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THE ENERGY BARRIERS TO ION TRANSPORT BY NONACTIN ACROSS THIN LIPID MEMBRANES

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SUMMARY

The experimental steady-state current–voltage relations for low concentrations of a neutral carrier and an ion may be fitted theoretically either by assuming a form for the potential dependence of the rate of transfer of complex across the membrane and adjusting the proposed nature of the association–dissociation reactions or by assuming equilibrium for the association and adjusting the potential dependence of the transfer process. Different dependences for the rate of transfer correspond, at least formally, to different shapes for the potential energy barrier which the complex must cross. By comparing measurements of the current–voltage relations for nonactin with Na^+, K^+, and NH_4^+, it is possible to distinguish between the effects of the various rates. For black lipid membranes made from glycerolmonooleate + *n*-hexadecane, the potential energy barrier is high with a narrow top, but the rate of association still becomes increasingly limiting for Na^+, K^+ and NH_4^+, in the order given. For bacterial phosphatidylethanolamine, with *n*-decane the barrier is much wider and no effect of the rate of association can be detected.

INTRODUCTION

A small particle moves through a viscous liquid or a solid by executing a sequence of jumps each with length comparable to atomic dimensions [1]. If the nature of the medium changes with position the rate and length of the jumps will vary in a manner which may be predicted, at least formally, by introducing a sequence of energy barriers with appropriate spacings and heights [2]. These barriers determine the local values for the diffusion constant and the solubility of the particle. If the particle is charged the rates at which the various jumps are taken will depend on electrostatic forces as well as on the nature of the barriers associated with each step. For an ion moving across a thin lipid membrane separating two aqueous phases the two most important electrostatic forces will be the externally applied field and the image force which will tend to pull the ion out of the membrane into the solutions. As a result of these forces the potential energy of the ion will have an additional component which

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changes slowly with position in comparison to the local barriers which determine the nature of the jumps. It is this slowly varying component of the energy of the particle which is termed the potential energy profile or the potential energy barrier.

While current–voltage relations may be calculated from a knowledge of the nature of the medium and the long-range forces acting on an ion, it is only possible to work backwards from the experimental data to the potential energy profile in certain cases and then only in terms of an appropriate model. The simplest example of such an approach is for the lipid-soluble ions, tetraphenylboride$^-$ and dipicrylamine$^-$, where it has been shown that the potential energy of an ion is at a maximum near the middle of the membrane [3, 4].

The analysis of data obtained with ion–carrier complexes must be more elaborate because of the finite rates at which the ion and carrier associate and dissociate. It will be shown here that some current–voltage relations can be explained either by adjusting the assumed potential energy profile or by adjusting the assumed dependence of the rates of association and dissociation on the applied potential*. However, by considering the current–voltage relations for complexes of a carrier with different ions, it should be possible to change the rates of association and dissociation and thus, if these are low enough to affect the current–voltage relation, to change that relation. It should thus be possible to distinguish between the effects of various potential energy profiles and the effect of slow association–dissociation reactions.

THEORY

There is ample evidence, frequently reviewed (e.g. refs 4 and 5) that the actins carry cations across black lipid membranes by forming one carrier–one ion complexes which cross from one side of the membrane to the other. Similarly the carrier model has been considered frequently in the literature [4, 6–14]. The notation used here is taken from ref. 11 where the assumptions are discussed which allow the use of the simple version of the model in Fig. 1. The evidence that these assumptions are appropriate for the actins has been collected together in a recent review [15].

The principal theoretical difficulty which must be overcome is that there is no completely adequate procedure by which to account for the variation in the total amount of carrier in the membrane as the potential and bulk-phase concentrations are changed. However, so long as the electrolyte activities are sufficiently small, a negligible proportion of the carrier in the membrane will be in the form of complex. It is then a reasonable first choice to assume that $N_s'+N_s''$, the concentration of free carrier in the membrane, is a constant, $2N_s$. If, in addition, the free carrier crosses the membrane as easily or more easily than complex, then the concentration of free carrier on each side of the membrane will be the same i.e. $N_s' = N_s''$. The equations describing the flux of a single species of ions M_i under these conditions are for the left interface

$$J_i = k'_{Ri}\, a'_i\, N_s - k'_{Di}\, N'_{is}, \qquad (1)$$

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* Variations with position of the diffusion constant should be far less important since the current–voltage relations are sensitive to the value of this constant only in the limited region where the potential energy of the ions is a maximum.

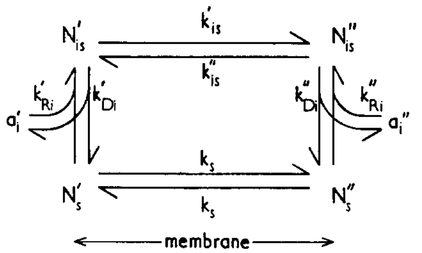

Fig. 1. The simple model for a neutral carrier of ions. Ions present in the left aqueous phase at activity a'_i combine with the neutral free carrier at surface concentration N'_s to form complexes at surface concentration N'_{is}. The rate of association is $k'_{Ri}a'_iN_s'$, and the rate of dissociation is $k'_{Di}N'_{is}$. Similar relations hold on the right. Both free carrier and complex may cross the hydrocarbon core of the membrane. These fluxes are assumed to increase linearly with the respective concentrations, e.g. the flux of complex from left to right is $k'_{is}N'_{is}$. The rate of exchange of free carrier and complexes across the unstirred layer separating the membranes from the bulk of the aqueous phases is assumed to be small relative to the fluxes across the membrane.

for the membrane interior

$$J_i = k'_{is}N'_{is} - k''_{is}N''_{is}, \tag{2}$$

and for the right interface

$$J_i = k''_{Di}N''_{is} - k''_{Ri}a''_iN_s. \tag{3}$$

The current is thus

$$\frac{I}{z_iF} = J_i = N_s\frac{k'_{Ri}a'_ik'_{is}/k'_{Di} - k''_{Ri}a''_ik''_{is}/k''_{Di}}{1+k'_{is}/k'_{Di}+k''_{is}/k''_{Di}} \tag{4}$$

where z_i is the charge per ion and the rest of the notation is defined in Fig. 1. More complex derivations which do not assume low electrolyte activity have been given for constant free carrier concentration in the membrane, called the "lipid-buffered carrier model" [11]; and for constant total concentration, the "membrane-bound carrier model" [11, 12]. For low electrolyte activities (essentially in the present instance $k_{Ri}a_i/k_s \ll 1$), these expressions reduce to Eqn 4. As the present data is restricted to low ion activities further discussion of the different approximations is deferred to a later paper.

If the flux of ions across the membrane is much less than the rates of association and dissociation, then from Eqns 1 and 3 $N'_{is} = (k'_{Ri}a'_i/k'_{Di})N_s$ and $N''_{is} = (k''_{Ri}a''_i/k''_{Di})N_s$. Thus the numerator of Eqn 4 is just the flux in the sometimes hypothetical case where the concentration of the complex on either side is not disturbed. If the flux is much larger than the rate of dissociation, $k_{is}/k_{Di} \gg 1$, then the rate-limiting step is the rate of association which may be seen clearly at large negative potentials, $k'_{is} \gg k''_{is}$, where $J = N_sk'_{Ri}a'_i$.

In order to convert the formal expression for the current into an actual prediction, it is necessary to specify the potential dependence of the rate constants. Various possibilities for similar rate constants have been considered by Haydon and Hladky [4]. It is assumed that the entire applied potential falls as a linear gradient across the hydrocarbon interior of the membrane [16] which extends from $-d/2$ to $d/2$. An ion enters the membrane either by adsorption of the ion–carrier complex preformed in the aqueous phase or as is more likely [17] by complexing with an adsorbed carrier which may change position within the membrane during this process. The portion of the field which assists or retards the complexing (or adsorption) will depend on how far into the hydrocarbon region the ion must move. Thus if the activated state in complexing (or in adsorbing) is located a distance ηd into the membrane,

$$k'_{\mathrm{Ri}} = k_{\mathrm{Ri}} \exp\,(-z_{\mathrm{i}} F \Delta V \eta / RT) \tag{5}$$

Similarly if the complex once formed sits a distance ξd into the membrane,

$$k'_{\mathrm{Di}} = k_{\mathrm{Di}} \exp\,(z_{\mathrm{i}} F \Delta V (\xi - \eta)/RT) \tag{6}$$

where the usual exponential dependence has been assumed for the potential dependence of a process with a single rate-limiting step.

The remaining portion of the field acts to move the complex from one side of the membrane to the other. It is possible to postulate a number of forms for the variation of the potential energy of the complex with position and thus a number of forms for the dependence of k'_{is} and k''_{is} on the applied potential. If the shape of the potential energy barrier near the middle of the membrane is determined by the image force then following the procedure outlined by Haydon and Hladky [4],

$$k'_{\mathrm{is}} = k_{\mathrm{is}} \exp\,\{-z_{\mathrm{is}} F \Delta V (0.5 - \xi)/RT - b(F \Delta V / RT)^2\} \tag{7}$$

and

$$k''_{\mathrm{is}} = k_{\mathrm{is}} \exp\,\{+z_{\mathrm{is}} F \Delta V (0.5 - \xi)/RT - b(F \Delta V / RT)^2\} \tag{8}$$

where b is calculated to be $1.1 \cdot 10^{-4}\, d$ for d in Å units. Analogous expressions, for a trapezoidal energy barrier, are derived in the appendix.

When the electrolyte activities on the two sides of the membrane are different, the potential at which the current vanishes is,

$$V_0 = (RT/F) \ln\,(a'_{\mathrm{i}}/a''_{\mathrm{i}}) \tag{9}$$

at least in all instances where Eqn 4 applies. It is possible to define a rectification ratio, r, as the ratio of the currents which flow if the applied potential is displaced an amount ΔV either way from the zero current value, V_0. Thus whenever Eqn 4 is valid and assuming formulas 5–9.

$$r = \frac{-I(\Delta V - V_0)}{I(-\Delta - V_0)} = \frac{1 + \dfrac{2k_{\mathrm{is}}}{k_{\mathrm{Di}}} \cosh\left\{\dfrac{z_{\mathrm{is}} F(V_0 + \Delta V)(0.5 - \eta)}{RT}\right\} \exp\left\{-b\left(\dfrac{F(V_0 + \Delta V)}{RT}\right)^2\right\}}{1 + \dfrac{2k_{\mathrm{is}}}{k_{\mathrm{Di}}} \cosh\left\{\dfrac{z_{\mathrm{is}} F(V_0 - \Delta V)(0.5 - \eta)}{RT}\right\} \exp\left\{-b\left(\dfrac{F(V_0 - \Delta V)}{RT}\right)^2\right\}} \tag{10}$$

This equation is the obvious extension of Eqn 15 from the paper by Stark [12].

METHODS

Membranes were formed from a 7-mM solution of glycerylmonooleate in *n*-hexadecane, 10 mg/ml phosphatidylcholine in *n*-decane, or 12.5 mg/ml phosphatidyl ethanolamine in *n*-decane. Glycerylmonooleate (samples from The Hormel Institute, Austin, Minn., NuChek Prep, Elysian, Minn. and Sigma, London were used interchangeably) was stored under vacuum at -20 °C. Egg phosphatidylcholine and phosphatidylethanolamine (Lipid Products, Redhill) and L-dioleoylphosphatidylcholine (gift of Dr J. Metcalfe) were stored in chloroform under nitrogen at -20 °C. Bacterial phosphatidylethanolamine (Chromatography Services Ltd, Birkenhead–Supelco 04-6040) was stored in chloroform in the manufacturers sealed ampoules at -20 °C.

The normal alkanes (Koch-Light Ltd, Colnbrook) were passed down columns of chromatographic alumina and were then stored in the dark. Water from a commercial still was redistilled in a pyrex and poly(tetrafluoroethylene) apparatus. Its pH on use was normally approx. 5.7. Bubbles produced by shaking the distillate did not persist for more than a second. The alkali salts (BHD, Poole, Analar grade) were roasted overnight at temperatures greater than 500 °C to remove organic impurities. The NH_4Cl (Fisons, Loughborough, Analytical Reagent) was used as purchased. The salt solutions were not buffered.

Nonactin (gift of Dr Barbara Stearns, Squibb) was dissolved in ethanol at various concentrations between 10^{-3} and 10^{-7} M. All such solutions were stable for months at room temperature.

The experimental cell used was of conventional design with a 1-mm hole bored in the side of a poly(tetrafluoroethylene) cup suspended inside a borosilicate glass or silica beaker. The electrodes were Ag–AgCl, either 1-mm diameter wire exposed for about 1 cm (for currents up to 10^{-7} A) or a thin sheet spot welded onto wire such that the area was greater than 2 cm^2 (currents up to 10^{-5} A). The potential was applied to one electrode while the other was held at virtual earth by an amplifier with current feedback (Analog Devices, Ltd 42K). The accuracy of the applied potentials was better than 0.5 mV or 1 % whichever is worse while that of the current measurement is approx. 1 %. In order to minimize the corrections necessary for changes in membrane area, in most experiments the potential was first held at 100 mV, then changed to a new value and the steady conductance noted. Currents for potentials in excess of 200 mV were measured relative to the current at 200 mV. Normally after a potential change the conductance reaches a constant value within 1 ms after a change in potential.

The experimental cell and associated glassware were cleaned with detergent followed by immersion in a dichromate–H_2SO_4 mixture. The electrodes were normally only rinsed, but after experiments involving high aqueous concentrations of the carrier they were dipped into conc. HNO_3, rinsed, and then rechloridized.

Membranes were formed in the cell using disposable Pasteur pipettes [18]. All experiments were carried out at temperatures between 21 and 23 °C. The monoglyceride membranes would withstand 200 mV for several minutes (or longer) and 100 mV for several tens of minutes and would remain unbroken at 0 mV for hours (on several occasions overnight). In the absence of nonactin the conductance per unit area was less than 10^{-9} Ω^{-1}/cm^2 in 0.1 M KCl, NaCl or NH_4Cl. The bacterial phosphatidylethanolamine produced exceptionally stable membranes which easily withstood 300 mV applied potential. With the remaining phospholipids it was possible to produce membranes which would last for hours at low applied potentials, but these would normally withstand 200 mV for only a few minutes, or less. In all cases the background conductances were less than 10^{-8} Ω^{-1}/cm^2 and usually less than 10^{-9} Ω^{-1}/cm^2.

In all of the experiments with glycerylmonooleate+*n*-hexadecane and some others, the nonactin has been added to the lipid phase. The time course of the conductance changes following addition of nonactin to the aqueous phases and the effect of stirring were discussed in an earlier paper [19]. While it is possible when using phospholipid membranes to reform membranes repeatedly from the same lipid material and thus eventually to reach an equilibrium distribution of the carrier [20], this procedure has not been practical with glycerylmonooleate.

RESULTS

Current–voltage relations for nonactin carrying Na^+, K^+ and NH_4^+ ions across glycerylmonooleate+*n*-hexadecane membranes are shown in Fig. 2 as plots of the conductance relative to the value at 100 mV,

$$G(\Delta V)/G(100) = I(\Delta V)/\Delta V/I(100)/100,$$

vs the applied potential, ΔV. A plot of $G(\Delta V)/G(0)$ would emphasize the differences in the data for the various ions and the differences between the theoretical curves, particularly 3 and 4, but it would also be considerably further removed from a direct

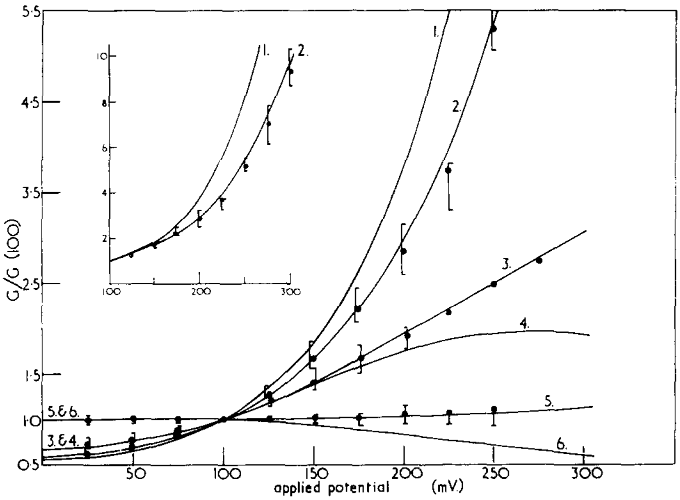

Fig. 2. The conductance of glycerylmonooleate+*n*-hexadecane membranes with nonactin as a function of the applied potential. The points, dashes, brackets, and bars are experimental where the extent of the brackets and bars indicates the complete spread of the experimental observations: [, Na^+;], K^+; I, NH_4^+. The points are the data from the "best" experiments, where "best" is determined by the stability of the membranes and the reproducibility of the measurements in successive determinations. The curves are theoretical plots of the conductances predicted by the following expressions for the current: 1, sinh $(0.5\ F\Delta V/RT)$; 2–6, Eqn 4 with $b = 0.005$ and with: 2, $k_{is}/k_{Di} = 0$; 3, $k_{is}/k_{Di} = 0.05$, $\eta = 0.1$; 4, $k_{is}/k_{Di} = 0.02$, $\eta = 0$; 5, $k_{is}/k_{Di} = 0.45$, $\eta = 0.1$; 6, $k_{is}/k_{Di} = 0.18$, $\eta = 0$.

presentation of the experimental data. The same data are replotted in Fig. 3 as $\log(I(\Delta V)/I(100))$ vs ΔV. Similar data for the bacterial phosphatidylethanolamine membranes are given in Fig. 4. Absolute values of the conductances extrapolated to $\Delta V = 0$ are presented in Table I.

The experiments with glycerylmonooleate membranes have been repeated frequently over a period of several years. The absolute conductances are proportional to nonactin concentration. The maximum scatter seen is approx. 50% but the results are reproducible to 20% within any one series of experiments for salt concentrations of 0.01 M or higher. The range of observed values for the relative conductances is indicated in Fig. 2. For NaCl the shape of the current–voltage relation is the same for electrolyte activities from 0.01 to 0.5 M while the conductance increases proportional to the activity. For KCl and NH_4Cl the departure of the shape of the curves reported from the limiting curves ($k_{Ri}a_i/2k_s \rightarrow 0$) is within the experimental error of the techniques described. From data obtained at higher electrolyte concentrations, which has been reported elsewhere [4, 15, 21], the values of $k_{Ri}/2k_s$ for KCl and NH_4Cl are both less than 5, and thus at the concentration used, 0.01 M, the theoretical expressions for low electrolyte activities are appropriate. It has been verified for 0.1 M NH_4Cl that the shape of the current–voltage curve and the absolute conductance do not depend on ionic strength.

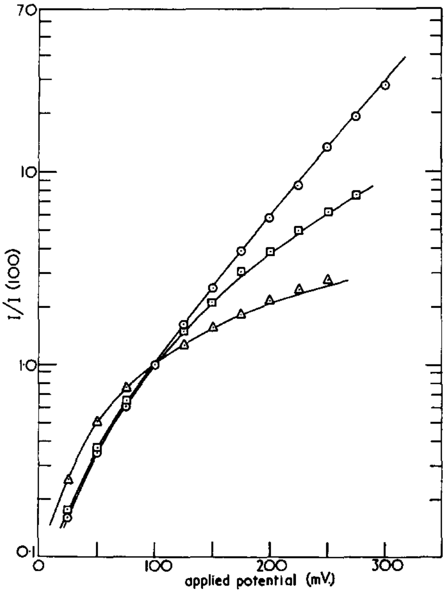

Fig. 3. Current–voltage curves for glycerylmonooleate+n-hexadecane membranes with nonactin as in Fig. 2. ○–○, Na^+; □–□, K^+; △–△, NH_4^+.

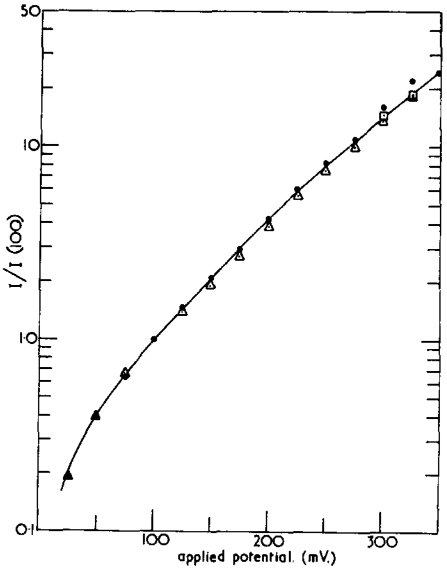

Fig. 4. The current–voltage relation for nonactin carrying Na^+ (1 M, ●–●), K^+ (0.1 M, □–□), and NH_4^+ (0.1 M, △–△) across bacterial phosphatidylethanolamine+n-decane membranes. Omitted data for K^+ are intermediate between those for Na^+ and NH_4^+. The three sets of data are the same within experimental error. The solid curve is calculated from the expression for the current across a high trapezoidal barrier, $I = G_0 \alpha \Delta\varphi \sinh(\Delta\varphi/2)/\sinh(\alpha\Delta\varphi/2)$ where $\Delta\varphi = F\Delta V/RT$, G_0 is the conductance at zero voltage, and $\alpha = 0.6d$ is the width of the flat top. This expression assumes that the barrier is high relative to $\Delta\varphi$ (see Appendix).

The current ratios for bacterial phosphatidylethanolamine show a scatter of about 5%. The ratios in 1 M and 6 m NH_4Cl are within 10% of those shown in Fig. 4. No attempt has been made to secure accurate values for the absolute conductances. The apparent scatter is 30–40%. The scatter of the data for each of the remaining lipids is slightly larger. With dioleoyllecithin membranes particular care was required to ensure that the current per unit area was measured, as the area could change considerably with applied potential or time.

DISCUSSION

Glycerylmonooleate+*n*-hexadecane membranes were chosen for a careful study of carrier kinetics first because they are at equilibrium with the bulk phases and thus have reproducible physical properties [22, 23], and secondly because they show only a small change in thickness with the applied potential (< approx. 3%, 0–200 mV). With all of the other membranes to be described here the specific capacity increased 10–20% within the same range of applied potentials. The corresponding changes in thickness require a considerable alteration in the composition of the hydrocarbon portion of the membrane [23], and possibly in the properties of the membrane–aqueous phase interface. The use of membranes with a mixture of lipid headgroups has been avoided since thinning may cause the selective depletion of whichever type of headgroup is less strongly adsorbed.

Absolute conductances have been determined carefully only for glycerylmonooleate+*n*-hexadecane membranes and for 0.1 M KCl with glycerylmonooleate +*n*-decane and egg lecithin+*n*-decane. The other values are listed in Table I as a general guide only. The conductivities for Na^+, K^+ and $NH_4{}^+$ at 0.1 M across glycerylmonooleate+*n*-hexadecane membranes are in the ratio of 0.009:1:4.9 while the ratios of $k_{Ri}k_{is}/k_{Di}$ are 0.008:1:7.2 for $\eta = 0.1$. For comparison the values on some phospholipid membranes [5] are 0.007:1:5. The agreement is satisfactory.

It should be noted that the fluxes across the monoglyceride membranes are much higher than those across the phospholipid membranes. It has been shown that this results primarily from the change in the dipole contribution to the surface potential [20, 24]. It is not, however, possible to say at this stage whether the higher flux results from a larger value of k_{is} (as would be produced by a change in dipole potential if the complex were adsorbed on the aqueous side of an intact dipole layer) or from a change in k_{Ri}/k_{Di} (as would result if the complex were adsorbed on the hydrocarbon side of an intact layer) or from some combination of both.

Glycerylmonooleate

The upward curvature of the current–voltage relations for nonactin carrying Na^+, K^+ and $NH_4{}^+$ across glycerylmonooleate+*n*-hexadecane membranes are clearly different. If it is assumed that

$$\frac{k'_{Ri}k'_{is}}{k'_{Di}} = \frac{k_{Ri}k_{is}}{k_{Di}}\exp\{-0.5F\Delta V/RT - b(F\Delta V/RT)^2\}$$

and (11)

$$\frac{k''_{Ri}k''_{is}}{k''_{Di}} = \frac{k_{Ri}k_{is}}{k_{Di}}\exp\{+0.5F\Delta V/RT - b(F\Delta V/RT)^2\}$$

TABLE I

THE CONDUCTANCES PRODUCED IN VARIOUS MEMBRANES BY ADDING NONACTIN AT CONCENTRATION c_s TO THE AQUEOUS PHASE

Values of $(G/A)/c_s$ in parentheses were obtained by adding nonactin to the lipid at a concentration $5000 \times c_s$ where the 5000 accounts, approximately, for the distribution of nonactin between the aqueous and lipid phases (Szabo et al. [18].) Accurate values of the specific capacity for egg lecithin (0.387 ± 0.005 μF/cm²) and for dioleoyl lecithin (0.395 ± 0.005 μF/cm²) have been determined by R. Fettiplace and J. Requena (unpublished). The surface potentials are taken from (a) Hladky and Haydon [20] and (b) Haydon (unpublished).

Lipid	Conductance/concentration of carrier, $(G_0/A)/c_s$ $((\Omega^{-1}/\text{cm}^2)/\text{molarity})$			Surface potential (−) (mV)	Specific capacity, C/A (μF/cm²)
	1 M Na^+	0.1 M K^+	0.1 M NH_4^+		
Glyceryl monooleate +*n*-hexadecane	(3900)	(43 000)	(210 000)	319–321^{a,b}	0.58 ± 0.004 (ref. 23)
Glyceryl monooleate +*n*-decane		10 000			0.38 ± 0.02
Bacterial L-α-phosphatidylethanolamine	1	40	170	480–520^{b}	0.42 ± 0.02
Egg L-α-phosphatidylethanolamine	5	115	240		0.41 ± 0.02
Egg lecithin	(7)	90	(270)	440–480^{a,b}	0.39 ± 0.02
Dioleoyl lecithin	5	225			
	(3–6)	(250)	(350)		0.40 ± 0.02

then the shape of the current–voltage relation for Na^+ is consistent with $k_{is}/k_{Di} \ll 1$, $b = 0.005$[*]. The potential energy barrier corresponding to $b = 0.005$ has a rounded top following the quadratic equation predicted on the basis of the image force, while the value of $b = 0.005$ is in good agreement with the approximate theoretical value of 0.0035. A high trapezoidal barrier with a flat top of width $\alpha = 0.35\,d$ would produce the same shape for the current–voltage relation (see Appendix).

For KCl and NH_4Cl the maximum observable upward curvatures are considerably less marked than for NaCl. The probable explanation is that the rate of dissociation of the complexes decreases for the changes from Na^+ to K^+ and from K^+ to NH_4^+ such that for K^+ and NH_4^+ the rate of crossing the membrane is no longer much slower than the rate of dissociation, i.e. k_{is}/k_{Di} is no longer much less than 1.

Various combinations of values for k_{is}/k_{Di} and η such as those in Table II may be used to fit the data. For comparison the curves for $\eta = 0$ and $\eta = 0.1$ are indicated in Fig. 2. The curve for $\eta = 0$ is, of course, the result of assuming that the rate of association does not depend on potential.

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[*] The current–voltage relation is approximately fitted by a curve with $k_{is}/k_{Di} = 0.01$, $b = 0$, but by comparison of the values of k_{is}/k_{Di} for K^+ and NH_4^+ with nonactin and Na^+, K^+ and NH_4^+ with trinactin (to be reported in a later paper), the value of k_{is}/k_{Di} for nonactin and Na^+ is expected to be smaller than required. The value of b chosen to achieve a fit to the nonactin–Na data (0–0.005) hardly affects any other parameter in the model.

TABLE II

The values of k_{is}/k_{Di} derived from Eqn 4 and the current–voltage curves for nonactin carrying K^+ and NH_4^+ across glycerylmonooleate+*n*-hexadecane membranes. These depend on the portion of the applied potential, η, presumed to act on the association reaction. Values in parentheses correspond to values of η for which no accurate fit is possible. The value of k_{is}/k_{Di} for a particular η is determined within 20 %.

η	k_{is}/k_{Di}	
	K^+	NH_4^+
0	(0.025)	(0.18)
0.05	0.03	0.28
0.075	0.04	0.3
0.1	0.05	0.45
0.15	0.09	(>1)
2	(0.14)	(∞)

It should be noted that the experimental data for K^+ and NH_4^+ require that the product of the rate constant for association and the carrier concentration, on the positive side of the membrane be allowed to vary with the applied potential. In a previous paper [19] it was shown that a change in the carrier concentration would require more than a minute to occur. Thus, since the current in the present experiments reaches its final value within a few milliseconds, the concentration of carrier may be presumed constant and the change in the product must correspond to a change in the rate constant. However, the data only suggest rather than require that the potential act on the rate in the manner indicated above. In particular the data do not exclude the possibility that the potential induces a change in the membrane which then alters the rate of association.

Bacterial phosphatidylethanolamine

The current–voltage relations for 1 M Na^+, 0.1 M K^+ and 0.1 M NH_4^+ in Fig. 4 are each in satisfactory agreement with the curves for K^+ presented by Hall et al. [25]. It would be possible using Eqns 4–8 with $k_{is}/k_{Di} = 0.07$ and $\eta = 0.15\,d$ to fit the shape of their experimental curve for K^+ though in agreement with Hall et al. [25] no value of k_{is}/k_{Di} is satisfactory for $\eta = 0$. Using $\eta = 0.15\,d$ and $k_{is}/k_{Di} = 0.07$, rectification ratios predicted from Eqn 10 would be in satisfactory agreement with the experimental values. However, if this value of k_{is}/k_{Di} were correct for K^+, different values and thus different curvatures for the current–voltage relations should be seen for different ions just as shown above using glycerylmonooleate membranes. As the experimental curves are essentially the same, it is concluded that $k_{is}/k_{Di} \ll 1$, and therefore that the shape of the current–voltage relation and the rectification both reflect a different potential energy profile and/or a different variation in the membrane properties with applied voltage than that found above for glycerylmonooleate. The current–voltage relations predicted by high-trapezoidal barriers are derived in the Appendix. The extent to which the data determine any specific barrier shape has been discussed at length by Hall et al. [25]. These data provide no evidence about the dependence of the association rate on potential since for these membranes this step is not rate limiting for accessible concentrations and potentials.

As noted earlier the conductances of phosphatidylethanolamine membranes are much lower than those across glycerylmonooleate membranes and it is now seen that the same is true for the ratios, k_{is}/k_{Di}. These results are consistent with either a decrease in the rate of crossing the membrane or an increase in the rate of dissociation of complexes as the surface potential becomes more positive. An unambiguous determination of the relative importance of these effects will require measurement of the concentration of complexes in the membrane.

Unsaturated phospholipids

The shapes of the current–voltage relations measured using dioleoyllecithin, egg lecithin, and egg phosphatidylethanolamine with Na^+, K^+ and $NH_4{}^+$ are all approximately the same as those reported for bacterial phosphatidylethanolamine. Thus in these instances it would appear that $k_{is}/k_{Di} \ll 1$ and that nonactin encounters a potential energy barrier similar to that given by Hall et al. [25]. It is premature to conclude that all phospholipid membranes present the same energy profile to the movement of nonactin, or that the membranes used here present the same profile to other carriers such as valinomycin. Nevertheless a similar shape of the current–voltage relations at low K^+ activity has been observed with monactin and valinomycin on dioleoyllecithin+n-decane membranes [17], and with valinomycin and membranes made from a variety of lecithins+n-decane [26]. If these curves can be correctly interpreted in the manner proposed by Hall et al., it would follow that, in these instances as well, $k_{is}/k_{Di} \ll 1$ in contrast to the result $k_{is}/k_{Di} \approx 0.25$ which was originally reported. Thus the available evidence would favour an interpretation of carrier transport across these membranes in which the rate-limiting step is transfer of the complex across the membrane interior. By contrast Stark et al. [17, 27] have presented good evidence that the rate of association is limiting for valinomycin and membranes formed from decane and one of the negatively charged phospholipids, phosphatidylinositol or phosphatidylserine. While the potential dependence of $k'_{Ri}k'_{is}/k'_{Di}$ and $k''_{Ri}\, k_{is}''/k''_{Di}$ for valinomycin and these membranes has still not been determined, the data from relaxation experiments [27] suffices to establish a lower limit for k_{is}/k_{Di}.

Ciani et al. [14, 28] have reported a different procedure for determining k_{is}/k_{Di} for trinactin and membranes formed from a mixture of glyceryldioleate and glycerylmonooleate in n-decane. In their method one species of cation was present at equal concentrations on the two sides of the membrane, a second species was added at various concentrations to one side, and the potential for zero current, V_0, was noted and plotted versus the logarithm of the activity of the second species. The resulting curve was then fitted using the expression [14]

$$V_0 = \frac{RT}{F} \ln \left\{ \frac{a_1'' + (P_2/P_1) a_2''}{a_1'} \right\} \approx \frac{RT}{F} \ln \left\{ 1 + \frac{P_2 C_2''}{P_1 C_1'} \right\} \tag{12}$$

where in the present notation the permeability ratio is given by

$$\frac{P_2}{P_1} = \frac{(k_{R2}\, k_{2s}/k_{D2})\{1 + (2k_{1s}/k_{D1}) \cosh (F\Delta V/2RT)\}}{(k_{R1}\, k_{1s}/k_{D1})\{1 + (2k_{2s}/k_{D2}) \cosh (F\Delta V/2RT)\}} \tag{13}$$

Ciani et al. have assumed that any parameters analogous to η and b in Eqn 10 are zero.

Using their procedure, Ciani et al. have reported $k_{is}/k_{Di} = 0.02$ for Na^+, 0.25 for K^+ and 0.3 for $NH_4{}^+$. Current–voltage relations similar to those for nonactin reported here have been given elsewhere [15] for trinactin and membranes made from glycerolmonooleate+n-hexadecane. From that data (or from the relaxation studies referred to there) it can be inferred that k_{is}/k_{Di} is about 0.5 for K^+ and 1.5 for $NH_4{}^+$ (and that $\eta \approx 0.05$). The different absolute values found in these two sets of experiments could merely reflect the different compositions of the membranes, but the difference in the values for $NH_4{}^+$ relative to K^+ is unexpected. As Ciani et al. neither discuss their experimental data nor describe the procedure for calculating the two adjustable constants for each ion, further comparison is difficult.

There is extensive data available at higher electrolyte activities which show clearly that in specific instances the current is not limited by the flux of complexes [4, 14, 15, 17, 21, 28]. These data and their interpretation should not be confused with the subject of this paper. As will be discussed at length in a paper to follow, the carrier model predicts that at high electrolyte activity, the current is limited by the rate at which the carrier can be returned from the negative to the positive side of the membrane [15].

APPENDIX: TRAPEZOIDAL ENERGY BARRIER

A potential dependence for the rate constant for transfer of the ion–carrier complex across the interior of a membrane may be calculated from the assumption that the movement occurs as diffusion in the presence of a particular potential energy profile. Examples of such calculations have been presented by Neumcke and Läuger [29], Haydon and Hladky [4] and Hall et al. [25]. The calculation given here for a trapezoidal barrier follows the simpler example given by Haydon and Hladky.

Ion–carrier complexes are formed and removed at positions $-\gamma$ and $+\gamma$ on either side of a symmetrical membrane of thickness d (Fig. 5). The potential energy, U, of a complex, as it crosses from one side to the other is given by

$$\frac{U}{RT} = \varphi = \left(\frac{2x}{d}\right)\left(\frac{\Delta\varphi}{2}\right) + \begin{cases} \varphi_0(1+2x/d)/(1-\alpha) & -\gamma < x < -\alpha d/2 \\ \varphi_0 & -\alpha d/2 < x < \alpha d/2 \\ \varphi_0(1-2x/d)/(1-\alpha) & \alpha d/2 < x < \gamma \end{cases} \tag{A-1}$$

where $\Delta\varphi = zF\Delta V/RT$ and ΔV is the applied potential, where $\varphi_0 = U_0/RT$ and U_0 is the height of the trapezoidal barrier and where αd is the width of the flat top of the barrier.

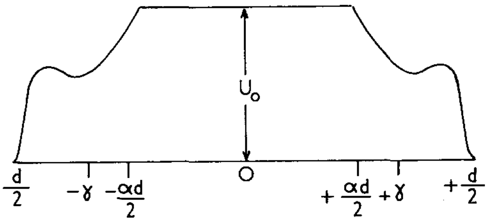

Fig. 5. The trapezoidal barrier to carrier transport. The barrier has a flat top of width αd and a height U_0. Ion–carrier complexes are adsorbed at $\pm\gamma$. The applied potential falls as a constant gradient across the entire core of the membrane which extends from $-d/2$ to $d/2$.

The Nernst–Planck equation after integration from $-\gamma$ to $+\gamma$ relates the flux to the concentrations of complexes on the two sides, $c(-\gamma)$ and $c(+\gamma)$, and to the potential energy profile,

$$J = -D\frac{c(\gamma)e^{\gamma\Delta\varphi/d} - c(-\gamma)e^{-\gamma\Delta\varphi/d}}{\displaystyle\int_{-\gamma}^{\gamma} e^{\varphi}dx} \tag{A-2}$$

The only calculation required is the evaluation of the integral, designated by Γ. After breaking the range of integration into three pieces, divided at $\pm\alpha d/2$, the integrals are standard with the result

$$\Gamma = (de^{\varphi_0}/\Delta\varphi)\left\{\frac{e^{\alpha\Delta\varphi/2}}{1-\Delta\varphi(1-\alpha)/2\varphi_0} - \frac{e^{-\alpha\Delta\varphi/2}}{1+\Delta\varphi(1-\alpha)/2\varphi_0}\right\} + \frac{e^{\gamma\Delta\varphi/d}}{\Delta\varphi/2-\varphi_0/(1-\alpha)} - \frac{e^{-\gamma\Delta\varphi/d}}{\Delta\varphi/2+\varphi_0(1-\alpha)}. \tag{A-3}$$

If the trapezoidal barrier is high, which is now assumed, then the term with e^{φ_0} dominates and

$$\Gamma = (2de^{\varphi_0}/\Delta\varphi)\sinh(\alpha\Delta\varphi/2). \tag{A-4}$$

With this value for Γ,

$$J = -\frac{D\Delta\varphi e^{-\varphi_0}}{2d}\,\frac{c(\gamma)e^{\gamma\Delta\varphi/d} - c(-\gamma)e^{-\gamma\Delta\varphi/d}}{\sinh(\alpha\Delta\varphi/2)} \tag{A-5}$$

This derivation has assumed that the movement of one complex is independent of the existence or movements of any other complexes (specifically that the applied potential creates a constant field). Under the same assumptions the flux from left to right is therefore given by Eqn A-5 with $c(\gamma)$ set equal to zero. Thus if k_{is} is defined so as to absorb the constants, including that for the change in notation from $c(-\gamma)$ to N'_{is}, and it is noted that $\gamma = d/2-\xi d$, then

$$k'_{is} = k_{is}(\alpha\Delta\varphi/2)\exp\{-(0.5-\xi)\Delta\varphi\}/\sinh(\alpha\Delta\varphi/2) \tag{A-6}$$

and similarly

$$k''_{is} = k_{is}(\alpha\Delta\varphi/2)\exp\{(0.5-\xi)\Delta\varphi\}/\sinh(\alpha\Delta\varphi/2) \tag{A-7}$$

There are two interesting special cases. Firstly for low electrolyte activities and equilibrium of the association–dissociation reactions, N'_{is} and N''_{is} may be expressed simply in terms of the concentration of the carrier and the activities of the electrolyte, with the result

$$I = N_s\frac{k_{Ri}k_{is}}{k_{Di}}\left(\frac{\alpha\Delta\varphi}{2}\right)\left(a'e^{-\Delta\varphi/2} - a''e^{\Delta\varphi/2}\right)\Big/\sinh\left(\frac{\alpha\Delta\varphi}{2}\right) \tag{A-8}$$

This equation should replace Eqns 10–12 from Hall et al. [25] which are incorrect.

Secondly, if $\alpha\Delta\varphi/2$ and $b\Delta\varphi^2$ are sufficiently small, then the exponential and $x/\sinh(x)$ may each be expanded in a power series,

$$e^{-b\Delta\varphi^2} = 1 - b\Delta\varphi^2 + \tfrac{1}{2}b^2\Delta\varphi^4 - \ldots \tag{A-9}$$

and

$$\left(\frac{\alpha\Delta\varphi}{a}\right) \Big/ \sinh\left(\frac{\alpha\Delta\varphi}{a}\right) = 1 - \frac{\alpha^2}{24}\Delta\varphi^2 + \frac{7\alpha^4}{5760}\Delta\varphi^4 - \ldots \tag{A-10}$$

Thus if $b = \alpha^2/24$, the two sets of expressions for k'_{is} and k''_{is} (A-6 and A-7 and 7 and 8) are indistinguishable for $\Delta\varphi$ sufficiently small. The discrepancy between them is less than 10% for $\alpha\Delta\varphi < 4$. Thus for $\alpha = 0.35$, which corresponds to $b = 0.005$ and to the nonactin–Na fluxes across glycerylmonooleate+*n*-hexadecane membranes, the predictions are essentially the same for all potentials up to 300 mV. The expressions for k'_{is} and k''_{is} in terms of b have been used in the main text to emphasize the agreement between the theoretical value of b on the basis of the image force and the value measured experimentally. For the phosphatidylethanolamine membranes, the image force is not an adequate explanation of the barrier shape. In addition for $\alpha = 0.6$ which predicts the measured current–voltage relation, it is possible to distinguish between the two shapes, parabolic and trapezoidal, by extending the current–voltage relation to potentials greater than approx. 175 mV.

REFERENCES

1 Einstein, A. (1907) Z. Elektrochem. 13, 41–42
2 Zwolinski, B. J., Eyring, H. and Reese, C. E. (1949) J. Phys. Chem. 53, 1426–1453
3 Ketterer, B., Neumcke, B. and Läuger, P. (1971) J. Membrane Biol. 5, 225–245
4 Haydon, D. A. and Hladky, S. B. (1972) Q. Rev. Biophys. 5, 187–282.
5 Eisenman, G., Szabo, G., Ciani, S., McLaughlin, S. and Krasne, S. (1973) in Progress in Surface and Membrane Science (Danielli, J. F., Rosenberg, M. D. and Cadenhead, D. A., eds), Vol. 6, pp. 139–241, Academic Press, New York
6 Markin, V. S., Krishtalik, L. I., Liberman, Ye. A. and Topaly, V. P. (1969) Biophysics 14, 272–282
7 Markin, V. S., Pastushenko, V. F., Krishtalik, L. I., Liberman, Ye. A. and Topaly, V. P. (1969) Biophysics 14, 487–500
8 Läuger, P. and Stark, G. (1970) Biochim. Biophys. Acta 211, 458–466
9 Markin, V. S. and Liberman, Ye. A. (1971) Dokl. Akad. Nauk. S.S.S.R. 201, 975–978
10 Markin, V. S. (1972) Dokl. Akad. Nauk. S.S.S.R. 202, 703–706
11 Hladky, S. B. (1972) J. Membrane Biol. 10, 67–91
12 Stark, G. (1973) Biochim. Biophys. Acta 298, 323–332
13 Ciani, S., Laprade, R., Eisenman, G. and Szabo, G. (1973) J. Membrane Biol. 11, 255–292
14 Ciani, S. M., Eisenman, G., Laprade, R. and Szabo, G. (1973) in Membranes – A Series of Advances (Eisenman, G. ed.), Vol. 2, pp. 61–177, Marcel Dekker, New York
15 Hladky, S. B., Gordon, L. G. M. and Haydon, D. A. (1974) Ann. Rev. Phys. Chem., in press
16 Everitt, C. T. and Haydon, D. A. (1968) J. Theor. Biol. 18, 371–379
17 Stark, G. and Benz, R. (1971) J. Membrane Biol. 5, 133–154
18 Szabo, G., Eisenman, G. and Ciani, S. (1969) J. Membrane Biol. 1, 346–382
19 Hladky, S. B. (1973) Biochim. Biophys. Acta 307, 261–269
20 Hladky, S. B. and Haydon, D. A. (1973) Biochim. Biophys. Acta 318, 464–468
21 Hladky, S. B. (1974) in Drugs and Transport Processes (Callingham, B. A. ed.), Macmillan, London, in press
22 Andrews, D. M., Manev, E. D. and Haydon, D. A. (1970) Spec. Discuss. Faraday Soc. 1, 46–56
23 Fettiplace, R., Andrews, D. M. and Haydon, D. A. (1971) J. Membrane Biol. 5, 277–296
24 Szabo, G. and Eisenman, G. (1973) Biophys. Soc. Abstr. 175a
25 Hall, J. E., Mead, C. A. and Szabo, G. (1973) J. Membrane Biol. 11, 75–97

26 Stark, G., Benz, R., Pohl, G. W. and Janko, K. (1972) Biochim. Biophys. Acta 266, 603–672
27 Stark, G., Ketterer, B., Benz, R. and Läuger, P. (1971) Biophys. J. 11, 981–994
28 Laprade, R., Ciani, S. M., Szabo, G. and Eisenman, G. (1972) Biophys. Soc. Abstr. 44a
29 Neumcke, B. and Läuger, P. (1969) Biophys. J. 9, 1160–1170