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Transport of Oppositely Charged Lipophilic Probe Ions in Lipid Bilayer Membranes having Various Structures

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Summary. A comparative study of the charge transport kinetics of oppositely charged lipophilic probe ions in lipid bilayer membranes of varying composition was carried out by using the charge pulse technique. The ions investigated were the chemical analogs tetraphenylborate, tetraphenylarsonium and tetraphenylphosphonium. Membrane structural aspects investigated were the type of solvent used in membrane formation, sterol content, and the nature of the principal lipid. The overall results indicate that the character of the transport process involving positive lipophilic probes is, in contrast to positively charged carrier complexes, very similar to that deduced in previous studies of negative lipophilic ions. The major effect on transport of lipophilic ions of both signs using different *n*-alkane solvents appears to be due to changes in the thickness of the membrane hydrocarbon region. Positive ion transport is relatively sensitive to the inclusion of sterols of several types in both monoolein and lecithin membranes, as compared with negative ion transport, suggesting that a combination of sterol-induced dipolar field and fluidity changes are involved. Results involving several variations in lipid structure, with the possible exception of hydrocarbon tail saturation, when interpreted in terms of dipolar field changes deduced under the assumption of charge independent fluidity effects, are consistent with monolayer surface potential measurements.

In recent years the influence of various structural features of lipid bilayers on transmembrane charge transport has been investigated with several lipophilic ions and charged carrier complexes [4, 5, 7, 10–12, 18, 29, 30]. These studies have yielded a great deal of information potentially significant to the interpretation of related types of research involving biological systems [28]. Nonetheless, a number of questions regarding the behavior of charged probes in membranes remain not fully answered. For instance, the radically different influence of membrane thickness on the kinetics of carrier ion complexes [4, 5, 7] and of lipophilic ions [4, 7, 10, 11, 18] is not easily understood. The same can be said for the

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different mode of action in membranes made from phosphatidylcholine/cholesterol mixtures of the two types of transport systems. It is clear that in explaining these results important influences not having to do with the sign of the electric charge must be called into account. Investigations which have utilized analogous probes differing principally in the sign of their respective charges [20, 21, 29, 30], on the other hand, have largely avoided the difficulties inherent in comparing results on lipophilic ions and charged carrier systems. However, until now such experiments have not included systematic investigations of the influence of membrane structure on ion transport.

The principal lipophilic probe substances whose charge transfer characteristics we have examined in this study are the negatively charged ion tetraphenylborate ($T\phi B^-$) and the positively charged ion tetraphenylarsonium ($T\phi A^+$). Some measurements were also made with tetraphenylphosphonium ($T\phi Ph^+$), which is very similar to $T\phi A^+$ in its attributes. The variations in membrane composition investigated can be classified as follows: (i) changes in the type of solvent used in the membrane forming solutions; (ii) alterations in the sterol content; and (iii) differences in the molecular structure of the principal lipids forming the membranes. We have in all cases been able to compare, at the very least, experimental values of the product $k_i\beta$ in the positive and negative charge transport systems, where k_i is the rate constant for translocation of the ion across the central energy barrier in the membrane and β is the partition coefficient for adsorption of the ion at the membrane surface. In addition, we have made comparisons of our values of the kinetic parameters with those previously obtained by using other charge transfer systems. For most of the experimental work we have used the charge-pulse technique [11], which has been shown to be efficient and reliable in studying the pertinent kinetics with a minimum of electrical perturbation.

Materials and Methods

Materials and Experimental Technique

Most of the bilayer membranes used in this study contained solvent and were formed from a solution of lipid in various *n*-alkanes (1–3 %) across a circular hole (either 1 or 2 mm diameter) in a Teflon septum, separating aqueous solutions of identical composition. Solvent-free membranes were obtained as described earlier [5]. In all experiments the aqueous phase consisted of unbuffered 0.1 M NaCl into which the conductivity inducing ions had been introduced. The temperature was held constant at 25 °C.

For membrane formation, different lipids were used. The monoglycerides had the following fatty-acid residues: oleoyl (Sigma, St. Louis, Mo.), linoleoyl and linolenoyl (Nu Check Prep., Elysian, Mich.). Dioleoyl phosphatidylcholine (1,2 dioleoyl-*sn*-glycerol-3-phosphorylcholine) and a phosphatidylcholine with mixed chains (L-1-oleoyl-2-stearoyl-3-phosphatidylcholine) were synthesized by K. Janko [8, 18]. An ether phosphatidylcholine (DL-1-O-oleyl-2-O-palmityl-3-phosphatidylcholine) was obtained from Calbiochem, San Diego, Calif. DL-dioleoylphosphatidylethanolamine and its ether analog were synthesized as described previously [8]. Sterols used consisted of the following: cholesterol (cholest-5-en-3β-ol, Eastman reagent grade), dihydrocholesterol (5α-cholestan-3-ol, Sigma), stigmasterol (cholest-5, 22-dien-27-ethyl-3β-al, Sigma), ergosterol (cholest-5,7,22-trien-24-methyl-3β-ol, Sigma) and epicholesterol (cholest-5-en-3α-ol, Merck analytical grade). The positively charged lipophilic ions (analytical grade) were obtained as chlorides from Fluka, Buchs, Switzerland, whereas the sodium salt of tetraphenylborate (analytical grade) was purchased from Merck, Darmstadt, Germany.

The charge-pulse method used throughout these studies has been discussed in some detail previously [11]. The membrane capacticance is charged to a voltage of approximately 10 mV by a short-lived current pulse (tens of nsec) through silver/silver-chloride electrodes. The membrane is then effectively isolated from the external circuit by a high resistance FET switch, so that the ensuing decay of the initial voltage results from charge transport processes across the membrane itself. The time course of the voltage is recorded as a display on a storage oscilloscope (Tektronix 5115/5A22), from which the appropriate time constants and relaxation amplitudes may be determined. Some supplementary measurements were also made in the course of these studies using the voltage jump (voltage clamp) technique, in which a fast rising voltage step is applied to the membrane through an external circuit and the time dependence of the subsequent circuit current is observed [19]. In principle, the same information on charge transfer kinetics is accessible in both the voltage-jump and charge-pulse methods. However, the latter proves to be a particularly simple technique in which extremely fast relaxations ($\tau \geqq 1$ μsec) may be observed and in which the applied voltage can be held to very small values. Consequently, our use of the voltage-jump method has been restricted to voltage-dependence investigations largely of a diagnostic nature, as explained below.

Theoretical Considerations

Previous relaxation studies [3, 5, 11, 13, 19] have provided ample evidence that the transport of lipophilic ions such as tetraphenylborate and dipicrylamine under the influence of a transmembrane potential proceeds in three distinct steps. These are (i) adsorption of the ion from the aqueous solution into the membrane surface region, (ii) translocation from one surface to the other across an energy barrier which is largely electrostatic in nature, and (iii) desorption into the aqueous phase on the opposite side of the membrane. This scheme has generally been assumed to also govern the transport of positive lipophilic ions [3, 20, 30], a contention which is strongly supported by the findings of this paper.

In general, in charge-pulse experiments involving the transport of lipophilic ions, the decay of the membrane voltage is governed by two exponentials, as follows:

$$V_{m(t)} = V_{m^0}(a_1 e^{-t/\tau_1} + a_2 e^{-t/\tau_2})$$

where τ_1 and τ_2 are the relaxation times of a faster process and a slower process, respectively, and a_1 and a_2 are the corresponding relaxation amplitudes. The complete set of equations relating these quantities to the kinetic parameters of the charge transport

process is as follows [11]:

$$\frac{1}{\tau_1} \equiv \lambda_1 = p + \sqrt{p^2 - 2k_{ma}k_i bN_t} \tag{1}$$

$$\frac{1}{\tau_2} \equiv \lambda_2 = p - \sqrt{p^2 - 2k_{ma}k_i bN_t} \tag{2}$$

$$a_1 = \frac{\lambda_1 - (k_{ma} + 2k_i)}{\lambda_1 - \lambda_2} \tag{3}$$

$$a_2 = 1 - a_1 \tag{4}$$

$$p = \frac{k_{ma}}{2}[1 + (1-\alpha)^2 bN_t] + k_i(1 + \alpha^2 bN_t) \tag{5}$$

$$b = \frac{z^2F^2}{4RTC_m}. \tag{6}$$

In these equations k_i is the rate constant for translocation of ions across the central barrier, k_{ma} is the rate constant for desorption to the aqueous phase, C_m is the specific capacitance of the membrane, and N_t is the total equilibrium concentration of the permeable ion in the membrane. N_t is related to the partition coefficient β, describing the equilibrium distribution of ions between membrane and water and to the rates of adsorption and desorption (rate constants k_{am} and k_{ma}) by

$$\frac{N_t}{2c} = \frac{k_{am}}{k_{ma}} = \beta. \tag{7}$$

The parameter α appearing in Eq. (5), which is the fraction of the total applied voltage that drops across the central barrier, can often be determined from voltage jump experiments, as discussed below. In all the experiments reported in this paper, it is sufficiently accurate to make the approximation $\alpha = 1$.

Positive probes. In practically all the charge pulse experiments we have performed involving the transport of positively charged lipophilic ions, only one relaxation was observed. This relaxation corresponds to τ_2 and is controlled by processes involving the translocation of charges across the membrane interior, interface processes being relatively rapid. Provided that aqueous diffusion at the membrane surface is fast, this situation can be described by the inequality $k_{ma} \gg k_i$, a condition which can be verified by supplementary voltage-jump experiments. Voltage-jump experiments also show that, in fact, diffusion polarization plays no significant role in the conduction processes with the positive ions.

In voltage-jump experiments, upon the application of the voltage step and following the very short transient pulse of current due to charging of the membrane capacitance, the early current due to translocation of adsorbed charge across the membrane interior can be observed. This "initial" current J_0 then decays exponentially to the final steady-state value J_∞. The final and initial currents are related in the limit of small applied voltage, by [19]

$$\frac{J_\infty}{J_0} = \frac{k_{ma}}{2k_i + k_{ma}}. \tag{8}$$

In almost all cases we have studied using $T\phi A^+$ or $T\phi Ph^+$, the initial current appears to be equal to the final current, i.e., upon application of the voltage step, the current (apart from the very short capacitive spike) is seen to immediately attain its final value. This absence of an

observed relaxation indicates one of two possible situations: either (i) the fast relaxation has a time constant too short to be resolved by the equipment, or (ii) $J_0 = J_\infty$, implying (from Eq. (8)) $k_{ma} \gg k_i$ (and also that diffusion polarization is not rate limiting). In either case the corresponding charge-pulse experiment may show only one relaxation, either because the fast process is too rapid to be resolved even by the charge pulse apparatus, or because (from the meaning of $k_{ma} \gg k_i$) solution ions can be supplied to and removed from the membrane as fast as they are translocated across the central energy barrier. That the latter situation leads to a single relaxation in the charge-pulse experiment can be seen analytically by evaluating Eqs. (1)–(7) in the limit $k_{ma} \gg k_i$. With $\alpha = 1$, the result is

$$\tau_2 = \frac{RT}{z^2 F^2} \frac{C_m}{c k_i \beta} \tag{9}$$

and

$$a_1 = 0, \quad a_2 = 1. \tag{10}$$

τ_2 may, under these conditions, be identified with the steady-state time constant of the membrane

$$\tau_m \equiv C_m / \lambda_m, \tag{11}$$

where λ_m is the steady-state specific conductance. (Compare Eq. (9) with the expression obtained by substituting into Eq. (11) the value of steady-state conductance given by Eq. (17) of Ketterer *et al.* [19] evaluated in the limit $k_{ma} \gg k_i$). It is clear that βk_i for the positive lipophilic ions can just as well be obtained from steady-state conductance measurements as from relaxation experiments. However, we have chosen the latter method in our studies, as it necessitates no separate measurement of membrane area (providing C_m is known) and yields conductance information at extremely low values of applied voltage.

In order to verify that the single relaxation observed in most of the charge-pulse experiments with positive ions corresponds to Eqs. (9) and (10) (as distinguished from the situation in which the fast relaxation is not time resolvable), additional voltage-jump experiments are used. Specifically, the behavior of the steady state conductance λ_m as a function of the reduced voltage $u = FV_m/RT$ is compared with the equation

$$\frac{\lambda_m(u)}{\lambda_{m0}} = \frac{2(1+A)\sinh(u/2)}{u\left(1 + f(u)\, A \cosh\left(\frac{\alpha u + u}{4}\right)\right)} f(u) \tag{12}$$

in which $A = 2k_i/k_{ma}$, $f(u)$ is a factor of the order unity which accounts for the voltage dependence of k_i arising from the shape of the central barrier [3, 11, 17, 24], and λ_{m0} is the steady-state conductance in the limit of zero applied voltage. Eq. (12) is obtained following a formalism similar to that employed in deriving Eq. (12) in Ref. 19. However, in the present case appropriate expressions for effective voltage ($\alpha u/2$ or $(1-\alpha)u/4$) have been used in the voltage-dependence factors for the translocation rate constants and for the interfacial rate parameters. Also, in the derivation, the modified rate constant $f(u)\, k_i$ has been substituted for k_i. For small values of A, Eq. (12) predicts that the behavior of the conductance ratio $\lambda_m(u)/\lambda_{m0}$ plotted *vs.* u is strongly superlinear, whereas at moderate values of A the curve flattens out. We have, in fact, found that for the positive lipophilic ions a superlinear behavior is observed, indicating that $k_{ma} \gg k_i$. Typical experimental results illustrating the voltage dependence of membrane conductance in the presence of $T\phi A^+$ are shown in Fig. 1.

In Fig. 1 several curves based on Eq. (12) are given. In the calculations we have assumed $\alpha = 1$. The barrier-shape function $f(u)$, which represents a small correction at moderate voltages [3, 17, 24], has been approximated by $f(u) = \exp(-\omega u^2)$ where ω is a function of

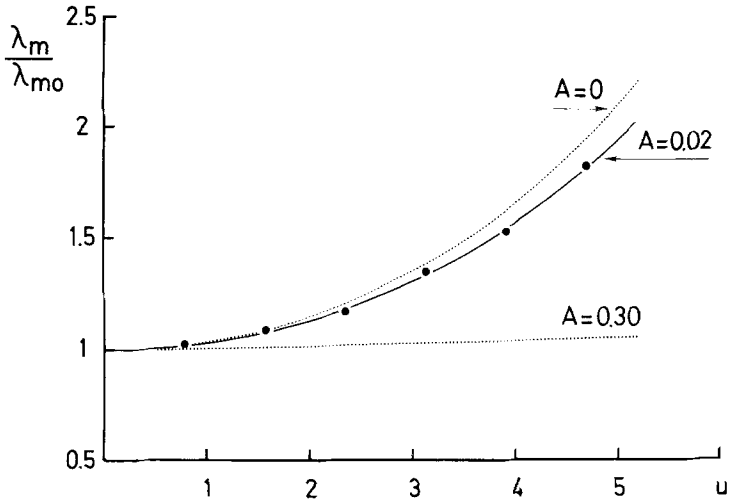

Fig. 1. Voltage dependence of membrane conductance in the presence of 10^{-4} M tetraphenylarsonium for membranes made from glycerolmonooleate dissolved in *n*-hexadecane. λ_m/λ_{m0} is the ratio of steady-state membrane conductance at reduced voltage $u=FV_m/RT$ to that obtained in the limit of zero applied voltage V_m. The smooth curves are plotted according to Eq. (12) for several values of the parameter $A=2k_i/k_{ma}$, assuming $\alpha=1$. The function $f(u)$ appearing in Eq. (12) has been approximated by $\exp(-\omega u^2)$, where $\omega=0.0055$ corresponds to a membrane thickness of 3.2 nm [3, 24]

membrane thickness d(*see* table of ω values in ref. *3*). With these assumptions, the voltage dependence predicted by Eq. (12) becomes sublinear for values of A greater than about 0.35. The value of A, for which the theoretical curve fits our data (indicating k_{ma} to be about 2 orders of magnitude larger than k_i), should be regarded only as an approximation owing to uncertainties associated with the appropriate values of $f(u)$. For instance, our experimental points could as well have been fitted by a curve for which $A=0$ and $\omega=0.008$ (rather than $\omega=0.0055$, as in Fig. 1). The main conclusion to be drawn from the voltage-dependence data is, however, unchanged; any reasonable $f(u)$ requires that A be small in order to obtain a superlinear behavior.

Negative probes. Charge-pulse experiments involving the transport of negative lipophilic ions have been discussed in detail in previous publications [4, 7, 10, 11, 18]. In our own experiments with TϕB$^-$, two relaxations with widely separated time constants are observed in all cases. The faster process corresponds to the translocation of ions across the central barrier, whereas the slow relaxation can be identified with one of several possibilities: either the surface process involving exchange of lipophilic ions between the aqueous phase and adsorption sites is very slow ($k_i \gg k_{ma}$) or diffusion in the aqueous phase near the membrane is rate limiting. A third possibility, which is only important at very low concentrations, is that the slow process reflects the "background" conductance arising from the small permeability of the membrane to metal cations from the aqueous phase. Previous studies [11] have indicated that in the case of TϕB$^-$ diffusion polarization does in fact enter into the slow process so that only the first relaxation can be analyzed in terms of the model of lipophilic ion transport described above. However, as this first process is generally much faster than all subsequent decay processes, we may derive information on the kinetics from the pertinent experimental parameters (τ_1 and a_1) using expressions which are given by evaluating Eqs.

(1)–(5) in the limit $k_i \gg k_{ma}$. Assuming $\alpha = 1$, values of k_i, N_t and β may be found from the following equations:

$$\tau_1 = \frac{1}{2k_i(1+bN_t)} \tag{13}$$

$$a_1 = \frac{bN_t}{1+bN_t} \tag{14}$$

as well as Eqs. (6) and (7).

Determinations of α. The parameter α which we have approximated by the value 1 throughout this paper has been estimated for negative ions on the basis of the voltage-dependent behavior of charge translocation in voltage-jump experiments [2, 3, 11]. For example, the initial conductance as a function of voltage may be compared with the following expression [11]:

$$\left(\frac{\lambda}{\lambda_0}\right)_{t=0} = \frac{\sinh(z\alpha u/2)}{z\alpha u/2} f(u) \tag{15}$$

where λ/λ_0 is the ratio of the conductance at reduced voltage u to that obtained in the limit $u = 0$. The voltage dependence of the relaxation time has also been used to determine α. Values of α which have been reported for 10^{-7} M TϕB$^-$ are as follows: 0.92 ± 0.04 in phosphatidylcholine membranes [11], 0.97 ± 0.01 in phosphatidylethanolamine membranes [2], and 0.99 in monoolein membranes [2]. Unfortunately, the procedures used to establish values of α for negative lipophilic ions cannot be applied in the case of the positive analog ions. Eq. (12), which gives the voltage dependence of membrane conductance in the steady state (equal to the initial conductance, in this case), is insensitive to α when A is small. However, it is unreasonable to expect that α for positive ions is smaller than α for negative ions of the same size. Rather, because the dipolar fields at the surface of the membrane would tend to force the planes of adsorption of positive ions closer to the interface, a larger fraction of the applied voltage might be expected to affect the translocation over the central barrier than in the case of negative ions. The magnitude of this effect is unimportant; α is assumed to have the value unity throughout this paper.

Results and Discussion

Influence of the Solvent-Thickness Effects

The effects on charge transfer kinetics in membranes made from lipids dissolved in various *n*-alkane solvents were examined. A number of previous studies [7, 8, 10, 15] have indicated that the major influence of a change of solvent is due to variations in the thickness of the hydrocarbon region of the membrane which arise, presumably, from differences in the fraction of solvent included. In the present experiments the solvents ranged from *n*-octane to *n*-hexadecane. "Solvent free" monoolein membranes made by the Montal-Mueller method [5, 6, 23] were also tested using the TϕB$^-$ probe. As has been discussed previously [5] in the case of solvent-free membranes, the concentration of permeant ions in the aqueous phase is

reduced by an undetermined amount due to an excess of lipids required in forming the membranes, so that values of β obtained represent only a lower limit. Thus, as only the product $k_i\beta$ could be determined in most measurements on positive lipophilic ions, there was little reason to study charge transport of $T\phi A^+$ or $T\phi Ph^+$ through solvent-free membranes.

The results of the charge-pulse measurements are summarized in Table 1. Also listed are the appropriate values of the specific capacitance C_m (taken from the literature [6, 8]) as well as ratios of the $k_i\beta$ values found for the negative ions $(k_i\beta)^-$ to the corresponding values found for the positive ions $(k_i\beta)^+$. The standard errors in the individual parameters of $T\phi B^-$ transport are comparable to those given in some detail in other papers [4, 11] reporting such measurements (less than $\pm 15\,\%$ for k_i and less than $\pm 25\,\%$ for β); the standard error for $(k_i\beta)^-$ was less than $\pm 20\,\%$. The standard errors in $(k_i\beta)^+$ obtained with the positive ion, because of problems with short term drift are larger in low conductance membranes than in those of high conductance; they ranged from about $\pm 7\,\%$ in membranes having $\tau_2 \simeq 50$ msec to about $\pm 20\,\%$ in those with $\tau_2 \simeq 5000$ msec.

Table 1. Kinetic parameters of $T\phi B^-$, $T\phi A^+$, and $T\phi Ph^+$ transport through membranes made from glycerol monooleate (GMO) and dioleoyl phospatidylcholine (PC) dissolved in different *n*-alkanes and in solvent-free GMO membranes[a]

Solvent	C_m/ nF · cm^{-2}	$T\phi B^-$				$T\phi A^+$ ($T\phi Ph$)$^+$		
		τ_1/ msec	a_1	k_i/ sec^{-1}	β/ 10^{-3} cm	τ_2/ msec	$k_i\beta$/ 10^{-7} cm · sec^{-1}	$(k_i\beta)^-/(k_i\beta)$
Glycerol monooleate membranes								
n-Octane	394	25	0.30	14	0.90			
n-Decane	390	24	0.28	15	0.80	1120(922)	30(37)	0.40×10^4
n-Dodecane	416	20	0.25	19	0.75	995	36	0.40×10^4
n-Tetradecane	469	14	0.27	27	0.90	667	61	0.40×10^4
n-Hexadecane	585	3.3	0.20	120	0.80	310(322)	168(158)	0.57×10^4
Solvent-free	745	2.2	0.092	210	>0.40			
Dioleoyl phosphatidylcholine membranes								
n-Decane	374	10	0.87	6.5	17	5180	0.063	1.8×10^7
n-Hexadecane	624	1.9	0.84	46	20	836	0.66	1.4×10^7

[a] Data for $T\phi Ph^+$ transport is given in parentheses. Ratios of $k_i\beta$ obtained with negative ions $(k_i\beta)^-$ to $k_i\beta$ obtained with positive ions $(k_i\beta)^+$ in membranes of the same composition are also given. The aqueous phase contained 0.1 M NaCl and the following concentrations of permeant ions: 10^{-7} $T\phi B^-$; 3×10^{-5} M $T\phi A^+$ or $T\phi Ph^+$ (GMO membranes); 10^{-2} M $T\phi A^+$ (PC membranes). $T = 25\,°C$. Data for $T\phi B^-$ in PC/*n*-decane membranes are taken from ref. 11. Values of specific capacitance C_m are taken from refs. 6 and 8.

The results given in Table 1 show that as membrane thickness decreases (increasing C_m) the translocation rate constant k_i for TϕB$^-$ increases, whereas the partition coefficient is essentially unchanged. The consequent increase in $(k_i\beta)^-$ is paralleled by a similar rise in values of $(k_i\beta)^+$ obtained with the positive lipophilic ions. The ratio $(k_i\beta)^-/(k_i\beta)^+$, on the other hand, is not much affected by the choice of solvent.

A calculation of the electrostatic energy of a charged particle within a slab of homogeneous dielectric must take into account the electrical interaction of the particle with adjacent dielectric media. On this basis, the energy (in units of kT) of a univalent ion in a membrane of thickness d can be shown [27] to be different from that of an identical ion in a membrane of thickness d^* by the amount

$$\Delta w(d) = h\left(\frac{1}{d^*} - \frac{1}{d}\right) \tag{16}$$

where h, a parameter depending on the dielectric constants of water (78.5) and hydrocarbon (2.1), has a value of about 17.8 nm [5, 7, 10]. The height of the dielectric barrier over which a lipophilic ion must move in crossing the hydrocarbon interior of the membrane thus changes by $\Delta w(d)$ when different solvents are used in the forming solution, if the only structural influence of the solvent is on the thickness of the hydrocarbon region. The expected dependence of the translocation rate constant k_i is given by

$$k_i/k_i^* = e^{-\Delta w(d)} \tag{17}$$

where k_i^* is the rate constant for a membrane of thickness d^*.

Values obtained from Eq. (17) referred to *n*-decane are given in Table 2. Values of d are computed from specific capacitances C_m (Table 1) by the parallel plate capacitor formula $C_m = \varepsilon_0 \varepsilon_m / d$. (It should be noted that, as the computation of $\Delta w(d)$ depends effectively on the difference between values of C_m of comparable magnitude, the values given in the third column of Table 2 are associated with relatively large errors.) The experimental data, which can be compared with the predictions of Eq. (17), are also tabulated for a series of lipophilic ions and charged carrier complexes. For TϕA$^+$ only the ratio $(k_i\beta)/(k_i\beta)^*$ can be given. One is able to conclude from Table 2 that for the lipophilic ions TϕB$^-$, DPA$^-$ and, most likely, TϕA$^+$ the thickness dependence of the dielectric barrier in the interior of the membrane is the chief effect of varying the solvent. There is no agreement with Eq. (17) for the charged carrier complexes MS in the PV-K$^+$ and valinomycin-Rb$^+$ systems. For TϕA$^+$ we must assume that the partition coefficient β is not influenced by the nature of the solvent in order for agreement with Eq. (17)

Table 2. Ratios of the rate constants (k_i, k_{MS}) for charge translocation in membranes dissolved in various *n*-alkanes and in solvent-free membranes to the rate constants in membranes dissolved in *n*-decane (k_i^*, k_{MS}^*) using various ions and carrier complexes[a]

Solvent	d/nm	$e^{-\Delta w(d)}$	k_i/k_i^*		$k_i\beta/(k_i\beta)^*$	k_{MS}/k_{MS}^*	
			DPA$^-$	TϕB$^-$	TϕA$^+$	PV-K$^+$	val-Rb$^+$
Monoolein membranes							
n-Octane	4.7	1.1	1.0	0.9		1.1	
n-Decane	4.8	1	1	1	1	1	1
n-Dodecane	4.5	1.3	1.3	1.3	1.2	1.1	0.7
n-Tetradecane	4.0	2.1	1.7	1.8	2.0	1.2	0.7
n-Hexadecane	3.2	6.4	6.6	8.0	7.3	1.1	0.9
Solvent-free	2.5	30	>12	>14		>1.2	>1.5
Dioleoyl phosphatidylcholine membranes							
n-Decane	5.0	1	1	1	1		
n-Hexadecane	3.0	10.7	13.8	7.1	10.5		

[a] For TϕA$^+$ ratios of the product $k_i\beta$ are given. Values of the thickness dependence factor $e^{-\Delta w(d)}$ referred to *n*-decane (*see* Eqs. (16) and (17)) are listed, along with values of thickness d computed, as explained in the text, on the basis of specific capacitances listed in Table 1. Ratios for TϕB$^-$ and TϕA$^+$ are based on data given in Table 1, for DPA$^-$ on data in ref. 10, for PV-K$^+$ on data in ref. 7, and for val-Rb$^+$ on data in ref. 5.

to be valid. Strong evidence in favor of this assumption is provided by a study of saturation behavior. In Fig. 2 we plot values of conductance of monoolein membranes formed from solutions containing different *n*-alkane solvents as a function of concentration c of TϕA$^+$ in the aqueous phase. The departure from linear behavior occurs at about the same value of c in each case, indicating that the number of ions N_t adsorbed into surface sites at any concentration does not depend on the solvent. Thus, as in the case of TϕB$^-$ (*see* Table 1), $\beta = N_t/2c$ is very likely insensitive to the nature of the solvent. The same has also been shown to be true for DPA$^-$ [10] and for the PV-K$^+$ [7] systems. This behavior is to be expected if only the thickness of the hydrocarbon region is altered. Haydon [16] has argued, for instance, that the packing of the polar head groups is largely unaffected by the presence of solvent.

That the applicability of Eq. (17) is not simply a matter of the sign of the charge of the translocating species has not until now been entirely clear. With respect to thickness effects, TϕA$^+$ appears to behave exactly like TϕB$^-$ and DPA$^-$. The different behavior of the charged carrier complexes has been discussed [7] in terms of the greater influence of these relatively

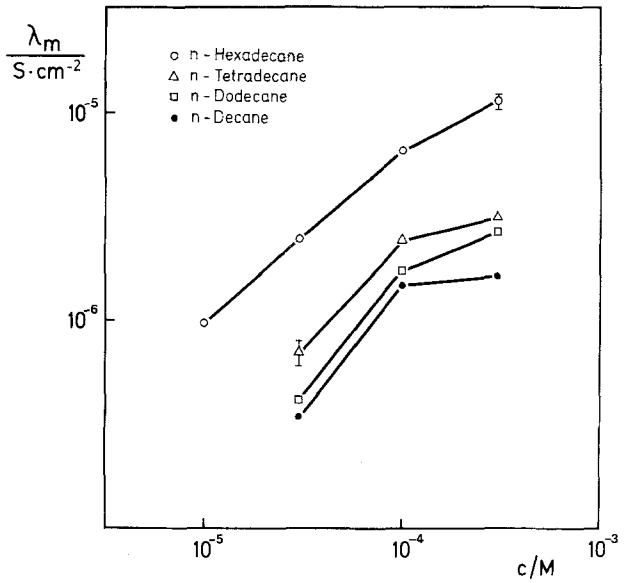

Fig. 2. Concentration dependence of steady-state membrane conductance λ_m in the presence of tetraphenylarsonium ($T\phi A^+$) for membranes made from glycerolmonooleate dissolved in various *n*-alkanes. *c* is the molar concentration of $T\phi A^+$ in the aqueous phase. Several typical error intervals are indicated

large sized entities on the fluidity in the interior of the membrane, as well as in terms of the location of the adsorption plane of charged complexes relative to the dipolar region. According to arguments based on the latter concept, the positive complexes may be adsorbed on the inner side of the dipolar layer at a depth where the potential barrier is not a strong function of position across the membrane. By contrast, all three of the relatively small lipophilic ions can be supposed to be adsorbed closer to the aqueous phase so that in translocating across the membrane they must overcome a potential barrier which is thickness dependent.

In any event we should expect the dipolar field at the surface of the membrane to be little affected by simple changes in the width of the hydrocarbon interior. Thus the product $k_i\beta$ which, as we shall see, is a measure of that field should change in going from the case of a negative lipophilic ion to that of an analogous positive ion by the same amount independent of thickness. This conclusion is supported by the results in the last column of Table 1, where the ratio $(k_i\beta)^-/(k_i\beta)^+$ is shown to have essentially a solvent-independent value in both monoolein and phosphatidylcholine membranes.

Sterol Dependence

Earlier experiments [4, 5, 7] have shown that the transport properties of lipophilic ions and carriers are strongly affected by the presence of cholesterol in monoolein membranes, whereas several other sterols have either smaller or negligible influence. Sterol content was also shown to cause radically different effects in lecithin as compared with monoolein membranes [4, 7, 10]. Table 3 summarizes the experimental findings on the sterol dependence of the transport parameters for the lipophilic ions $T\phi B^-$ and $T\phi A^+$. Essentially the same sterols were investigated as in references 4 and 7. Results for both glycerol monooleate and dioleoyl-phosphatidylcholine membranes are shown. The solvent used in all cases in the forming solution was *n*-hexadecane in order to minimize sterol-induced thinning effects [4]. The highest sterol mole fractions (referred to total lipid) which could be successfully investigated were restricted by the very long time constants manifested in some sterol-rich membranes (using $T\phi A^+$) or by the limited solubility of some sterols (especially ergosterol and stigmasterol). Standard errors are essentially the same as those summarized in the preceding section.

As has been noticed in other studies [4, 5, 7, 29, 30], the presence of cholesterol in monoolein membranes enhances the transport of negative ions across the interior region, whereas it suppresses the transport of positively charged species. These effects are borne out by the results summarized in Table 3. Past experiments [4] had also detected weak sterol-induced effects only on negative ion transport in dihydrocholesterol-monoolein membranes and negligible effects of all tested sterols in lecithin membranes. Our results, which clearly show $T\phi A^+$ to be much more sensitive to the presence of sterol than other probes used, are able to round out this picture. Cholesterol and, to a progressively lesser extent, dihydrocholesterol, ergosterol, and stigmasterol have an influence on interior transport of $T\phi A^+$ in monoolein membranes. We also find moderate (and comparable) effects in lecithin membranes containing cholesterol, dihydrocholesterol, and stigmasterol. As in most of our previous experiments on other probes (the exception being the PV-K^+ complex [7]), the partition coefficients β measured for $T\phi B^-$ are insensitive to the presence of sterol.

The inclusion of sterol molecules in the bilayer can be expected to influence the transport of charged particles across a membrane of constant thickness in two ways: (1) by altering the dipolar field in the interfacial regions, and (2) by affecting the microviscosity in the hydrocarbon interior. Changes in the dipolar field can, in principle, affect both the partition

Table 3. Kinetic parameters of $T\phi B^-$ and $T\phi A^+$ transport through membranes made from glycerol monooleate/sterol mixtures and dioleoylphosphatidylcholine/sterol mixtures dissolved in *n*-hexadecane[a]

Sterol	x	$T\phi B^-$					$T\phi A^+$	
		τ_1/ msec	a_1	k_i/ sec^{-1}	β/ 10^{-3} cm	$k_i\beta$/ 10^{-3} cm·sec^{-1}	τ_2/ msec	$k_i\beta$/ 10^{-7} cm·sec^{-1}
Glycerolmonooleate membranes								
	0	3.0	0.22	130	0.90	117	310	168
Cholesterol	0.17	3.3	0.21	120	0.85	102	455	114
	0.33	1.8	0.19	230	0.75	173	1048	50
	0.40						1887	28
	0.50	0.74	0.20	540	0.80	432	8666	6
Dihydrocholesterol	0.33						796	65
	0.40						1250	42
	0.50						3130	17
	0.67	1.4	0.21	280	0.85	238		
Ergosterol	0.17						528	98
	0.33						780	67
	0.50	2.8	0.23	140	0.95	133	770	68
	0.67						864	60
Stigmasterol	0.17						506	103
	0.33						393	132
	0.50						544	96
	0.80	2.5	0.24	150	1.0	150	540	96
Epicholesterol	0.50						286	182
	0.80	3.2	0.24	120	1.0	120	261	199
Dioleoylphosphatidylcholine membranes								
	0	1.9	0.84	46	20	920	192	0.85
Cholesterol	0.33						283	0.58
	0.50	2.4	0.75	76	9	680	269	0.61
	0.67						367	0.45
Dihydrocholesterol	0.33						353	0.46
	0.50	2.4	0.83	36	16	580		
	0.67						453	0.36
Ergosterol	0.50	1.2	0.80	86	13	1100	191	0.86
Stigmasterol	0.50	2.0	0.83	46	16	740	288	0.57
Epicholesterol	0.67	2.9	0.82	31	16	500	230	0.71

[a] The mole fraction x of sterol in the membrane-forming solution (referred to total lipids) is given in the first column. The aqueous phase contained 0.1 M NaCl and the following concentrations of permeant ions: 10^{-7} M $T\phi B^-$, 3×10^{-5} M $T\phi A^+$ (monooleate membranes), 10^{-2} M $T\phi A^+$ (phosphatidylcholine membranes). $T=25$ °C. Data on $T\phi B^-$ transport in glycerolmonooleate membranes are taken from ref. 4. In the computation of β and $k_i\beta$, the values of specific capacitance C_m given in Table 1 for sterol-free/*n*-hexadecane membranes were used.

coefficient for adsorption of lipophilic ions β and the translocation rate parameter k_i. An expression used in the past [4] to describe the behavior of the product $k_i\beta$ when a mole fraction x of sterol is included in the membrane is

$$\beta k_i/\beta_0 k_{i0} = \exp(-zx\delta F\Delta V_D/RT)\exp(-zx(1-\delta)F\Delta V_D/RT). \quad (18)$$

Here β_0 and k_{i0} are the values of β and k_i in the absence of sterol ($\beta_0 k_{i0} \equiv (\beta k_i)_0$), z is the valency of the translocated ion, δ is the fraction of the distance across the dipolar region (measured from the aqueous side) at which the ions are adsorbed, and ΔV_D is the change in the dipolar field when the sterol content is changed from $x=0$ to $x=1$. This formulation assumes that the dipolar field changes linearly with sterol content. The first exponential term in Eq. (18) represents the influence of sterol on β, the other exponential represents the influence on k_i.

Benz and Cros [4] have been able to fit their results on the transport of the lipophilic ions dipicrylamine and tetraphenylborate in monoolein-cholesterol membranes using Eq. (18), assuming $\Delta V_D = 70$ mV. This value, representing the difference in dipole potential between pure monoolein and pure cholesterol membranes, is consistent with surface potential measurements on monolayers formed from these substances [1, 16]. To explain the independence of β on sterol content, it was assumed that the adsorption plane for both ions is on the aqueous side of the dipolar layer, i.e., $\delta = 0$. For this special case Eq. (18) reduces to

$$k_i\beta/(k_i\beta)_0 = k_i/k_{i0} = \exp(zFx\Delta V_D/RT). \quad (19)$$

In Table 4 values calculated from Eq. (19) evaluated for $\Delta V_D = 70$ mV are displayed alongside appropriate experimental values for monoolein membranes having various sterol compositions in the presence of a series of lipophilic ions as well as the valinomycin-Rb^+ carrier complex. In each case shown (with the exception of $T\phi A^+$) the partition coefficient has been shown to be independent of sterol composition. In order to explain the cholesterol dependence of the translocation rate constant k_s of the uncharged free carrier in the val-Rb^+ system, it has been assumed that the changes in both k_s and the charged complex rate constant k_{MS} are membrane fluidity effects; the variations with cholesterol content of k_R and the k_i's for the negative ions, on the other hand, have been assumed to be dipolar field effects. It can be seen that for cholesterol-monoolein membranes Eq. (19) adequately describes the cholesterol dependence of the rate constants in the DPA^-, $T\phi B^-$, and val-Rb^+

Table 4. Ratios of the rate constants (k_i, k_{MS}, k_s, k_R) for charge translocation in membranes formed from solutions (in *n*-hexadecane) containing various sterols to the rate constants for membranes formed from sterol-free solutions ($k_i^0, k_{MS}^0, k_s^0, k_R^0$)[a]

Sterol	x	$e^{2.7x}$	DPA$^-$ k_i/k_i^0	TϕB$^-$ k_i/k_i^0	TϕA$^+$ $(k_i\beta)^0/k_i\beta$	Valinomycin-Rb$^+$		
						k_{MS}^0/k_{MS}	k_s^0/k_s	k_R^0/k_R
Glycerolmonooleate membranes								
Cholesterol	0.17	1.6	1.2	0.9	1.5	1.2	1.1	1.2
	0.33	2.4	1.6	1.8	3.4	1.6	1.8	1.8
	0.50	3.9	3.2	4.2	28	3.2	3.0	2.4
	0.67	6.1	5.5	5.3		5.5	4.8	5.0
	0.80	8.7	8.1	8.5		8.2	7.3	7.5
Dihydrocholesterol	0.50	3.9	2.3		4.0			
	0.67	6.1		2.2	9.9			
	0.80	8.7	3.6	2.7				
Ergosterol	0.50	3.9	0.9	1.1	2.5			
	0.67	6.1			2.8	1.1	0.9	0.6
	0.80	8.7	0.8	1.0				
Stigmasterol	0.50	3.9			1.8			
	0.80	8.7	1.1	1.2	1.8	1.0	0.9	0.6
Epicholesterol	0.50	3.9	0.8		0.9	1.0	1.1	0.5
	0.80	8.7	0.9	0.9	0.8	1.0	1.4	0.6
Dioleoylphosphatidylcholine membranes								
Cholesterol	0.33		0.9		1.5			
	0.50		1.0	1.6	1.4			
	0.67		1.1		1.9			
Dihydrocholesterol	0.33				1.8			
	0.50			0.8				
	0.67				2.4			
Ergosterol	0.50			1.9	1.0			
	0.80		1.0					
Stigmasterol	0.50			1.0	1.5			
Epicholesterol	0.67			0.7	1.2			
	0.80		1.2					

[a] For the valinomycin-Rb$^+$ complex the inverse ratio is given. For TϕA$^+$ the inverse ratio of the product $k_i\beta$ is given. The factor given in the third column is the ratio of rate constants predicted by Eq. (19) for each mol fraction x of sterol (referred to total lipid) using $\Delta V_D = 70$ mV. Ratios for TϕA$^+$ and for TϕB$^-$ in phosphatidylcholine membranes are based on data listed in Table 3; the remainder are based on data given in ref. 4.

systems. The correlation with k_s and k_{MS} is very likely accidental. Only the behavior of $k_i\beta$ for TϕA$^+$ has a radically different behavior.

The greater sensitivity on sterol content of the positive ion suggests

that fluidity changes may influence the transport of $T\phi A^+$ much more readily than they influence transport of the negative ions $T\phi B^-$ or DPA^-. However, the not unreasonable possibility that the negative and positive lipophilic probes in monoolein membranes are roughly equivalent with respect to sterol-induced fluidity effects cannot be excluded by our data. Suppose we assume as a working hypothesis that both $T\phi B^-$ and $T\phi A^+$ are adsorbed in the dipolar regions of the membrane and that their translocation rates are equally affected by sterol-induced changes in membrane viscosity. Then, in a way similar to that discussed by several authors [3, 22, 29, 30], we can calculate for each sterol mole fraction a dipolar potential change

$$\Delta V_D(x) = \frac{RT}{2F} \ln \frac{(k_i\beta)^+ (k_i\beta)_0^-}{(k_i\beta)^- (k_i\beta)_0^+} \tag{20}$$

as well as a factor $f(x)$ representing the influence of viscosity of the membrane interior on "intrinsic" conductance

$$f(x) = \sqrt{(k_i\beta)^+ (k_i\beta)^- / (k_i\beta)_0^+ (k_i\beta)_0^-}. \tag{21}$$

Values derived in this way for monoolein membranes containing cholesterol mole fractions $x = 0.17$, 0.33, and 0.50 are $\Delta V_D(x) = 3$, 21, and 60 mV and $f(x) = 0.8$, 0.7, and 0.4, respectively. These numbers are found to be not inconsistent with values obtained by Szabo [29, 30] using several different cations and anions, especially if it is recalled that in the previous work no correction for sterol-induced membrane thinning was applied. The dipolar potential changes are not simply proportional to x, nor is there any reason why this should be expected. For instance, Pickar and Hobbs[1], on the basis of alternating current studies with lecithin/sterol membranes, have concluded that the sterol component may deform or otherwise interact with the dipolar region in such a way as to reduce the dipolar potential changes at low sterol concentrations.

Table 4 shows that only moderate effects occur in monoolein membranes made with dihydrocholesterol, and that ergosterol and stigmasterol induce still weaker modifications. Epicholesterol produces no significant changes. The possibility that certain sterols are partially or totally excluded from the black area of the membrane cannot be discounted, but there is little supporting evidence for this. Direct measurements on the

1 Pickar, A.D., Hobbs, J. 1978. Sterol dependence of surface and interior electrical properties of lipid bilayers in the presence of pentachlorophenol. Alternating current studies. (*unpublished*)

proportion of sterol in the bilayer have only been done in the case of cholesterol [25]. Also, studies which show that a number of sterols are as readily incorporated into liposomes as is cholesterol has been done with egg lecithin [14]. Similar studies with monoolein-sterol mixtures would be of great value. Of particular interest in the new data we present in Table 4, however, is that a sensitive probe such as $T\phi A^+$ does in fact show that, besides cholesterol, at least dihydrocholesterol significantly modifies monoolein membranes. Moreover, the effect of this sterol as measured in terms of $\Delta V_D(x)$ and $f(x)$ (derived from Eqs. 20 and 21) are not vastly different from cholesterol: at $x=0.67$ we obtain values of 40 and 0.5 mV, respectively, for the values of these parameters.

In phosphatidylcholine membranes the $T\phi A^+$ results also show some moderate sterol-induced influences which appear to be absent with the negative lipophilic ions. Interestingly, the effectiveness of both dihydrocholesterol and stigmasterol are comparable to that of cholesterol. It is possible that in the case of DPA^- and $T\phi B^-$ dipolar and fluidity effects essentially cancel one another in PC membranes. This conjecture is similar to conclusions based on the AC studies reported in Pickar & Hobbs, 1978.[2] In that work cholestanol and 7-dehydrocholesterol, which are sterols whose nuclei are, compared to cholesterol, more and less saturated, respectively, were tested. Cholestanol was shown to suppress conductance of a negative-charged probe in the membrane interior; 7-dehydrocholesterol enhanced it, whereas cholesterol had relatively little effect. This behavior has been explained on the basis of differences among the three sterols in the relative influence of dipolar potential changes compared with fluidity effects.

Effects of Lipid Structure

Table 5 summarizes the results of our studies in which several structural features of the principal membrane lipids were varied. These features are: (i) degree of saturation in the fatty-acid tail, (ii) nature of the head group, and (iii) type of linkage between the hydrocarbon chain and glycerol backbone. All lipids tested were electrically neutral and *n*-decane was used in all cases as the solvent. Ion concentrations were chosen for which membrane transport was in the linear range. The magnitudes of the experimental error were comparable to those in other

2 *Ibid.*

Table 5. Kinetic parameters of $T\phi B^-$ and $T\phi A^+$ (or $T\phi Ph^+$) transport through membranes made from different lipids dissolved in *n*-decane[a]

Fatty acid residues	C_m/ nF cm^{-2}	$T\phi B^-$					$T\phi A^+$ ($T\phi Ph^+$)	
		τ_1/ msec	a_1	k_i/ sec^{-1}	N_t/ p mol · cm^{-2}	β/ 10^{-3} cm	τ_2/ msec	$k_i\beta$/ 10^{-7} cm sec^{-1}
Monoglyceride membranes								
Oleoyl (Δ^9-$C_{18:1}$)	390	24	0.28	15	0.16	0.80	1120(922)	30(37)
Linoleoyl ($\Delta^{9,12}$-$C_{18:2}$)	464	4.1	0.76	32	1.1	0.54	(30)	(400)
Linolenoyl ($\Delta^{9,12,15}$-$C_{18:3}$)	576	2.1	0.75	61	20	1.0	(*see* note below*)	(35,000)
Phosphatidylcholine (PC) membranes								
Dioleoyl (Δ^9-$C_{18:1}$)	374	10	0.87	6.5	3.3	16.5	2840	0.034
1-Oleoyl-2-stearoyl (Δ^9-$C_{18:1}$, $C_{18:0}$)	370	7.5	0.73	17	1.4	6.8	320	0.30
1-0-Oleyl-2-0-palmityl (Δ^9-$C_{18:1}$, $C_{16:0}$)	352	490	0.83	0.25	1.2	5.8	2830	3.2
Phosphatidylethanolamine (PE) membranes								
Dioleoyl (Δ^9-$C_{18:1}$)	372	3.0	0.23	128	0.12	0.59	2010	0.048
Di-0-oleyl (Δ^9-$C_{18:1}$)	357	45	0.40	6.6	0.27	1.3	980	9.5

[a] (Data for $T\phi Ph^+$ are given in parentheses.) The aqueous phase contained 0.1 M NaCl and the following concentrations of permeant ions: $T\phi A^+$ (and/or $T\phi Ph^+$) 3×10^{-5} M in glycerol monooleate membranes, 10^{-4} M in other monoglyceride and in diether PC and PE membranes, 10^{-2} M in other PC and PE membranes; $T\phi B^-$ 10^{-7} M in all membranes, except glycerolmonolinoleneate (10^{-6} M). $T = 25$ °C. Data on $T\phi B^-$ transport in dioleoylphosphatidylcholine membranes are taken from ref. 11. Values of specific capacitance C_m are taken from references 5 and 8. Detailed parameters for $T\phi Ph^+$ transport in glycerolmonolinoleate membranes* are $\tau_1 = 0.36$ msec, $\tau_2 = 1.8$ msec, $a_1 = 0.33$, $k_i = 195\ sec^{-1}$, $N_t = 3.5$ pmol · cm^{-2}, $\beta = 0.018 \times 10^{-3}$ cm.

parts of this study. With one exception, only one relaxation could be resolved in the measurements on $T\phi A^+$ and $T\phi Ph^+$. For the particular case where two $T\phi Ph^+$ relaxations could be resolved (18:2 monoglyceride), the time constants were not sufficiently separated to permit us to use Eqs. (13) and (14) in the analysis; therefore the rate parameters, listed in the Table heading, were obtained from the data using Eqs. (1)–(6). At $c = 10^{-4}$ M, the ion concentration at which these particular results were obtained, the surface charge transfer process was shown not be diffusion limited.

Our results given in Table 5 for the three monoglycerides show that as the number of double bonds in the hydrocarbon region increases the

charge transport, as measured by k_i, is enhanced for both negative and positive lipophilic ions. It is to be expected that the structurally related characteristics responsible for the differences among the kinetic parameters for the three monoglycerides are membrane thickness and interior fluidity. Dipolar potentials are unlikely to be a strong function of the number of double bonds in the fatty acid tail. In fact, one can show that the variation in k_i for TϕB$^-$ can be almost entirely accounted for by differences in thickness by using Eqs. (16)–(18) and the capacitance values listed in Table 5. The changes in partition coefficient β are, as might be expected, not very extreme and may possibly be due to small differences in packing of the polar head groups. The dependences of TϕB$^-$ transport on saturation in the lipid hydrocarbon region is very similar to that previously observed with DPA$^-$ in phosphatidylcholine membranes dissolved in *n*-hexadecane [7]. As k_i was observed not to be strongly affected by the number of double bonds in those (essentially) constant thickness membranes, it was concluded that fluidity effects were relatively small. The partition coefficient also should, in principle, not be affected by fluidity.

In view of the conclusions just cited, the corresponding findings with positively charged probes are interesting. Even when corrected for thinning effects, $k_i\beta$ for TϕPh$^+$ increases by about 5× in going from the monoglyceride with a $C_{18:1}$ hydrocarbon tail to a $C_{18:2}$ tail; a further increase of about 30× is obtained in going to the $C_{18:3}$ tail. This contrasting behavior of positive probes as compared with negative ions has also been observed in previous studies using charged-carrier complexes [5, 7]. The translocation rate parameter k_{MS} for PV-K$^+$ increased by about an order of magnitude for each step in going from $C_{18:1}$ to $C_{18:2}$ to $C_{18:3}$ phosphatidylcholine in *n*-hexadecane membranes. The translocation rates for the valinomycin-Rb$^+$ system were also found to depend in a similar, but much less severe, way on saturation in the hydrocarbon region of monoglyceride in *n*-decane membranes, after correction for thinning.

It has seemed reasonable to suppose that the apparently greater sensitivity of the carrier complexes to fluidity effects is possibly due to their larger size relative to TϕB$^-$ or DPA$^-$, as well as to differences in their chemical interactions with the membrane interior. In the context of this explanation the TϕPh$^+$ results are surprising. With regards to both size and chemical properties, TϕPh$^+$ (and TϕA$^+$) are very similar to TϕB$^-$, so that their perturbing effects and their mobilities in the hydrocarbon interior should be expected to be about the same.

Table 6. Values of $k_i\beta$ (DPA^-, $T\phi B^-$, $T\phi A^+$, $T\phi Ph^+$) and k_{MS} ($PV\text{-}K^+$) for membranes made from different lipids dissolved in *n*-decane[a]

Lipid	V_M/ mV	DPA^- $k_i\beta$/ $cm \cdot sec^{-1}$	$T\phi B^-$ $k_i\beta$/ 10^{-3} $cm \cdot sec^{-1}$	$T\phi A^+$ (or $T\phi Ph^+$) $k_i\beta$/ 10^{-7} $cm \cdot sec^{-1}$	$PV\text{-}K^+$ $k_{MS}\beta$/ $cm \cdot sec^{-1}$	V'_P/ mV
Glycerolmonooleate	320	1.8	12	(30)	14	108
Phosphatidylcholines (PC)						
Dioleoyl-PC	440	16	107	0.034	0.96	224
1-Oleoyl-2stearoyl-PC	390	8.8	118	0.30	0.33	197
1-O-Oleyl-2-O-palmityl-PC	290	0.74	1.5	3.2	2.8	110
Phosphatidylethanolamines (PE)						
Dioleoyl-PE	420	28	75	0.048	0.088	215
Di-O-Oleyl-PE	310	5.6	8.8	9.5	3.4	119

[a] Values of surface potentials V_M of monolayers made from corresponding lipids are also listed. Data listed for $T\phi B^-$, $T\phi A^+$, $T\phi Ph^+$ are based on results shown in Table 5, for DPA^- and $PV\text{-}K^+$ on results given in ref. 7. Monolayer data are from ref. 16 and 26. Estimates of dipolar potentials V'_P based on the ($k_i\beta$) for $T\phi B^-$ and $T\phi A^+$ (or $T\phi Ph^+$) and Eq. (22) are given in the last column.

Nevertheless, this assumption, which we have previously used as a working hypothesis in discussing the sterol data, may not be strictly correct. Dipolar changes among the lipids with different hydrocarbon saturation, due possibly to differences in polar head group packing, cannot reasonably be expected to be large enough to account for the different dependence of negative and positive transport on this aspect of membrane structure. Apparently the positive probe is much more sensitive to changes in membrane fluidity than its negative counterpart, at least in unsaturated lipid structures. The reason for the difference is not clear at this time, especially in view of the limited information we have been able to obtain on the kinetics of the positive probe.

Alterations in the type of polar head group or in nature of the linkage between the hydrocarbon tail and glycerol backbone can be expected to affect the dipolar potential at the membrane surface. Potential changes, in turn, should affect the values of the product $k_i\beta$. Table 6 lists values of $k_i\beta$ obtained for several charged probes in a series of membranes where these structural features of the component lipid have been varied. For purposes of comparison, surface potentials V_M obtained from monolayers made from the several lipids are also shown. Thinning effects are not

important for this series, as can be seen from the capacitance data in Table 5. We first note that in going from monoolein to PC or PE membranes, the negative lipophilic probes behave in a similar way and in sharp contrast to $T\phi A^+$ or to the PV-K^+ complex. If only dipolar changes are involved, the negative ion data alone yield a difference of about 50–70 mV between the dipolar potentials of 18-carbon chain monoglyceride and PC or PE membranes (using Eq. (19) with $x=1$), which is somewhat smaller than the dipolar changes indicated by the monolayer data. The reason for this may reside in the fact that ion mobilities are unlikely to be the same in monoglyceride and PC or PE membranes.

A somewhat better correlation of transport behavior as a function of lipid type with monolayer data is had by assuming that fluidity effects are approximately the same in $T\phi B^-$ and $T\phi A^+$ in any given membrane type. We can then obtain an approximation V_P' for the membrane dipolar potential using

$$V_P' = \frac{RT}{2F} \ln \frac{(k_i\beta)^-}{(k_i\beta)^+} \tag{22}$$

where $(k_i\beta)^-$ and $(k_i\beta)^+$ are the values of $k_i\beta$ obtained for $T\phi B^-$ and $T\phi A^+$ (or $T\phi Ph^+$), respectively. One must take care not to identify V_P' with the actual dipolar potential which probably exists at the membrane surface as, among other things, Eq. (22) does not take into account differences in hydration energies of negative and positive ions [3, 22]. However, differences in V_P' from one type of lipid to another are likely to be significant.

A comparison of values of V_P' (listed in the last column of Table 6) with the monolayer compensation potentials V_M strongly suggests that the analog probes $T\phi B^-$ and $T\phi A^+$ (or $T\phi Ph^+$) are probably good indicators of the changes in dipolar potential associated with changes in lipid type. For example, results obtained with lipids of the diacyl ester form correlate well with results obtained with lipids of the diether type (1-O-oleyl-2-O-palmityl-PC and di-O-oleyl-PE). Comparing the mixed chain PC with the ether PC, V_P' differs by 87 mV and V_M differs by 100 mV; for the PE's, V_P' differs by 96 mV and V_M differs by 110 mV. Interestingly, if calculations of V_P' are carried out using $k_{MS}\beta$ for the PV-K^+ system rather than $(k_i\beta)^+$, the correlation with the monolayer data, though worse, is not outrageously bad. Previous studies [7] have indicated that the PV-K^+ complex, because of its relatively large size, probably perturbs the state of the membrane interior. The resultant

fluidity changes, however, are apparently less important than dipolar potential changes in determining membrane conductance differences among membranes made from various lipids.

Conclusions

This is one of a series of papers which have been concerned with the relative importance of various compositional features of lipid bilayer membranes on the transport of lipophilic ions and carrier complexes across these structures. Among other aspects of membrane makeup which have been considered are the nature of the solvent in the forming solution, the sterol content, the degree of saturation in the hydrocarbon tail of the principal lipid, and the type and linkage of the polar head groups. Previous studies have shown the behavior of the positively charged carrier systems to be more complicated than that of the negative lipophilic ions. In the present work we have made a systematic investigation of structural influences on the kinetics of charge transport, using the positive lipophilic ions tetraphenylarsonium and tetraphenylphosphonium ($T\phi A^+$ and $T\phi Ph^+$) as well as their negative structural analog tetraphenylborate ($T\phi B^-$).

The major conclusion we have been able to reach is that $T\phi A^+$ and $T\phi Ph^+$ are considerably more like $T\phi B^-$ and the negative ion dipicrylamine (DPA^-) with respect to transport behavior in bilayers than are the positive carrier complexes. For example, results involving solvent-related thickness changes lead one to conclude that lipophilic ions, both positive and negative, are adsorbed within and probably towards the aqueous side of the polar layer in the membrane, whereas the carrier complexes adsorb toward the hydrocarbon side. In sterol containing membranes the results are not inconsistent with a characterization of $T\phi A^+$ and $T\phi B^-$ as oppositely charged, but chemically analogous probe ions. That the positive species is much more sensitive to sterol content can be explained on the basis of a combination of sterol-induced dipolar potential and fluidity changes. However, another possibility, viz., that the positive lipophilic ion is inherently more sensitive to fluidity effects, is supported by some of the results involving variations in the main component lipid. Whereas, for example, DPA^- and $T\phi B^-$ are relatively unaffected by variations in the degree of saturation in the hydrocarbon tail, $T\phi A^+$ is highly sensitive to the fluidity changes which, on the basis of previous studies, can be assumed to accompany such structural

variations. With respect to other variations in lipid structure, on the other hand, there is strong evidence that $T\phi B^-$ and $T\phi A^+$ are good analog probes. For instance, differences in dipole potentials among membranes made from various lipids, which are inferred from the charge transport data assuming fluidity effects to be independent of the sign of the charge, correlate well with monolayer surface potential measurements.

A great deal of potentially useful information on the details of hydrophobic ion transport in membranes has been gathered in the course of these studies. In particular, the influence of a full range of structural features on the electrical behavior of the series of lipophilic ions $T\phi A^+$, $T\phi Ph^+$, and $T\phi B^-$ is now known. These ions appear to function as fairly good analog probes in most cases, though in view of the lack of complete kinetic data, the evidence is, to an extent, circumstantial. The need for a new family of synthetic probes for which the kinetics of both the electrically positive and negative species are readily accessible to experiment is evident. A program of investigation bearing on this aim is currently underway in our laboratory.

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