the validity of the present model for describing the transport process.

The fit of Eqs. (4) and (6) to the data of Fig. 6 yields the values of the "equilibrium permeability ratios," and these have been listed in column 4 of Table 2. If these "equilibrium permeability ratios" are independent of the lipid composition of the membrane, then they should agree with the permeability ratios measured in PE/dec membranes; and they do, as can be seen by comparing the values in columns 2 and 4 of Table 2. It is this particular internal consistency which favors the present, extended model since, as shown in the Appendix, this agreement of "equilibrium permeability ratios" for PE/dec and GDO/dec bilayers is not obtained if the ion-carrier complexation reaction is assumed to be independent of the transmembrane potential.

Comparison of the Selectivities of Nonactin, Trinactin and Tetranactin and their "Kinetic" and "Equilibrium" Components

Having characterized tetranactin's permeation-mediating properties in bilayers we are now in a position to compare these properties with

those of the less methylated nonactin homologues, nonactin and trinactin, and thereby draw some conclusions about the effects of methylation on the ion-selectivity and permeability-inducing properties of nonactin-type molecules. In this section, the same type of analysis has been carried out for the nonactin and trinactin data as was done for tetranactin above; and so only the results will be presented.[8]

The Permeability Properties Induced by the Macrotetralide Actins in the "Equilibrium Domain" (as Assessed in PE/dec Membranes)

Conductance-voltage behaviors. The conductance-voltage behaviors observed for PE/dec bilayers in the presence of nonactin, trinactin, and tetranactin are illustrated by the data points in Fig. 7, where it is seen that for a particular carrier, the data for all of the cations are almost identical. Although we could have fitted the data for each carrier by Eq. (3) choosing values for the width of the "diffusion barrier" of $\alpha \approx 0.70$ for nonactin and $\alpha \approx 0.76$ for trinactin and tetranactin, all three sets of data can also be adequately fitted by the solid lines in these figures drawn using a single value of $\alpha = 0.72$ and very small kinetic effects for the most permeant trinactin and tetranactin ion-carrier complexes, corresponding to values of $\tilde{w}_i < 0.025$ for all of the ions. (Recall again that these small values of $\tilde{w}_i$ will have a negligible effect on the selectivity calculations.)[9]

Zero-current potential behaviors. The zero-current potential behaviors of PE/dec membranes in the presence of nonactin or trinactin for ionic mixtures are illustrated in Fig. 8. The dashed curves in these figures have been drawn according to Eq. (4) using the (constant) permeability ratios for the cations relative to NH_4^+ listed in column 2 of Tables 4 and 5, and

8 The conductance-voltage data analyzed here for trinactin in GDO/dec bilayers for all the cations except for Li^+ and NH_4^+ have been taken directly from Laprade *et al.*, 1975. The remaining conductance-voltage and zero-current potential data for nonactin and trinactin presented in this section are newly obtained and are in good agreement (within a factor of two) with data obtained previously by other investigators for the same lipids (Laprade *et al.*, 1975; Hall *et al.*, 1973; Hladky, 1974).

9 Although a literal acceptance of the best-fit values of α tempts one to speculate that there might be an increase in the width of the "diffusion barrier" in going from nonactin to trinactin and tetranactin, it is more likely that this difference is artifactual, being due to an increase in membrane area with applied voltage and a faster equilibration of nonactin than of trinactin or tetranactin between the aqueous phase and the membrane. Thus, in the case of nonactin one might be more sensitive to the increase in membrane area at high voltage than for trinactin or tetranactin, this effect leading to a larger total current for a given voltage in the case of nonactin and, thus, a slightly smaller estimate for α.

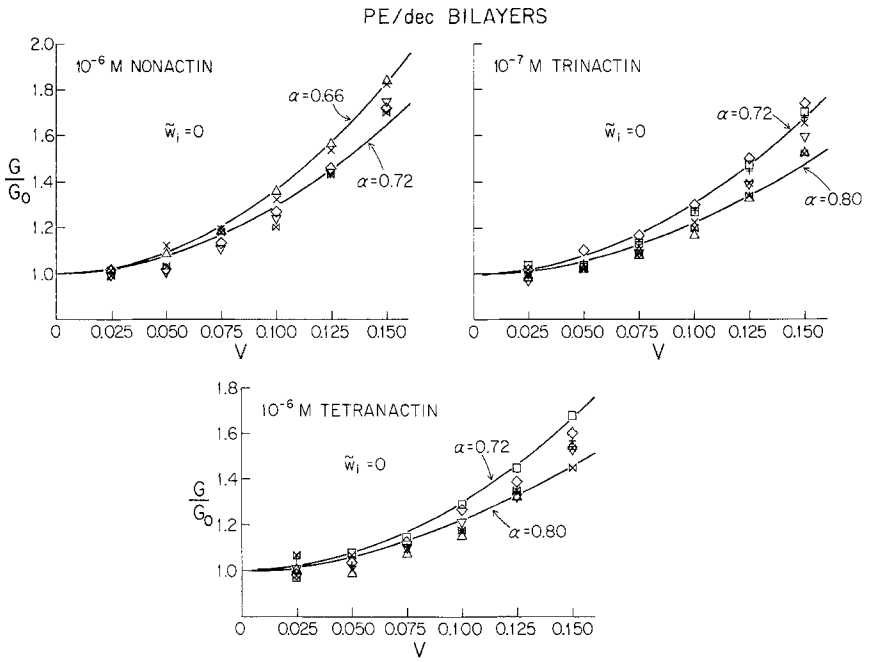

Fig. 7. The conductance-voltage behavior of nonactin, trinactin and tetranactin in PE/dec bilayers. These data were obtained and are plotted in the same manner as those of Fig. 5 (left). Although the data points can be described in all cases by curves corresponding to the "equilibrium domain" of Eq. (3) within the values of the parameter α ranging from the limits 0.66–0.72 for nonactin to 0.72–0.80 for trinactin and tetranactin (as was illustrated for tetranactin at the left of Fig. 5), we believe the truest representation corresponds to a value of $\alpha=0.72$ with the departures in the case of trinactin and tetranactin being due to very small kinetic effects ($\tilde{w}_i$ always less than 0.025). (We have set $\beta=\frac{\alpha}{2}$ in order to determine the largest values of $\tilde{w}_i$ which would be necessary to fit this data assuming $\alpha=0.72$; for $\frac{\alpha}{2}<\beta\leqq 0.5$, the values extracted for $\tilde{w}_i$ would be even smaller.)

it can be seen that the experimental data points are in good agreement with these theoretical curves, consistent with the expectations for the "equilibrium domain". Comparing these "equilibrium permeability ratios" for nonactin and trinactin with those given for tetranactin in column 2 of Table 2 leads to the conclusion that the equilibrium selectivity among cations is different for each of the carriers (mobility differences being almost certainly negligible). That these changes in selectivity are a systematic function of carrier methylation is more readily seen in the left-most plot of Fig. 12 in which the "equilibrium" permeability ratios relative to Cs have been plotted as a function of methylation for nonactin, trinactin and tetranactin. The permeabilities of Na, K, Rb, NH_4, and Tl

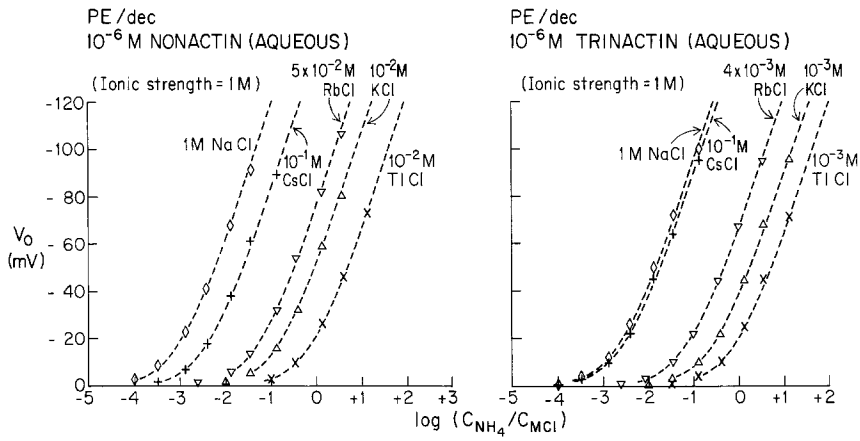

Fig. 8. Membrane potentials mediated by nonactin (left) and trinactin (right) in PE/dec bilayers in ionic mixtures. These data were obtained and plotted in the same manner as those of Fig. 6 (left). The dashed lines have been drawn according to the "equilibrium domain" expectations of Eq. (4) for the (constant) values of permeability ratios listed in column 2 of Tables 3 (for nonactin) and 4 (for trinactin)

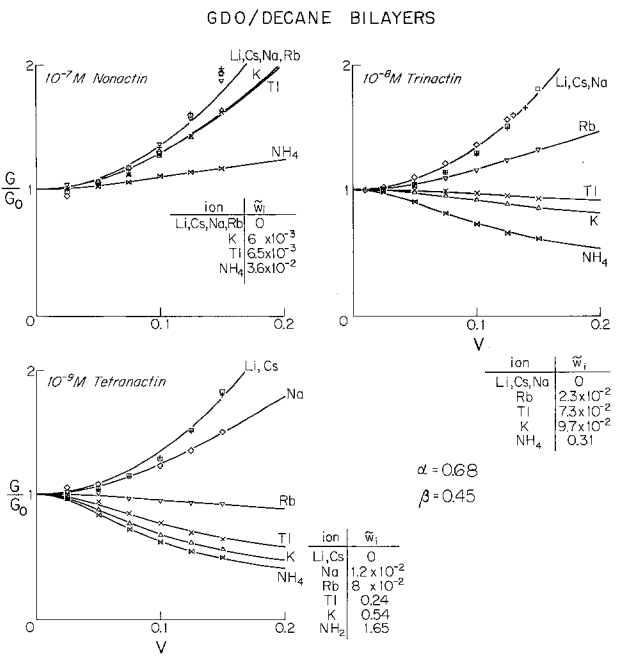

Fig. 9. The conductance-voltage behavior of nonactin, trinactin, and tetranactin in GDO/dec bilayers. The data here are obtained and plotted in the same manner as was done in Fig. 5 (right) for the indicated carrier concentrations. Note that all curves have been fitted with the same values for α (0.68) and for β (0.45) and with the values of $\tilde{w}_i$ summarized in the inserts and in Table 4. The symbols are the same as in Fig. 4*b*

all increase relative to that for Cs; and the effect of increasing methylation on the "equilibrium permeabilities" mediated by these carriers is to increase the selectivity of the carrier for smaller cations relative to that for larger cations. The most striking change is in the permeability ratio for Na and Cs which inverts between trinactin and tetranactin. The significance of this will be discussed later.

The Permeability Properties Induced by the Macrotetralide Actins in the "Kinetic domain" (as Assessed in GDO/dec Bilayers)

Conductance-voltage behaviors. The conductance-voltage behaviors observed for GDO/dec bilayers in the presence of nonactin, trinactin, and tetranactin are plotted as data points in Fig. 9. The solid curves in Fig. 9 have been drawn according to Eq. (2) for the best-fit values of $\tilde{w}_i$ listed in Tables 2 and 3, using the same values, $\alpha = 0.68$ and $\beta = 0.45$, for this lipid as were found above for tetranactin. The values of α and β appear to be reliably the same for nonactin, trinactin and tetranactin as discussed in the Appendix.

Systematic trends can be seen, as a function of carrier methylation, in the degree to which the conductance-voltage behaviors deviate from the behavior expected for the limiting case of the "equilibrium domain". Thus, with increasing methylation these curves show increasing kinetic limitations as is most clearly illustrated in Fig. 10, where we have plotted the "kinetic factor", $\tilde{w}_i$, as a function of the methylation of the carrier molecule. Clearly, increasing methylation of these carriers causes systematic increases in $\tilde{w}_i$ and thus causes increasingly striking departures from the "equilibrium domain" in this lipid.

Table 3. Values of $\tilde{w}_i$ for nonactin and trinactin in GDO/dec bilayers[a]

Ion	Nonactin $\tilde{w}_i$	Trinactin $\tilde{w}_i$
Li	0	0
Na	0	0
K	0.0061	0.097
Rb	0	0.023
Cs	0	0
Tl	0.0065	0.073
NH_4	0.036	0.31

[a] $\alpha = 0.68$; $\beta = 0.45$.

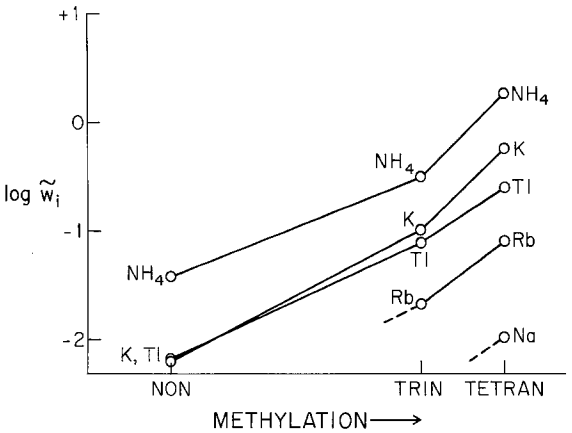

Fig. 10. Effect of carrier methylation on the magnitude of the parameter $\tilde{w}_i$. The ordinate is the logarithm of $\tilde{w}_i$ (i.e., the ratio of the rate constant, $\tilde{A}_{is}^{*}$, for translocation of the ion-carrier complex across the membrane to the rate constant, $\tilde{K}_i^B$, for dissociation of the complex near the membrane-solution interface), calculated from the conductance-voltage data for GDO/dec bilayers. The abscissa ranks the indicated nactins in their order of increasing methylation. The ion-carrier complexes not represented in this figure had values of $\tilde{w}_i \approx 0$

Zero-current potential behaviors. The zero-current potential behaviors observed, in ionic mixtures, for GDO/dec bilayers in the presence of nonactin and trinactin are illustrated by the data points in Fig. 11, left and right, respectively. The dashed curves have been drawn according to Eq. (4) using the "low-voltage permeability ratios" fitted to the data at low potentials and listed for nonactin and trinactin in column 3 of Tables 4 and 5, respectively. It can be seen that the deviations from the ideal "Nernst" behavior (illustrated by the dashed curve) are greater the more methylated is the carrier. In addition, there are changes in the "low-voltage permeability ratios" upon methylating the carrier, which are most readily seen in the middle plot in Fig. 12 where these ratios have been plotted (relative to Cs) as a function of carrier methylation. These changes are quite different from the systematic trends observed for the "equilibrium" selectivity plotted in the left of Fig. 12. Indeed, the effects of added methyl groups on the "low-voltage permeability ratios" induced in GDO/dec bilayers can be nonmonotonic (e.g., the permeabilities for K and Tl relative to Cs first increase in going from nonactin to trinactin but then decrease in going from trinactin to tetranactin) and bear no obvious relationship to ion size. Of course, this complex behavior results from the fact that in GDO/dec membranes the permeability ratio changes reflect competing effects of methylation on both the "equilibrium" and the "kinetic" components of the selectivity as discussed below.

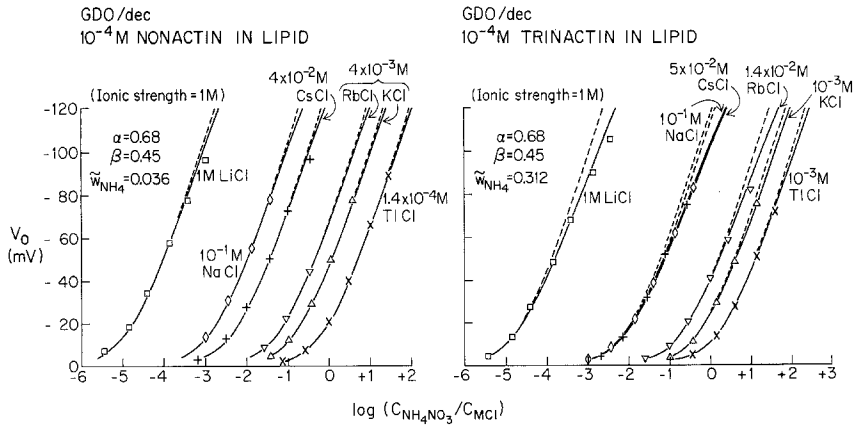

Fig. 11. Membrane potentials mediated by nonactin (left) and trinactin (right) in GDO/dec bilayers in ionic mixtures. These data were obtained and plotted in the same manner as those of Fig. 6 (right). The broken lines have been drawn according to Eq. (4) with the (constant) "low-voltage" permeability ratios listed for nonactin and trinactin in column 3 of Tables 3 and 4, respectively. The solid curves have been drawn according to Eq. (4) with the "apparent" permeability ratios being given by Eq. (5) for the best-fit values of "equilibrium" permeability ratios listed in column 4 of Tables 3 and 4 for nonactin and trinactin, respectively, and the values of $\tilde{w}_i$, α and β deduced from the conductance-voltage data (*cf.* Table 3)

Table 4. Permeability ratios for nonactin in PE/dec and GDO/dec bilayers

Ion	PE/dec $\left(\frac{P_{NH_4}}{P_i}\right)$	GDO/dec $\left(\frac{P_{NH_4}}{P_i}\right)_{V\to 0}$	GDO/dec $\left(\frac{P_{NH_4}}{P_i}\right)_{Eq.}$
Li	–	6.99×10^4	8.33×10^4
Na	1.02×10^3	6.58×10^2	8.00×10^2
K	7.14	5.78	6.67
Rb	19.2	14.9	18.2
Cs	2.38×10^2	1.89×10^2	2.27×10^2
Tl	1.32	1.32	1.54
NH_4	[1]	[1]	[1]

Table 5. Permeability ratios for trinactin in PE/dec and GDO/dec bilayers

Ion	PE/dec $\left(\frac{P_{NH_4}}{P_i}\right)$	GDO/dec $\left(\frac{P_{NH_4}}{P_i}\right)_{V\to 0}$	GDO/dec $\left(\frac{P_{NH_4}}{P_i}\right)_{Eq.}$
Li	–	5×10^4	1.4×10^5
Na	4.35×10^2	1.08×10^2	3.03×10^2
K	3.45	1.67	3.03
Rb	13.2	4.35	11.1
Cs	3.45×10^2	1.0×10^2	2.78×10^2
Tl	1.23	0.55	1.09
NH_4	[1]	[1]	[1]

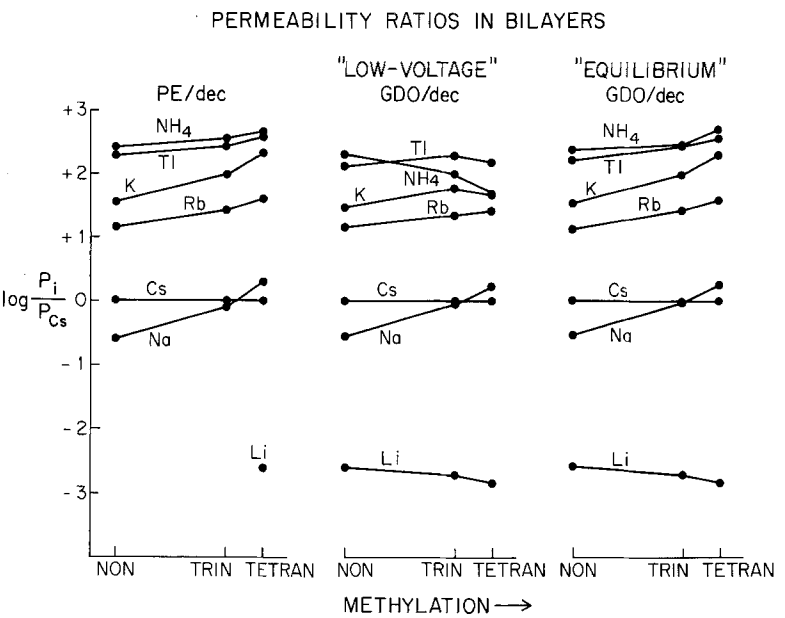

Fig. 12. The effect of methylation on the "apparent", "low-voltage", and "equilibrium" selectivities for the nactins in PE/dec bilayers (left) and GDO/dec bilayers (middle and right). The abscissa in each figure ranks the indicated nactins in their direction of increasing methylation. In the left figure, the ordinate plots the logarithm of the indicated permeability ratio (P_j/P_i) observed in PE/dec bilayers, which under the present assumptions corresponds to the "equilibrium permeability ratio" $(P_j/P_i)_{Eq.}$, while the middle figure plots the "low voltage permeability ratio" $(P_j/P_i)_{V\to 0}$ observed in GDO/dec bilayers and the right figure plots the "equilibrium permeability ratio" determined for GDO/dec bilayers using Eq. (5) and the values of $\tilde{w}_i$ given in Table 4 as well as the values $\alpha=0.68$ and $\beta=0.45$ (as determined from the conductance-voltage data)

In order to evaluate separately these effects of carrier methylation for GDO/dec bilayers, we have carried out the same type of decomposition of the permeability ratios into "equilibrium" and "kinetic" components as was done for tetranactin. The solid curves in Fig. 11 have been drawn to give the best fit values of the "equilibrium permeability ratios" listed in the 4th column of Tables 4 and 5 for nonactin and trinactin, respectively, using the values of $\tilde{w}_i$, α and β extracted from the conductance-voltage data. These "equilibrium permeability ratios" have been plotted (relative to Cs) as a function of carrier methylation for GDO/dec membranes at the right of Fig. 12. Comparing the right-most and left-most plots in Fig. 12 illustrates that, for each of the three carriers, the calculated "equilibrium permeability ratios" for GDO/dec bilayers are virtually identical to the "equilibrium permeability ratios" directly observed in

PE/dec bilayers. Thus, the *difference* seen in GDO/dec *vs.* PE/dec bilayers for the effect of carrier methylation on the "apparent permeability ratios" can be rationalized by proposing that the sole effect of methylation is to increase the magnitude of the "kinetic" component of permeation, this component being observable in GDO/dec but not in PE/dec bilayers.

Discussion

The results of the present paper illustrate that the carrier-mediated, selective ion permeation of a bilayer, deduced from zero-current potential measurements, is composed of a mixture of two components, an "equilibrium" selectivity and a component dependent on the kinetics of the ion-carrier complexation reaction. The way in which these two components contribute to the "apparent permeability ratios" observed in a given lipid is described by the expression

$$\left(\frac{P_j}{P_i}\right)_{\text{app.}} = \left(\frac{P_j}{P_i}\right)_{\text{Eq.}} \frac{1+2\tilde{w}_i[f(\phi)]}{1+2\tilde{w}_j[f(\phi)]} \tag{6}$$

$$f(\phi) = \phi \frac{\cosh \beta\phi}{\sinh \frac{\alpha\phi}{2}}. \tag{7}$$

This expression has two extreme limits. In the purely "equilibrium" limit, the voltage-dependent "kinetic" terms, $2\tilde{w}_i[f(\phi)]$ and $2\tilde{w}_j[f(\phi)]$, are very small compared to 1, so that

$$\left(\frac{P_j}{P_i}\right)_{\text{app.}} = \left(\frac{P_j}{P_i}\right)_{\text{Eq.}} = \frac{\bar{K}_j \tilde{A}^*_{js}}{\bar{K}_i \tilde{A}^*_{is}}. \tag{9}$$

That is, the "apparent" permeability ratios will equal the "equilibrium domain" permeability ratios which in turn equal the product of a selectivity due to the ratio of equilibrium constants for the heterogeneous ion-carrier complexation reaction in the membrane and a (mobility ratio) selectivity due to possible differences in the rate constants for translocation of the ion-carrier complexes across the membrane interior (Ciani *et al.*, 1969, 1973*a*). In the purely "kinetic" limit, the "kinetic" terms are large compared to 1, and

$$\left(\frac{P_j}{P_i}\right)_{\text{app.}} = \left(\frac{P_j}{P_i}\right)_{\text{Eq.}} \times \left(\frac{\tilde{w}_i}{\tilde{w}_j}\right) = \frac{\bar{K}^F_j}{\bar{K}^F_i}. \tag{10}$$

In this extreme case the "apparent permeability ratios" solely reflect the relative (forward) rate constants for complexation of the carrier in the

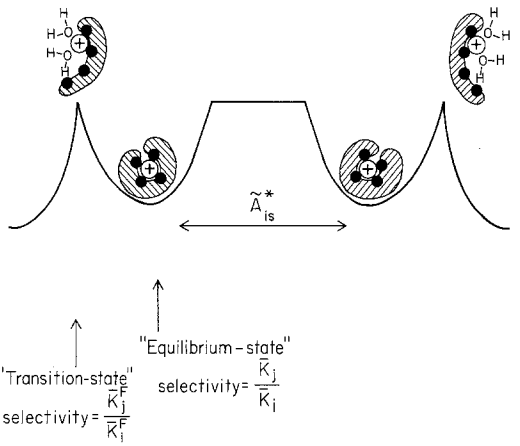

Fig. 13. The "states" of the ion-carrier complex. Hypothetical conformations of the ion-carrier complex have been schematized for two points along the potential energy profile (described in Fig. 1) for carrier-mediated transport through the membrane. The outermost complexes schematize a possible conformation for the "transition state" for the heterogeneous ion-carrier complexation reaction. This state would occur at the peak along the potential energy profile corresponding to the rate-limiting step for complexation, the locus of this peak being termed the "reaction plane." The possibility that some water molecules are coordinated to the ion in this "transition state" has been included. The innermost complexes schematize a possible conformation for the "equilibrium state" for the heterogeneous ion-carrier complexation reaction. This state would occur at the "well" along the potential energy profile corresponding to the minimum energy position for the complex near the membrane-solution interface

membrane with the different ions near the membrane-solution interface. From Eq. (6) it can be seen that this "kinetic" effect will be emphasized by increasing the membrane potential to sufficiently large values. In addition, the results of the present study (as well as the results of other investigators cited in the Introduction) indicate that both structural changes in the carrier and changes in the lipid composition of the bilayer can also influence the relative amounts of "equilibrium" *vs.* "kinetic" contributions to the "apparent permeability ratios."

What sort of physical picture of the system might correspond to these different limiting cases of selectively in ion permeation? Fig. 13 is a schematic illustration of intuitively reasonable conformations of the ion-carrier complex at a point corresponding to the "reaction plane" near the membrane-solution interface and in the "well" slightly further into the interior corresponding to the minimum energy position for the completely sequestered ion-carrier complex. The outer-most figures are schematic representations of partially-formed ion-carrier complexes representing the "transition-state" for complex formation between the ion

and carrier at the plane of the reaction, and the ion selectivity associated with the carrier in this "transition-state" is given, according to Eq. (10), by $\bar{K}_j^F/\bar{K}_i^F$, that is the ratio of the forward rate constants for complex formation. In the extreme case of the purely "kinetic" limit, the apparent selectivity observed for membrane transport can be viewed, therefore, as the selectivity intrinsic to the carrier molecule in the conformation (here schematized as partially closed) which characterizes this "transition state."[10]

The center figures are schematic drawings of the "equilibrium state" of the ion-carrier complex once it is formed inside the membrane (but before it diffuses through the membrane interior). The selectivity of the carrier in this state is given by $\bar{K}_j/\bar{K}_i$, that is the ratio of the equilibrium constants for the heterogeneous reaction between the ion (initially in the aqueous phase) and the carrier (in the membrane). In the present model the "equilibrium permeability ratio" equals the product of this "equilibrium state" selectivity and the (mobility) selectivity given by the ratio $\tilde{A}_{js}/\tilde{A}_{is}$ [cf. Eq. (9)].[11] Thus, for "isosteric" complexes (i.e., for complexes for which $\tilde{A}_{js}^* = \tilde{A}_{is}^*$), the "equilibrium permeability ratios" simply reflect the "equilibrium state" selectivity of the carrier in the membrane, given by $\bar{K}_j/\bar{K}_i$.

The Effects of Lipid Composition and of Carrier Methylation in Modulating the Relative Amounts of "Equilibrium" vs. "Kinetic" Components in the "Apparent Permeability"

That changing the lipid composition affects the "apparent permeability" has been demonstrated by the differences seen between PE/dec and GDO/dec bilayers. Both the conductance-voltage and the zero-current potential behaviors of PE/dec membranes in the presence of the macrotetralide actins are consistent with the expectations for the purely "equi-

10 Of course, in this state the ion may still be partially hydrated as well and thus the hydration energy term associated with the removal of water and contributing to the ion selectivity may also be different than that for the completely dehydrated equilibrium state of ion-carrier complex formation.

11 Because the present model utilizes a Nernst-Planck formalism to represent the translocation of the ion-carrier complex across the membrane interior, the rate constant, $\tilde{A}_{is}^*$, for this translocation includes the potential energy difference between the plateau of the barrier and the energy "well" for the ion-carrier complex inside the membrane as well as a diffusion constant for the rate of movement of the complex along the plateau of the "diffusion barrier" (*see* Eq. (24) of Ciani, 1976). Thus, one cannot readily describe this translocation step in terms of a "transition state" as was done for the ion-carrier complexation reactions (which utilized an Eyring formalism).

librium" limit of these two types of measurements in this lipid. By contrast, the conductance-voltage and zero-current potential behaviors of GDO/dec bilayers in the presence of trinactin or tetranactin and the more permeant cations are consistent with a significant contribution of the kinetic factor $\tilde{w}_i$ to the apparent selectivity.

The enhancement of this kinetic factor in GDO/dec as compared to PE/dec bilayers could be due either to an increase in the rate constant, $\tilde{A}_{is}^{*}$, for translocation of the complex across the membrane or a decrease in the rate constant, $\bar{K}_i^B$, for its dissociation, and thus it is of some interest to determine whether any of the known differences in the physical properties of these two lipids could lead to either of these changes in rate constants. It has been shown previously (Szabo & Eisenman, 1973; Hladky & Haydon, 1973; and A.D. Bangham, personal communication) that a difference of approximately 160 mV exists in the surface dipolar potentials of these two lipids with the inside being relatively less positive in GDO/dec than in PE/dec bilayers. If the locus of the ion-carrier complexation reaction occurs at a plane in the membrane which is interior to the surface dipoles, then the direction of the surface potential difference between these two lipids will tend to produce a slower rate of dissociation, and thus a larger value of $\tilde{w}_i$, in GDO/dec than in PE/dec bilayers. If ion-carrier complexation takes place outside the polar head groups, then the surface dipole potential in GDO/dec bilayers will act to increase the rate of translocation of the ion-carrier complex, and thus again to increase the value of $\tilde{w}_i$, relative to that in PE/dec bilayers. Although the observed increase is clearly expected in terms of the measured differences in the surface dipole potentials of these two lipids, the present experiments cannot distinguish between these alternative ways of producing the increase.

Methylation of the macrotetralide actins also affects the degree to which the selectivity observed for a particular bilayer composition reflects "kinetic" *vs.* "equilibrium" components. As illustrated in Fig. 10, the effect of methylation is to increase $\tilde{w}_i$ and thereby to increase the relative contribution of the kinetics of ion-carrier complexation to the "apparent" selectivity of ion permeation. Intuitively, it is reasonable to expect the added methyl groups either to have little effect on the translocation rate constant ($\tilde{A}_{is}^{*}$) for the ion-carrier complex or, if anything, to decrease it. Indeed, the relaxation studies of Feldberg and Kissel (1975) and of Benz and Stark (1975) indicate that for nonactin, monactin, dinactin and trinactin this rate constant is virtually identical. We can therefore infer that methylation increases $\tilde{w}_i$ predominantly by decreasing the rate con-

stant for dissociation of the ion-carrier complex ($\bar{K}_i^B$), as was also indicated by the relaxation studies of Feldberg and Kissel (1975) and Benz and Stark (1975) for nonactin, monactin, dinactin, and trinactin.

The Effect of Methylation on the "Equilibrium Permeability Ratios" and on the "Equilibrium-state" Selectivity Intrinsic to the Carrier

Evidence has been presented elsewhere (Kilbourn, Dunitz, Pioda & Simon, 1967; J.D. Dunitz, *personal communication;* Szabo *et al.*, 1969; Eisenman *et al.*, 1969; Iitaka, Sakamaki & Nawata, 1972; Ciani *et al.*, 1973*a*) which indicates that, with the exception of the NH_4 complexes, the assumption of "isostericity" appears to hold well for nonactin-type carriers. Therefore, the conclusions presented here for the "equilibrium permeability ratio" selectivity will also be assumed to apply to the "equilibrium-state" selectivity intrinsic to the carrier molecule in the membrane.

For the "equilibrium permeability ratios," the added methyl groups generally appear to increase the preference for smaller cations relative to larger ones as illustrated in Fig. 12 (left and right). The trend is particularly clear with Na *vs.* Cs. Only the results for the Li ion deviate from this generalization. Furthermore, although nonactin and trinactin both have the "equilibrium permeability" sequence $K > Rb > Cs > Na > Li$, with the addition of one more methyl group to form tetranactin, an actual change in the sequence of selectivity among the alkali cations occurs in that Na is preferred over Cs. This trend has been observed and discussed previously for both the permeation selectivity (Szabo *et al.*, 1969) and the equilibrium selectivity (Eisenman *et al.*, 1969) of nonactin-type molecules and can be rationalized by the Eisenman "field strength model" for ion selectivity (Eisenman, 1961, 1969; Krasne & Eisenman, 1973), assuming that the added methyl groups increase the negative "field strengths" of the oxygen ligands via Taft induction effects or Kirkwood-Westheimer field effects (*see* Murrell, Kettle & Tedder, 1965), analogous to the mechanisms proposed for the effects of methylating organic acids in increasing their pK_a's.[12] Two possibilities seem most likely for interpreting the behavior seen for Li^+. The first is that the permeant species is actually a partially-

12 Although one might argue that methylation decreases the optimum cavity size of the carrier, it is not only intuitively difficult to see why such an effect would result from methyl groups added external to the ring, but the x-ray crystallography for complexes with K and Na show almost identical cation-ligand distances for nonactin (Kilbourn *et al.*, 1967; Dunitz, *personal communication*) and tetranactin (Iitaka *et al.*, 1972). If anything, these data indicate that the tetranactin cavity may tend to be slightly larger in the complex than that of nonactin.

hydrated Li ion so that the actual cation-water complex might be comparable to or even larger than the Cs ion. In this case the trend seen for Li would be consistent with the general effect of methylation in increasing the selectivity for smaller ions relative to that for larger ions. The other interpretation is based upon a second effect expected as the ligand "field strength" is increased for ion complexers containing multiple ligands which pack closely about the ions. For such complexers, methylation should increase not only the electrostatic energy of ion-ligand attraction but also that of ligand-ligand repulsion. Model calculations of both ion-ligand and ligand-ligand electrostatic interactions (S. Krasne, *unpublished*)[13] indicate that for multipolar ligands, the electrostatic contribution of ligand-ligand repulsion should have a steeper dependence upon distance than that of ion-ligand attraction, and therefore that, as the ligand "field strength" is increased, the repulsive energy will begin to dominate over the attractive energy first for the smallest ions (that is, for the smallest ligand-ligand distances). Thus, if methylation does increase the "field strengths" of the ligands, at some point a reversal is expected in the trend of preference for smaller cations relative to larger ones, and this reversal should start with the smallest ion, as is observed for these nonactin homologues. Quantum chemical calculations, recently begun (Kostetsky, Ivanov, Ovchinnikov & Shchembelov, 1973; Pullman & Schuster, 1974) may be expected to shed light on this question, as should recent studies with laser Raman spectroscopy (Phillies, Asher & Stanley, 1975).

A final detail in the effect of methylating these carriers is that the change in "equilibrium permeability ratios" for Tl and NH_4 (relative to Cs) parallels that for the comparably-sized Rb ion. To the extent that these changes in "equilibrium permeability ratios" for Tl and NH_4 reflect parallel changes in the "equilibrium-state" selectivities intrinsic to these carriers, and to the extent that these equilibrium selectivities can in turn be divided into a "simple coulombic" fraction dependent of ion radius and an "excess energy" fraction dependent on factors (e.g., partial covalence, hydrogen bonding) other than ion radius [as proposed by Eisenman (1969) and Krasne and Eisenman (1973)], we can infer that the

13 These calculations were based on a partial point charge model (*see* Eisenman, 1969; Krasne & Eisenman, 1973) for eight $C=0$ ligands cubically arrayed with their long axes oriented towards the central cation. Their packing was determined by first arranging four ligands tetrahedrally about the central cation in closest contact (using Pauling cation radii) and then arranging the other four ligands as close to the central ion as possible after the first tetrahedral set of ligands was in place. (This general scheme of packing is in accord with that observed in the x-ray crystallography data (Iitaka *et al.*, 1972; Kilbourn *et al.*, 1967; Dunitz, *personal communication*) for nonactin and tetranactin with several alkali cations.)

equilibrium effects of methylating these carriers are mainly on the "simple coulombic" interactions.

The Selectivity of the "Kinetic" Component

As pointed out in the Introduction, one of the aims of studies, such as this one, on structure-selectivity relationships is to provide a basis for inferring intimate details of the molecular "structures"[14] involved in ion-binding sites in biological systems. For reasons analogous to those discussed for the present carriers, the selectivities observed in many biological phenomena may reflect the "kinetics" of ion-site complexation rather than the equilibrium selectivity of the site. The relative roles of such factors in the selectivities of biological "channels" and enzyme kinetics have been discussed in detail by Hille (1975), who has suggested extending equilibrium selectivity considerations to include the selectivities of the "transition states" in the particular process under consideration. In the present study, the selectivity of the "transition state" of ion-carrier complex formation has been shown by Eq. (10) to equal the ratios of the forward rate constants of ion-carrier complexation. "Transition state" selectivities have been calculated for the present carriers from Eq. (10) using the values of $(P_i/P_{NH_4})_{Eq.}$ and $\tilde{w}_{NH_4}/\tilde{w}_i$ obtained in GDO/dec bilayers and have been plotted (relative to NH_4) as a function of carrier methylation in Fig. 14. Taking the data for tetranactin (which is the most complete) as typifying that of the other two molecules, it can be seen that both the sequence and the magnitude of selectivity differ for the "transition" and "equilibrium" states of the complex (*cf.* Figs. 14 and the right-most plot in Fig. 12).[15] Whereas the "equilibrium-state" has the selectivity sequence $NH_4 > Tl > K > Rb > Na$, the "transition state" has the sequence $Tl > Rb > K > NH_4 > Na$.

Although we do not know the physical origin of this selectivity change, we can speculate on the difference in the position of NH_4 and the simi-

14 We use the term "structure" loosely here to imply such molecular details of the ion binding sites as the chemical types, spatial array and electron distributions of the ligands. To the extent that one can discriminate, for example, a carbonyl- from an ether-type ligand based upon such information, one can hope to establish ways from known model compounds to deduce the chemical composition and "structure" of an unknown site (*see*, for example, Krasne & Eisenman, 1973; Eisenman & Krasne, 1975).

15 Because the values of $\tilde{w}_i$ for the least permeant ion-carrier complexes are too small to be assessed accurately, the data in Fig. 14 are not complete enough to determine whether carrier methylation produces any trends in the "transition-state" selectivities; indeed, the small changes observed are less than the accuracy with which we can deduce this selectivity.

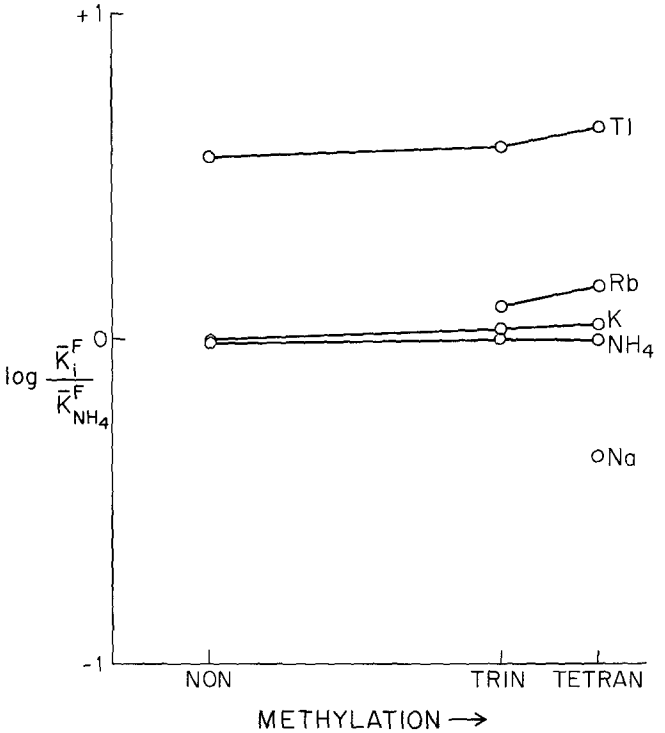

Fig. 14. The "transition state" selectivities of nonactin, trinactin, and tetranactin. The ordinate gives the ratio of the forward rate constant for ion-carrier complexation in the membrane for the indicated cation relative to that for NH_4. The abscissa is the nonactin-type carrier ranked from left to right according to its degree of methylation. The ratios of forward rate constants have been calculated according to Eq. (10) using the values of $(P_i/R_{NH_4})_{Eq.}$ and $\tilde{w}_{NH_4}/\tilde{w}_i$ calculated from the zero-current potential and conductance-voltage data obtained for each ion-carrier complex in GDO/dec bilayers

larity in the position of Tl in these two states. In the "equilibrium-state" complex, NH_4 is more selected than the comparably-sized group *Ia* cation, Rb. This "supra *Ia*" selectivity has been inferred previously to result from a tetrahedral coordination geometry of the ligands (Eisenman, 1969; Eisenman & Krasne, 1975). In the "transition state" complex, NH_4 is less preferred than Rb and thus this "sub-*Ia*" position might be taken to imply a lack of tetrahedrality for the ligands in this state. In contrast to the change observed for NH_4, Tl is "supra-*Ia*" (relative to the comparably-sized Rb ion) in both the "equilibrium-state" and "transition-state" complexes. This result is consistent with the postulate (*see*, Krasne & Eisenman, 1973) that the "supra-*Ia*" position for Tl observed for the macrotetralide actins (as well as for polyether molecules) results from the interaction of Tl with ether ligands in the complex, regardless of their

coordination. As for the reduction in the magnitude of selectivity seen for the "transition-state" complex, such a difference is intuitively consistent with the postulate that fewer ligands from the carrier are involved in coordinating the cations in the "transition-state" along with the possibility that the ions are still coordinated to some water molecules in this state.

Parameters Describing the "Diffusion Barrier" and the "Reaction Plane"

It was shown in the results that for each membrane composition the data for all of the ions and carriers could be described using a single value of α for the "diffusion barrier" and a single value of β for the "reaction plane." In GDO/dec bilayers $\alpha=0.68$ and $\beta=0.45$; while in PE/dec bilayers, $\alpha=0.72$ and β could not be determined. These values gave an internally consistent fit of all of the experimental conductance-voltage and zero-current potential data and led to the observation that the "equilibrium permeability ratios" induced by a particular carrier are independent of the lipid composition.[16]

The observation that the shape of the "diffusion barrier" is independent of the differences between these carriers is reasonable if indeed this energy barrier is determined by "image forces" (*see*, for example, Neumcke & Läuger, 1969), for these forces should be mainly affected by the charge of the ion-carrier complex and the dielectric constant and thickness of the membrane. Although a trapezoidal energy barrier is only a caricature of the parabolic-shaped barrier theoretically expected (Neumcke & Läuger, 1969; Haydon & Hladky, 1972) for image forces, the trapezoidal barrier has thus far been found (*see*, for example, Hladky, 1973) to give either an indistinguishable fit or, where distinguishable (as in PE/dec bilayers), a better fit to the data than the theoretically expected parabola.[17]

16 That these carriers should have the same "equilibrium" selectivities in GDO/dec or PE/dec membranes is not surprising since it has been previously shown (Eisenman *et al.*, 1969) by two-phase salt extraction experiments that the equilibrium selectivities of nonactin, monactin, dinactin and trinactin are relatively insensitive to changes in the organic phase over the range of dielectric constants of from 2 to 9, and such a result is expected for "isosteric" complexes.

17 The small differences between the value for PE/dec bilayers of $\alpha=0.72$ reported here to fit all of the nactins and $\alpha=0.6$ reported by Hladky (1973) for nonactin complexes may simply result from small differences in the experimental procedures. The most likely source of this difference results from the increase in area and decrease in membrane thickness which occurs (White, 1970) upon increasing the transmembrane potential. Since our initial membrane areas were smaller than Hladky's, the error expected to be produced by this change in area is likely to be different (*see* White, 1972). Considering this artifact as well as the different experimental procedures for obtaining the conductance-voltage curves (Hladky used steady-state measurements for step changes in voltage and we used a ramp of voltage), we would consider this small discrepancy between the two values to be reasonable.

Finally, the similarity of the "reaction plane" for nonactin, trinactin, and tetranactin and for the different ions implies that the "transition-state" for formation of the ion-carrier complex occurs at the same plane in the membrane for each of these carriers. This observed dependence on transmembrane potential of the complexation reaction is in agreement with the findings of Andersen and Fuchs (1975) for tetraphenylborate and of Feldberg (*personal communication*) for the macrotetralide actins that the instantaneous currents produced by these charged species sense only a fraction of the applied potential, implying that the equilibrium position of the charged species lies somewhat within the membrane. Interpretation of the physical meaning of this "reaction plane" is not straightforward, however. The fact that the chemical reactions between the ion and carrier appear to sense 10% of the applied potential cannot be used to imply, for example, that complex formation takes place inside the hydrocarbon region of the membrane since the electric field inside the membrane may not be constant, and also some fraction of the potential drop may occur across the regions of the polar headgroups and may even extend into the aqueous phase (if, for example, the dielectric constant of the water in the region of the polar headgroups were significantly lower than that of the bulk aqueous phase). In order to determine the actual position in the membrane at which complexation takes place, one would have to know the potential profile across the membrane. In addition, when one considers that these ion-carrier complexes are about 12 Å in diameter, compared to hydrocarbon thicknesses of the bilayer of about 40 Å, it seems reasonable to consider that some fraction of the trans-membrane potential occurs across the carrier itself, and it becomes somewhat meaningless to think literally in terms of the carrier, itself, occupying a two-dimensional "plane" in the membrane.[18]

Conclusions

1. The selectivity observed for carrier-induced ion permeability across a membrane results from a mixture of two selectivity components. The

18 The small dependence of membrane area and thickness on the transmembrane potential (White, 1970) noted in the preceding footnote may also lead to a smaller estimate for β than that which actually describes the plane of complex formation in the membrane. If one were interested in knowing the "true" plane of ion-carrier complex formation, one should make steady-state measurements of conductance (to insure that the carrier has re-equilibrated with any new membrane area exposed) at different applied potentials and simultaneously make the careful types of capacitance measurements carried out by White (1970, 1972) in order to monitor and correct for changes in the membrane's dimensions.

first is an "equilibrium permeability ratio" which depends upon the product of the binding constants for the heterogeneous ion-carrier complexation reaction and the rate constants for translocation of the ion-carrier complexes across the membrane. For "isosteric" complexes, such as the present ones, these "equilibrium permeability ratios" reduce to the equilibrium-state selectivity intrinsic to the carrier in the membrane. The second component contributing to the overall selectivity in ion permeability of the membrane is the selectivity of the transition state for ion-carrier complexation, which depends upon the forward rate constants for ion-carrier complexation and which represents the selectivity intrinsic to the carrier molecule in its conformation at the peak of the activation energy barrier for the ion-carrier complexation reaction.

2. The lipid composition of the membrane affects the membrane's "apparent" carrier-mediated selectivity by modulating the relative amounts of equilibrium-state *vs.* transition-state components contributing to the "apparent permeability ratios". The degree to which each of these components contributes to the observed selectivity is reflected in the kinetic parameter $\tilde{w}_i$ (which is the ratio of the rate constant for translocation of the complex across the membrane to the rate constant for ion-carrier dissociation near the interface); and the effect of lipid composition in altering the value of $\tilde{w}_i$ can be rationalized in terms of the differences in the surface dipole potentials associated with the different bilayer compositions. In addition, increasing the transmembrane potential increases the degree to which the "apparent permeability ratios" reflect the selectivity of the transition-state complex.

3. The steady-state permeability properties induced by tetranactin, which has one more methyl group than trinactin, have been examined in bilayers and compared with those induced by nonactin and trinactin. Tetranactin changes the permeability properties of bilayer membranes in the same regular manner as the less methylated nonactin homologues. Indeed, in terms of its conductance-mediating properties and its selectivity among monovalent cations, the behavior of tetranactin is simply what would be expected from extrapolating the trends in these behaviors heretofore observed in the series nonactin, monactin, dinactin and trinactin.

4. Methylation of the nactins affects their "apparent permeability ratios" in two ways. First, increased methylation causes an increase in the "equilibrium permeability ratios" for smaller cations relative to larger ones (with the exception of Li). This effect of methylation is consistent with the expectations of a "field strength" model for selectivity and

"inductive" or "field" effects of added methyl groups. Second, increased methylation of the carrier slows down its rate of dissociation, as reflected in an increase in the values of $\tilde{w}_i$, thereby enhancing the contribution of the "transition-state" selectivity to the "apparent permeability ratios" measured for the membrane.

5. The results of the present paper have been analyzed according to the expectations (derived by Ciani, 1976) for an extended model of the energy profile for membrane transport which has two more parameters than the simplest Eyring model introduced by Läuger and his colleagues, one parameter specifying the width of the plateau of the diffusion barrier and the other specifying the plane of the reaction. The present results indicate that the plateau of the diffusion barrier occupies about 70% of the membrane thickness and is approximately the same for all of the ion-carrier complexes, consistent with the expectation that this barrier shape is determined by electrostatic image forces. Furthermore, this parameter is approximately the same for PE/dec and GDO/dec membranes. The plane of the reaction has been determined in GDO/dec bilayers for the most permeant ion-carrier complexes and appears to sense 10% of the transmembrane potential for all of these ion-carrier complexes in this lipid. In addition, the extended model leads to the intuitively reasonable result that the "equilibrium permeability ratios" are independent of the lipid. This result is not obtained (*see* the Appendix) if the rates of the ion-carrier complexation reaction are assumed to be independent of the applied potential.

We gratefully acknowledge Dr. K. Ando and Dr. W. Simon for the gifts of tetranactin and Dr. Hans Bickel and Ms. Barbara Stearns for the gifts of nonactin and trinactin. In addition, we thank Dr. Sergio Ciani for many helpful discussions throughout the course of this work as well as for his willingness to undertake the theoretical analysis which provides the basis for interpretation of the present results. Lastly, analysis of the various parameters of the model was accomplished using the facilities of the UCLA Campus Computing Network with considerable help from Mr. Chris Clausen, to whom we are gratefully indebted.

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Appendix

The Method of Analysis of the Conductance-Voltage and Zero-Current Potential Data for GDO/dec Bilayers

In order to fractionate the "apparent permeability ratios" in GDO bilayers into their "equilibrium" and "kinetic" components it was neces-

sary to determine values for the parameters α and β which specify the width of the "diffusion barrier" and the position of the "reaction plane," respectively. For any experimental data there are a large number of values for the parameters $\tilde{w}_i$, α, and β which will give equally acceptable fits since, over the experimentally accessible voltage range of these experiments, a variation in the value of one parameter can frequently be compensated for by a variation in one or both of the other parameters. It was therefore necessary to use the limiting behaviors expected theoretically for the conductance-voltage behaviors along with "intuitive," but physically reasonable, assumptions about these parameters in order to analyze the data. These assumptions are:

1. The value of α is independent of the complexed ion.
2. If the curve for G/G_0 *vs.* voltage which is the most increasing function of voltage is seen for complexes with two or more of the least strongly complexing cations, these ions may be taken to be in the "equilibrium domain" for their complexes with the carriers in this lipid.
3. The value of β is independent of the complexed ion.
4. The "equilibrium" component of the carrier-induced NH_4/Cs selectivity in GDO/dec bilayers is approximately the same as it is in PE/dec bilayers.

The purpose of this appendix is to present the logic by which we analyzed, for GDO/dec bilayers, the conductance-voltage data in symmetrical, single-salt solutions and the membrane-potential data at zero current in asymmetrical, two-cation mixtures. The justification for the above assumptions and the degree to which the results are sensitive to these assumptions will be discussed.

Determination of α, the Width of the "Diffusion Barrier" Plateau

As illustrated in Eqs. (38) and (39) of Ciani (1976), in the limit in which $\tilde{w}_i \approx 0$, that is, in the "equilibrium domain," the normalized conductance is given by

$$\frac{G}{G_0} = \frac{\alpha \sinh \frac{\phi}{2}}{\sinh \frac{\alpha \phi}{2}} \qquad 0 \leqq \alpha \leqq 1. \tag{3}$$

Thus, in this limit the parameter α can be unambiguously determined from the conductance-voltage data. In order to assess α, therefore, we must determine which, if any, of the ion-carrier complexes are in the "equilibrium domain" in GDO/dec bilayers.

According to Eq. (3), the normalized conductance (G/G_0) in the "equilibrium domain" either will be independent of voltage (for $\alpha=1$) or an increasing function of voltage (for $\alpha<1$). Furthermore, if the width of the "diffusion barrier" for a particular carrier is reasonably independent of the complexed ion, as was shown above to be approximately true for PE/dec bilayers (and as is expected to be the case if this parameter is due to "image forces"), then the normalized conductance will be the same for all ions in the "equilibrium domain" and will be a more increasing function of voltage than for complexes in the "kinetic domain."[19] Examining Fig. 9, we can see that for each carrier there are two or more ions which obey these "equilibrium domain" criteria, Li and Cs for tetranactin, Li, Cs and Na for trinactin and Li, Cs, Na and Rb for nonactin. Not only are these presumed "equilibrium domain" data similar for the different ion complexes of a particular carrier but also the data for the different carriers are similar, all of them being adequately fit by the theoretical curve for Eq. (3) with $\alpha=0.68$.[20]

Although we have not proved independently that the data for these particular ion-carrier complexes represent the "equilibrium domain," and thus that $\alpha=0.68$ accurately represents the width of the "diffusion barrier," any attempt to explain the agreement between the conductance-voltage data for all of these complexes other than as a consequence of being the expected "equilibrium domain" behavior would be less parsimonious and would involve arbitrary and fortuitous compensations of different kinetic parameters and barrier widths.

Determination of β, the "Reaction Plane" for the Ion-Carrier Complex

Over the range of transmembrane potentials for which GDO/dec bilayers are stable, the parameters $\tilde{w}_i$ and β cannot be independently

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19 Since $\beta \geqq \frac{\alpha}{2}$, then, from Eq. (2), a value of $\tilde{w}_i > 0$ will always have the effect of decreasing the conductance below that expected if $\tilde{w}_i = 0$.

20 The computer-determined best-fit of Eq. (3) to each set of "equilibrium domain" data points for GDO/dec bilayers produces the range of values for $\alpha=0.64$ to 0.70, the values becoming larger in going from nonactin to tetranactin. Although this trend may represent real differences in α or may be due to a small effect of kinetics in the more methylated species, the variation among these data is comparable to the experimental error in their measurement, and therefor we have chosen to fit all of the data with a single value of $\alpha=0.68$. Even if α actually varies from 0.64 to 0.70 for different ion-carrier complexes, this would have an insignificant effect on the values of the "equilibrium permeability ratios" and kinetic parameters extracted and compared in the body of this paper.

determined simply by fitting Eq. (2) to the experimental data, since many combinations of $\tilde{w}_i$ and β will produce an adequate (and visually indistinguishable) fit to the data. We have therefore had to make certain intuitively reasonable assumptions for disentangling these two parameters. First, we assumed that, for a particular carrier, the plane of the reaction is the same for all of the ions. Second, we assumed that the "equilibrium permeability ratios" for one pair of ions, NH_4 and Cs, is the same in PE/dec and GDO/dec bilayers.[21] This second assumption, along with the values of $(P_{NH_4}/P_{Cs})_{Eq.}$ and $(P_{NH_4}/P_{Cs})_{V\to 0}$ deduced from zero-current potential measurements in PE/dec and GDO/dec bilayers, respectively, and the values $\tilde{w}_{Cs}\approx 0$ and $\alpha=0.68$ deduced above, allows us to calculate $\tilde{w}_{NH_4}$ from the rearrangement of Eq. (8)

$$\tilde{w}_{NH_4}=\frac{\alpha}{2}\left[\frac{(P_{NH_4}/P_{Cs})_{Eq.}}{(P_{NH_4}/P_{Cs})^{GDO}_{V\to 0}}\left(1+\frac{4\tilde{w}_{Cs}}{\alpha}\right)-1\right]. \tag{11}$$

Knowing $\tilde{w}_{NH_4}$ allows us to calculate β by fitting Eq. (2) to the conductance-voltage data for NH_4 in GDO/dec bilayers (again using the value $\alpha=0.68$). This value of β can then be used in fitting Eq. (2) to all of the other conductance-voltage data, thereby obtaining values of $\tilde{w}_i$ for each of the other cations.

Interestingly, one result of this procedure was that β was found to be approximately equal to 0.45 for each of the carriers. (The actual values obtained being $\beta_{non}=0.45$, $\beta_{trin}=0.44$, $\beta_{tetran}=0.45$). This particular equality would be somewhat fortuitous if the assumption that $(P_{NH_4}/P_{Cs})_{Eq.}$ is the same in GDO/dec and PE/dec bilayers were incorrect. Furthermore, as discussed in the text, this set of assumptions gives the particularly simple result that the "equilibrium permeability ratio" deduced for *each* ion-carrier complex in GDO/dec bilayers agrees with that measured directly in PE/dec bilayers. Had out assumption that this equality held for NH_4 and Cs been incorrect, and thereby led us to wrong values for β, it is extremely unlikely that we could have obtained this particularly simple result for all the remaining ion-carrier complexes.

In order to illustrate that the calculated selectivities are not, for some reason, insensitive to the value chosen for β, we have fitted the tetranactin-mediated conductance-voltage data for GDO/dec bilayers with values of β ranging from 0.44 to 0.5, leading to different computed values of $\tilde{w}_i$;

21 This particular pair of ions was chosen for the following reasons: Cs was used because $\tilde{w}_{Cs}\approx 0$ allowing $\tilde{w}_{NH_4}$ to be assessed from Eq. (11), as shown below. NH_4 was used because it has the largest value for $\tilde{w}_i$ of the ions examined, and the larger the value of $\tilde{w}_i$, the less sensitive is the extracted value of β to an error in this parameter [*see* Ciani, 1976, Eq. (40)].

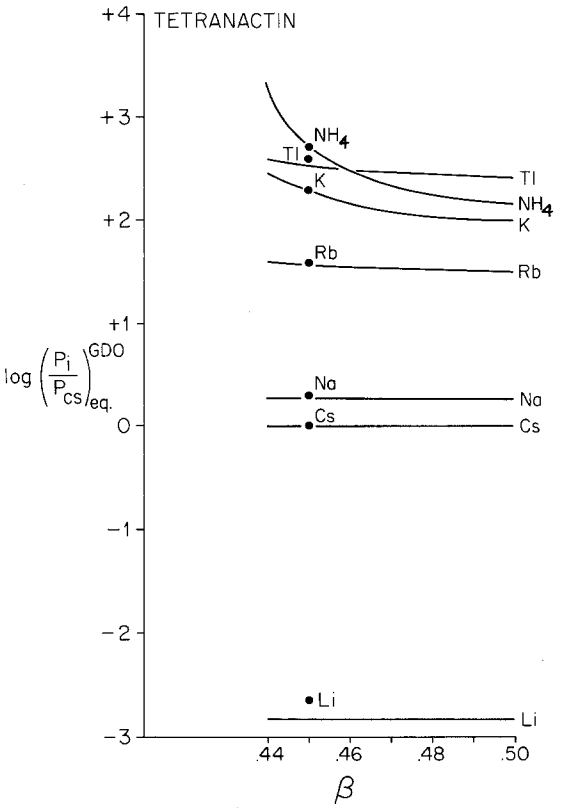

Fig. 15. The influence of the value assumed for the "reaction plane" on the "equilibrium permeability ratios" calculated for GDO/dec bilayers and tetranactin. The zero-current potential data of Fig. 6 (left) for GDO/dec membranes in the presence of tetranactin has been fit according to Eq. (4) with the values of $(P_j/P_i)_{app.}$ given by Eqs. (6) and (7). The values of $\tilde{w}_i$ (or $\tilde{w}_j$) were determined fitting Eq. (2) to the conductance-voltage data of Fig. 5 (right) for values of β ranging from 0.44 to 0.50, with $\alpha=0.68$. Different values of β yielded different values for $\tilde{w}_i$ (or $\tilde{w}_j$), and these were substituted in turn into Eq. (6) (along with the value of β used in fitting the conductance-voltage data and the value $\alpha=0.68$) to yield the values $(P_i/P_{Cs})^{GDO}_{Eq.}$ plotted as curved lines in the present figure. For comparison, the values of $(P_i/P_{Cs})_{Eq.}$ for PE/dec bilayers in the presence of tetranactin (from Fig. 12, left) are plotted as points at the value of $\beta=0.45$ used for fitting the GDO/dec data in the text. Notice that the calculated "equilibrium permeability ratios" are very sensitive functions of the value chosen for β. Furthermore, only for the case $\beta=0.45$ does one obtain the intuitively expected result that the "equilibrium permeability ratios" calculated for GDO/dec bilayers agree with those for PE/dec bilayers

we have then used these new values of $\tilde{w}_i$ and β (still using $\alpha=0.68$) to fit the zero-current potential data (which also can be adequately fit by these new combinations of parameters), thereby extracting different values for the "equilibrium permeability ratios." Fig. 15 illustrates how these calculated "equilibrium permeability ratios" vary as a function of the

value of β chosen and for comparison also plots the ("equilibrium") permeability ratios observed for PE/dec bilayers as data points at the value of $\beta = 0.45$. First of all, it is readily apparent that the "equilibrium permeability ratios" calculated for GDO/dec bilayers are exceedingly sensitive to the value chosen for β. Secondly, it is only when $\beta = 0.45$ that we obtain the particularly simple relationship that the calculated "equilibrium permeability ratios" in GDO/dec bilayers are equal to the observed ("equilibrium") permeability ratios in PE/dec bilayers.[22] As was the case for the assignment of α, we have not *proved* that β is the same for nonactin, trinactin and tetranactin and all of the complexed cations, nor that the "equilibrium permeability ratios" are the same in PE/dec and GDO/dec bilayers for these carriers. But these results are both physically reasonable and appealingly parsimonious.

References

Andersen, O., Fuchs, M. 1975. Potential energy barriers to ion transport within lipid bilayers. Studies with tetraphenylborate. *Biophys. J.* **15**:795

Benz, R., Stark, G. 1975. Kinetics of macrotetralide-induced ion transport across lipid bilayer membranes. *Biochim. Biophys. Acta* **382**:27

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

Ciani, S. 1976. The influence of molecular variations of ionophore and lipid on the selective ion permeability of membranes. II. A theoretical model. *J. Membrane Biol.* **30**:45

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

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

Ciani, S., Gambale, F., Gliozzi, A., Rolandi, R. 1975. Effects of unstirred layers on the steady-state zero-current conductance of bilayer membranes mediated by neutral carriers of ions. *J. Membrane Biol.* **24**:1

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

Eisenman, G. 1961. On the elementary atomic origin of equilibrium ionic specificity. *In:*

22 Note that $\beta = 0.5$ corresponds to the case in which the ion-carrier complexation reaction occurs at the membrane-solution interface, so that the rate constants for this reaction are independent of the transmembrane potential. In this case the "equilibrium permeability ratios" calculated for GDO/dec bilayers differ significantly from those measured in PE/dec bilayers, as seen in Fig. 15. The contrast between this result and the parsimony obtained by assuming $\beta = 0.45$ is the major argument, based on the data of the present paper, in favor of using an extended model which includes a voltage-dependence of the complexation reaction.

Symposium on Membrane Transport and Metabolism. A. Kleinzeller and A. Kotyk, editors. p. 163. New York, Academic Press

Eisenman, G. 1969. Theory of membrane electrode potentials: An examination of the parameters determining the selectivity of solid and liquid ion exchangers and of neutral ion–sequestering molecules. *In:* Ion-Selective Electrodes. R.A. Durst, editor. National Bureau of Standards Special Publications **314**:1–56

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., 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., Krasne, S. 1975. The ion selectivity of carrier molecules, membranes and enzymes. MTP International Review of Science, Biochemistry Series. C.F. Fox, editor. Vol. 2, pp. 27–59. Butterworths, London

Eisenman, G., Krasne, S., Ciani, S. 1975. The kinetic and equilibrium components of selective ionic permeability mediated by nactin- and valinomycin-type carriers having systematically varied degrees of methylation. *In:* Annals of the New York Academy of Sciences. International Conference on Carriers and Channels in Biological Systems. **264**:34

Eisenman, G., Szabo, G., Ciani, S., McLaughlin, S.G.A., Krasne, S. 1973. Ion binding and ion transport produced by lipid soluble molecules. *Prog. Surf. Membr. Sci.* **6**:139

Feldberg, S.W., Kissel, G. 1975. Charge pulse studies of transport phenomena in bilayer membranes: I. Steady-state measurements of actin and valinomycin mediated transport in glycerol monooleate bilayers. *J. Membrane Biol.* **20**:269

Hall, J.E., Mead, C.A., Szabo, G. 1973. A barrier model for current flow in lipid bilayer membranes. *J. Membrane Biol.* **11**:75

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

Hille, B. 1975. Ionic selectivity of Na and K channels of nerve membranes. *In:* Membranes–A Series of Advances. G. Eisenman, editor. Vol. 3, Ch. 4. Marcel Dekker, New York

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

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

Hladky, S.B. 1974. The energy barriers to ion transport by nonactin across thin lipid membranes. *Biochim. Biophys. Acta* **352**:71

Hladky, S.B., Haydon, D.A. 1973. Membrane conductance and surface potential. *Biochim. Biophys. Acta* **318**:464

Iitaka, Y., Sakamaki, T., Nawata, Y. 1972. The molecular structure of tetranactin and its alkali metal ion complexes. *Chem. Lett.* 1225

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

Kostetsky, P.V., Ivanov, V.T., Ovchinnikov, Yu.A., Shchembelov, G. 1973. The nature of the metal-ligand bonding in the complexes of ionophores with alkali metal ions. A quantum mechanical study of the N, N-dimethyl acetamide and methyl acetate interaction with Na^+ and Li^+. FEBS Letters **30**:205

Krasne, S., Eisenman, G. 1973. The molecular basis of ion selectivity. *In:* Membranes–A Series of Advances. G. Eisenman, editor. Vol. 2, pp. 277–328. Marcel Dekker, New York

Laprade, R., Ciani, S.M., Eisenman, G., Szabo, G. 1975. The kinetics of carrier-mediated ion permeation in lipid bilayers and its theoretical interpretation. *In:* Membranes–A Series of Advances. G. Eisenman, editor. Vol. 3, Ch. 2. Marcel Dekker, New York

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

Markin, V.S., Kristalik, L.I., Liberman, E.A., Topaly, V.P. 1969. Cell Biophysics. Mechanism of conductivity of artificial phospholipid membranes in the presence of ion carriers. *Biofizika*, No. 2 **14**:256

Murrell, J.N., Kettle, S.F.A., Tedder, J.M. 1965. Valence Theory. Ch. 16. John Wiley and Sons, Ltd., London

Neumcke, B., Läuger, P. 1969. Nonlinear electrical effects in lipid bilayer membranes. II. Integration of the generalized Nernst-Planck equation. *Biophys. J.* **9**:1160

Phillies, G.D.J., Asher, I.M., Stanley, H.E. 1975. Electrostatic and steric effects in the selective complexation of cations. *Science* **188**:1027

Pressman, B.C., Harris, E.J., Jagger, W.S., Johnson, J.H. 1967. Antibiotic-mediated transport of alkali ions across lipid barriers. *Proc. Nat. Acad. Sci.* **58**:1949

Pullmann, A., Schuster, P. 1974. Model studies on the binding of metal cations to macrocyclic ligands. The interaction of Li^+ with carbonyl groups. *Chem. Phys. Lett.* **24**:472

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

Szabo, G., Eisenman, G. 1973. Enhanced cation permeation in glyceryl oleate bilayers. *Biophys. Soc. Abstr.* 175*a*

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

Szabo, G., Eisenman, G., Ciani, S.M., Laprade, R., Krasne, S. 1973. Experimentally observed effects of carriers on the electrical properties of membranes. The equilibrium domain. *In:* Membranes—A Series of Advances. G. Eisenman, editor. Vol. 2, p. 179. Marcel Dekker, New York

White, S. 1970. Thickness changes in lipid bilayer membranes. *Biochim. Biophys. Acta* **323**:7

White, S. 1972. Analysis of the torus surrounding planar lipid bilayer membranes. *Biophys. J.* **12**:432