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NONELECTROLYTE DIFFUSION THROUGH LECITHIN–WATER LAMELLAR PHASES AND RED-CELL MEMBRANES

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SUMMARY

The longitudinal diffusion of a homologous series of monoamides through lecithin–water lamellar phases with aqueous channel widths of 16–27 Å has been studied. The diffusion coefficients relative to water of the hydrophilic amides, formamide and acetamide, depend logarithmically on solute molar volume, as previously demonstrated in human red cells. Aqueous diffusion of amides in red-cell membranes is similar to that in a lecithin–water phase of aqueous channel width less than 16 Å, the smallest channel width used. Partition coefficients of the lipophilic amides, valeramide and isovaleramide, between lecithin vesicles and water are 1.64 and 1.15 at 20 °C. These data enabled us to compute a valeramide diffusion coefficient of $6.5 \cdot 10^{-7}$ $cm^2 \cdot s^{-1}$ at 20 °C in the lipid region of a lamellar phase containing 30% water about one order of magnitude greater than the diffusion coefficient of spin-labelled analogs of phosphatidylcholine. The discrimination between the permeability coefficients of valeramide and isovaleramide is more than twice as great in the human red cell as between lipid diffusion coefficients in a phase containing 8% water. This suggests that the lipid region of the human red cell is more highly organized than lipid in the lecithin–water lamellar phase.

INTRODUCTION

Interpretation of studies of nonelectrolyte diffusion across the membranes of living cells depends upon comparison with data obtained in model systems of simpler structure and known chemical composition. Lipid–water lamellar phases provide unique advantages for this purpose because the dimensions of the aqueous and lipidic regions of the phase have been established by X-ray scattering and may be varied in a controlled fashion by changing the composition of the mixture.

We have measured the longitudinal diffusion of a series of monoamides through lecithin–water lamellar phases with aqueous channel widths of 16–27 Å. The amides studied ranged from formamide to valeramide. The diffusion coefficient of

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the hydrophilic amides relative to that of water has been shown to depend logarithmically on solute molar volume, as has previously been demonstrated in red cells [1].

To study diffusion in the lipidic region of the phase, we have measured the partition coefficient of valeramide and isovaleramide between water and lecithin thus making it possible to compute diffusion coefficients for these solutes in the phase lipids. As the water content of the system is decreased, the lipidic phase shows a discrimination between the diffusion of these two isomers similar to that exercised by the human red-cell membrane.

METHODS

The extraction of lecithin from egg yolk, the preparation of lecithin–water phases and the determination of phase-water content have been described previously [2]. The method of determining diffusion coefficients has been modified to permit measurement using smaller quantities of phase. The diffusion experiments were carried out in a capillary of 0.02 ml capacity filled with the phase. A drop of approx. 0.06 μl of radioactive tracer (250 μCi/ml) was introduced at one end of the capillary using a syringe and needle. This operation was performed under the microscope to permit accurate placing of the drop. The capillary was then sealed with a small plug of modelling clay at the radioactive end and with a coating of paraffin at the other end.

The capillary was kept at constant temperature in a water bath at 20.6 °C, controlled to within 0.05 °C, for from 2 days to 2 weeks depending on the diffusing substance and the water content of the phase. At the end of this time the capillary was broken to give three segments approx. 4 mm in length. The precise length of each segment was measured with a microscope. Each segment was placed in a scintillation counter vial with 10 ml Bray's solution and crushed. The remaining portion of the capillary was also counted.

The experimental boundary conditions were identical with those described in the previous paper [2] and the same solution to the diffusion equation applies, namely,

$$C(x,t) = [M/(\pi Dt)^{\frac{1}{2}}] \exp(-x^2/4Dt) \tag{1}$$

where $C(x,t)$ is the linear concentration of radioactive tracer at the point x at time t; M is the amount of tracer deposited at time $t = 0$ in the place $x = 0$; D is the diffusion coefficient of the diffusing tracer. Integration of Eqn 1 gives, for the amount of diffusing substance contained between x_1 and x_2 at time t,

$$C = \int_{x_1}^{x_2} dx\, C(x,t) = M(\mathrm{erf}[x_2/(4Dt)^{\frac{1}{2}}] - \mathrm{erf}[x_1/(4Dt)^{\frac{1}{2}}]) \tag{2}$$

Substituting the measured values of the quantities C, x_1, x_2 and M and the diffusion time, t, it is possible to determine the value of D which gives the best fit for each of the capillary segments. However, D may also be determined more directly and conveniently using probability paper, a type of graph paper used in statistical analysis. When plotted on this paper, Eqn. 2 gives a straight line, from whose slope D may be determined.

At each phase-water content, the diffusion coefficients of water and the five amides were measured in the same phase preparation. Each diffusion measurement

was made in quintuplet. [^{14}C]valeramide and [^{14}C]isovaleramide were obtained from International Chemical and Nuclear Corporation (Irvine, Calif.). All other radioisotopes used were obtained from New England Nuclear Corporation (Waltham, Mass.).

The radioactive tracers used were in aqueous solution with approx. 0.1 M carrier. The high carrier osmolality could lead to water movements causing errors in the determination of the diffusion coefficients. The extent of this effect was estimated by measuring the diffusion coefficient of acetamide using the standard solution of isotope to which unlabelled acetamide to a final concentration of 1.0 M had been added. This 10-fold increase in carrier concentration gave a decrease of approx. 15% in the measured diffusion coefficient. Assuming that the dependence of the measured diffusion coefficient on carrier concentration is roughly linear, the error in our determination due to this effect is probably less than 2%.

Measurement of partition coefficients

Partition coefficients between aqueous solutions of amides and hydrocarbon mixtures of hexadecane, 1-octadecene and 1,7-octadiene were determined using ^{14}C-labelled amides. The 1,7-octadiene was obtained from Aldrich Chemical Co. (Milwaukee, Wis.) and the others from Eastman Kodak Co. (Rochester, N.Y.). They were used as supplied without further purification. Equilibration was complete within 2 h at 21 °C. Samples were taken from both phases and counted.

Partition coefficients in lecithin were measured using aqueous dispersions of lecithin extracted as described above. The lecithin was dispersed by simple stirring with the aid of a magnetic stirring bar at a concentration of approx. 0.04 g/ml in solutions containing both labelled and unlabelled amide at neutral pH. Equilibrium was attained in 1 h at 20 °C and the dispersion was then centrifuged at 127 000 $\times g$ for 1.5 h at 20 °C. Aliquots of supernatant and pellet were counted in Brays' solution and the water content of a separate pellet sample determined by drying at 90 °C. To ensure uniformity of aliquots, the pellet was thoroughly stirred before sampling. Under these conditions of centrifugation the pellet contained approx. 50% water. A pellet was prepared in the same conditions in the absence of isotope and added to the supernatant samples before counting to ensure uniform quenching in all samples.

Initially it was expected that the partition coefficients would be very small so the experiments were designed to minimize errors. To this end, [^{3}H]glycerol, a hydrophilic molecule whose ether–water partition coefficient [3] is 0.00066, was added to the solution containing the ^{14}C-labelled amide before dispersing the lecithin. The experiments were carried out as described above with the difference that errors due to sample size of supernatant and pellet were eliminated as the partition coefficient could be determined from the ratio of the ratios of ^{3}H and ^{14}C in supernatant and in pellet. Initial measurements revealed that the partition coefficients were much larger than expected and the [^{3}H]glycerol was therefore no longer used. The results obtained with and without glycerol were in good agreement.

The partition coefficients in lecithin of valeramide and isovaleramide were determined at concentrations of 0.1, 1, 10 and 100 mM unlabelled substance. The partition coefficient did not vary with concentration indicating that no adsorption of solute occurred. As a check of the measurement technique, the partition coefficient of acetamide was determined and found to be too small to be measured (less than 10^{-4}).

RESULTS AND DISCUSSION

The measured diffusion coefficients* for water and the monoamides are given in Table I together with the fractional water content, Φ_w and aqueous channel width, d_{aq}, obtained from the data of Lecuyer and Dervichian [4]. Two general trends can be observed. Going down the columns the aqueous channel width increases, as does the diffusion coefficient. Going across the rows, the size of the permeating solute increases while the channel width stays constant. In this case the diffusion coefficient decreases. Both these trends can be understood in terms of steric restraints on molecules diffusing through narrow channels.

TABLE I

DIFFUSION COEFFICIENTS IN LIPID LAMELLAE

Water fraction Φ_w	Aqueous channel width, d_{aq}(Å)	Diffusion coefficients $\times 10^6$ ($cm^2 \cdot s^{-1}$)					
		Water	Form-amide	Acet-amide	Propion-amide	Valer-amide	Isovaler-amide
0.08	16.4	0.17	0.055	0.020	0.019	0.015	0.010
0.14	18.0	0.50	0.13	0.070	0.050	0.034	0.028
0.16	18.8	0.69	0.25	0.12	0.090	0.070	0.050
0.18	19.8	0.77	0.26	0.14	0.090	0.066	0.056
0.21	21.2	1.30	0.51	0.31	0.19	0.13	0.11
0.26	24.0	2.70	1.18	0.68	0.43	0.18	0.18
0.30	26.8	3.30	1.70	1.00	0.61	0.32	0.30

The diffusion coefficients in Table I were obtained assuming that the path length for diffusion was equal to the measured geometrical length of the capillary. As the lamellar phase is undoubtedly tortuous, the calculated values are lower bounds for the actual diffusion coefficient. However, the tortuosity factor may be eliminated in any single-phase preparation by dividing the diffusion coefficient for the amide by that of water to obtain the relative diffusion coefficients.

Horowitz and Fenichel [5] have pointed out that the aqueous diffusion coefficients of small solutes are related to the molar volume of the solute. Subsequently Sha'afi et al. [1] and Solomon and Gary Bobo [6] showed that the logarithm of the permeability coefficient relative to water is linearly dependent on molar volume, not only for the permeation of hydrophilic solutes through red-cell membranes, but also for permeation through antibiotic-induced aqueous channels in lipid bilayers. The precise logarithmic relationship is purely empirical though the general trend is presumably due to the steric restraints in narrow channels.

Fig. 1 shows that a logarithmic relation also holds for the relative diffusion

* The diffusion coefficients of water given here differ somewhat from those obtained previously [2]. Work in progress has shown that slight changes in the method of preparation of the phases give rise to marked differences in the aqueous diffusion coefficients. However, for a given method of phase preparation, the diffusion coefficients are reproducible and the ratios of the diffusion coefficients are generally similar, independent of phase-preparation method. The discrepancies are probably due to variations in phase homogeneity and will be discussed in a paper currently in preparation.

coefficients of hydrophilic amides in lecithin–water lamellar phases and that the slope is dependent on aqueous channel width. The points for propionamide, valeramide and isovaleramide are not included in Fig. 1 because these solutes are lipophilic; their behavior will be discussed in a later section. For comparison, the line for the human red cell taken from ref. 6 is also shown in the figure. The slope of the red-cell line is greater than that for the lamellar phase of lowest water content studied.

The comparison of the red-blood-cell data with that obtained in lamellar phases suggests the interesting possibility of determining whether the red-blood-cell membrane behaves, with respect to the diffusion of the amide series, like a phase of a certain channel size. This comparison would be evident if the straight line referring to the red blood cell in Fig. 1 were intermediate in slope to the lines for the lamellar phases of various water contents. This is not the case and it is in practice not possible to prepare homogeneous lamellar phases at water content below about 8%, which corresponds to an aqueous channel width of about 16 Å, the smallest we have used. The data therefore only permit the statement that aqueous diffusion in the red-blood-cell membranes is similar to that in a lecithin–water phase of aqueous channel width less than 16 Å, an observation which is consistent with the equivalent pore diameter of 8.4 Å characteristic of the human red cell [6].

The lipid–water spacings of the lecithin–water lamellar phase obtained by

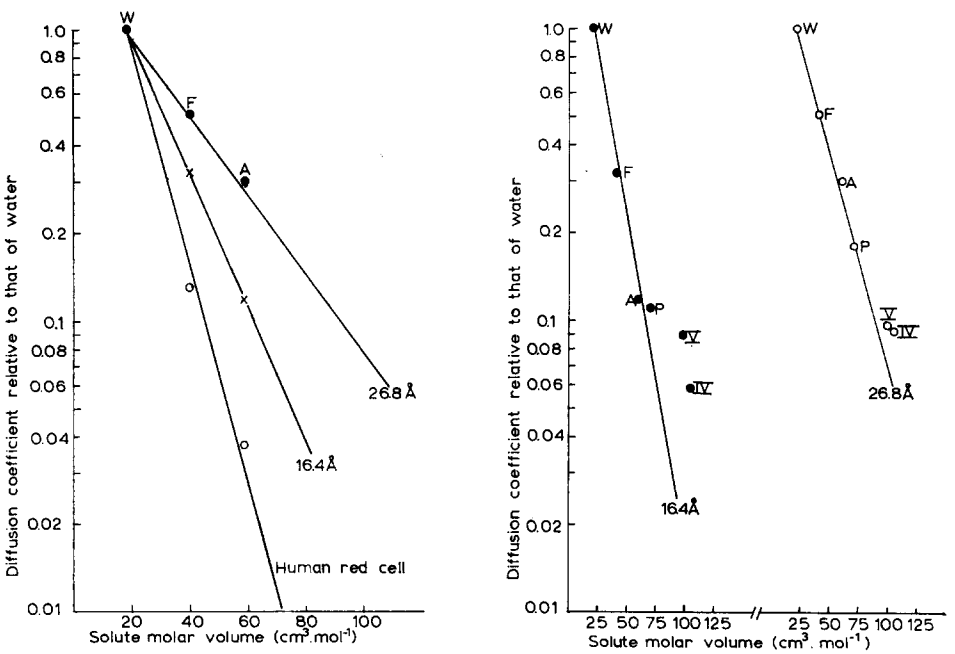

Fig. 1. Diffusion coefficients relative to water of hydrophilic amides in lecithin–water lamellar phases and in human red-cell membranes as a function of solute molar volume. The symbols are: W, water; F, formamide; A, acetamide.

Fig. 2. Diffusion coefficients relative to water of hydrophilic and lipophilic amides as a function of solute molar volume. The additional symbols are: P, propionamide; V, valeramide and IV, isovaleramide.

Lecuyer and Dervichian [4] from X-ray diffraction measurements are based on the assumption that the choline phosphate groups of the lecithin molecules are included in the aqueous region of the phase. The aqueous channel widths used here are therefore representative of channels encumbered by bulky polar groups. It is not known whether this might also be the case for aqueous pathways through the red-cell membrane. In any case, it is apparent that in the context of the measurements presented here, the lecithin–water system does not differ qualitatively from the red-blood-cell membrane and that no special effects due to the presence of cholesterol and structural proteins need be invoked.

Partition coefficients of lipophilic amides

The partition coefficients of propionamide, valeramide and isovaleramide have been determined in hydrocarbon mixtures of alkanes and alkenes with chain lengths of 8–18 carbons; the results are given in Table II. The ratio of the partition coefficient of valeramide to that of isovaleramide is independent of the solvent, ranging from 1.33 to 1.40 with an average of 1.35.

TABLE II

PARTITION COEFFICIENTS OF LIPOPHILIC AMIDES

	Mixture 1	Mixture 2	Mixture 3
Components of solvent (g)			
Hexadecane	47	47	47
1-Octadecene	53	33	0
1,7-Octadiene	0	20	53
Solute	Partition Coefficient* $k(\times 10^3)$		
Water	0.036	0.173	0.300
Propionamide	0.074	0.234	0.555
Valeramide	1.16	3.02	7.17
Isovaleramide	0.874	2.15	5.39
$k_{\text{valeramide}}/k_{\text{isovaleramide}}$	1.33	1.40	1.33

* The increase of k with increased 1,7-octadiene concentration may be a result of the antioxidant commonly used in the reagent to prevent oxidation of the conjugated double bond.

Partition coefficients were also measured between lecithin vesicles and water. They were unexpectedly high, 1.64 ± 0.05 for valeramide and 1.15 ± 0.05 for isovaleramide at 20 °C. These figures are independent of concentration over the range from 0.1 mM to 100 mM and it may therefore be concluded that they reflect a true solubility and not an adsorption effect.

The ratio of the partition coefficients of valeramide and isovaleramide in lecithin vesicles is 1.4, which is very close to the value obtained in the different hydrocarbon mixtures (Table II). It is interesting that the discrimination between isomers is the same in the liquid mixtures as in the much more structured vesicular system. The invariance of the ratio in these different systems suggests that the same ratio would apply for the red-cell membrane although the magnitude of the partition coefficients might be very different.

Diffusion in the lipid phase

From their extremely small ether–water partition coefficients [3], it can be assumed that water, formamide ($k_{ether} = 0.0014$) and acetamide ($k_{ether} = 0.0025$) diffuse exclusively in the aqueous region of the phase. As shown in Fig. 2, the logarithm of the diffusion coefficient relative to that of water of these two molecules lie on a straight line when plotted against the molar volume of the diffusing molecule. Propionamide, which has a higher partition coefficient, ($k_{ether} = 0.013$) is slightly above the line in some experiments and on the line to within experimental error in others. Valeramide and isovaleramide, which diffuse to a significant degree in the lipid region of the phase are displaced from the line, the displacement being greater at low phase-water content than at high phase-water content.

The diffusion of a molecule through two parallel channels (in this case, the aqueous and lipidic regions of the phase) can be expressed in terms of the two diffusion coefficients in the channels, the partition coefficient of the diffusing molecule between channels and the partition rate. In the experiments described here, partition which occurs over distances of angstroms takes place much more rapidly than diffusion in either channel, which is measured over distances of millimeters. When this is the case, it may be shown (see Appendix) that the measured diffusion coefficient, D_m is given by:

$$D_m = \frac{D_w \Phi_w + k\, D_l \Phi_l}{\Phi_w + k\, \Phi_l} \tag{3}$$

where D_w and D_l are the diffusion coefficients ($cm^2 \cdot s^{-1}$) in the aqueous and lipid regions respectively, k is the lecithin–water partition coefficients and Φ_l is the fractional lipid content of the phase in grams.

Using this equation, the diffusion coefficients for valeramide and isovaleramide in the lipid region of the phase may be calculated from the measured diffusion coefficients, the partition coefficients and D_w. The diffusion coefficient, D_w of either molecule may be obtained by extrapolation of the straight line in Fig. 2 to the molar volume of that molecule. In doing so we are assuming that diffusion in water of these molecules is the same function of molar volume as it is for hydrophilic molecules.

The calculated diffusion coefficients for valeramide and isovaleramide in the lipidic region of the phase as a function of phase-water content are given in Fig. 3. It is seen that the diffusion coefficients increase with phase-water content. At low water content the isomers have different diffusion coefficients, valeramide diffusing 1.5 times as fast as its isomer. This difference decreases with increasing phase-water content and disappears at the highest water content studied.

The partition coefficients used were those measured in lecithin vesicles and we have assumed that this partition coefficient does not vary with phase-water content. If this is not the case, the changes in the relative diffusion coefficients of valeramide and isovaleramide could result from changes in partition coefficient with phase-water content. However, the following argument makes this unlikely.

At low phase-water content, $\Phi_w \approx 0.1$, assuming that the partition coefficient is of order unity, Eqn 3 approaches

$$D_m \approx D_l,$$

which is independent of partition coefficient. It is therefore likely that the difference in the measured lipid diffusion coefficient of the isomers observed at low phase-water content does not reflect a difference in partition coefficient.

The difference in measured lipid diffusion coefficients of the isomers observed at low phase-water content, which disappears as the water content is increased, can be correlated with electron spin resonance studies [7–9]. Jost et al. [8] and Rigaud et al. [9] have shown that the fluidity of the hydrocarbon chains in the lipidic region of the phase increases with phase hydration. Thus the discrimination between the measured diffusion coefficients of the isomers could be ascribed to the rigidity of the hydrocarbon chains through which they are diffusing.

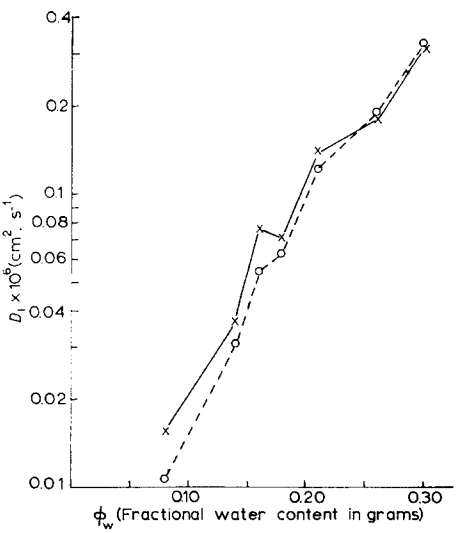

Fig. 3. Lipid diffusion coefficient of valeramide (×–×) and isovaleramide (○- - -○) as a function of phase-water content in lecithin–water lamellar phases.

Comparison with other systems

The ratio of D_l for valeramide to that for isovaleramide is 1.48 at $\Phi_w = 0.08$, the system with the lowest water content studied. In the human red cell, the ratio of the permeability coefficients of these same molecules is 3.75, more than twice as great. Hence, if the relation between rigidity and discrimination suggested above is correct, the lipids in the red-cell membrane are more highly structured than they are in the lipid–water system even at very low water content.

Lipid diffusion coefficients and membrane viscosities have been measured in a number of other biological systems and model membranes. In order to compare the present results with those of others, it is necessary to estimate the effect of tortuosity to compute a diffusion coefficient from D_l. The following procedure has been used to make a qualitative estimate of the tortuosity in the hydrophilic path which is also

the tortuosity in the lipid path*. In the limit, as Φ_w approaches 1.0, the measured diffusion coefficient of the hydrophilic solutes must approach the diffussion coefficient in bulk water. The measured diffusion coefficient of each hydrophilic solute in Table I, including water, has been divided by the free diffusion coefficient and the resulting ratios have been plotted on a logarithmic scale as a function of Φ_w. All of these curves must converge at the same point on the $\Phi_w = 1.0$ line and they must approach this line with zero slope. These constraints are sufficient to permit an estimate that the tortuosity will lie between 1.5 and 3.0, and we have taken a tortuosity of 2 as a reasonable figure. Using this tortuosity, the lipid diffusion coefficient for valeramide is $3.1 \cdot 10^{-8}$ cm$^2 \cdot$ s^{-1} at $\Phi_w = 0.08$ and rises by a factor of 20 to $6.5 \cdot 10^{-7}$ cm$^2 \cdot$ s^{-1} at $\Phi_w = 0.30$. The same estimated tortuosity may be applied to the data of Rigaud et al. [2] for the diffusion of benzene in lecithin–water lamellae prepared in a similar way, leading to a diffusion coefficient of $1.0 \cdot 10^{-6}$ cm$^2 \cdot$ s^{-1} at $\Phi_w = 0.08$ at 22 °C, which rises by a factor of less than 4 to a value of $3.7 \cdot 10^{-6}$ cm$^2 \cdot$ s^{-1} at $\Phi_w = 0.30$. Having no polar head it is easy for benzene to diffuse through the core of the lipidic region of the phase where the electron spin resonance studies of Jost et al. [8] and Rigaud et al. [9] have shown that the chains are more disordered than near the lipid–water interface. The much larger effect of decreased water content on the diffusion coefficient of valeramide is consistent with a much greater degree of order in the region through which the more polar valeramide diffuses, as compared to the case of the lipidic core.

In other biological and model systems, results obtained by electron spin resonance by McConnell and his colleagues [7, 11–13] are usually expressed in terms of diffusion coefficients, whereas those obtained by photodichroism by Cone [14] and fluorescence depolarization by Cogan et al. [15] and Rudy and Gitler [16] are given in terms of viscosity. It is instructive to make a qualitative comparison of the results despite the different methods applied to different probe molecules. For this purpose the Stokes–Einstein relationship has been used to compute approximate viscosities from diffusion data. The dimensions and the anisotropy of the probe molecules differ widely and no allowance is made for the ordered structure of the membrane, nor for the fact that the microviscosity is dependent on the position of the probe in the membrane. Notwithstanding the approximate nature of these apparent viscosities given in Table III, some interesting comparisons may be made. Our apparent viscosity of 0.1 poise for a lamellar phase with $\Phi_w = 0.30$ is in reasonable agreement with the value of 0.4 poise computed from Devaux and McConnell's [12] data on the diffusion of a spin-labelled analog of phosphatidylcholine in an oriented egg lecithin bilayer containing 30% water. The viscosity of 0.02–0.08 poise in the central core of the lipid phase permeated by benzene may be compared to the viscosity of 0.03 poise for hexadecane [17] at 20 °C. The diffusion coefficient for

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* The diffusion of hydrophilic solutes transversely across the lipid region makes a negligible contribution to the measured diffusion coefficient. The diffusion coefficient of these solutes across the lipid region should be less than that of water across lipid bilayers which is about $5 \cdot 10^{-10}$ cm$^2 \cdot$ s^{-1} [10]; preliminary measurements in this laboratory (Tong, S., Poznansky, M. J. and Solomon, A. K., unpublished) give a value of about $1 \cdot 10^{-11}$ cm$^2 \cdot$ s^{-1} for the diffusion coefficient of acetamide across an egg lecithin bilayer. These diffusion coefficients are smaller by many orders of magnitude than the diffusion coefficients given in Table I for the hydrophilic solutes.

TABLE III

DIFFUSION COEFFICIENTS AND APPARENT VISCOSITIES IN MODEL SYSTEMS AND BIOLOGICAL MEMBRANES

System	Probe	Temperature (°C)	Diffusion coefficient $\times 10^8$ ($cm^2 \cdot s^{-1}$)	Apparent viscosity (poise)	Note
Phosphatidylcholine	perylene	20		1.5	a
Phosphatidylcholine (plus cholesterol)	perylene	20		15	b
Phosphatidylcholine	spin-label analog of phosphatidylcholine	25	1.8	0.4	c
Dipalmitoyllecithin	spin-label androstane	41	1–3		d
Lamellar lecithin–water phases					
$\Phi_w = 0.08$	valeramide	20.6	3.1	2.0	e
$\Phi_w = 0.30$	valeramide	20.6	0.65	0.1	e
Lamellar lecithin–water phases					
$\Phi_w = 0.08$	benzene	22	0.97	0.08	f
$\Phi_w = 0.30$	benzene	22	0.376	0.02	f
Sarcoplasmic reticulum vesicles	spin label analog of phosphatidylcholine	20	3.6	0.9	g
Red-cell membrane	perylene	20		1.7	h
Visual receptor	rhodopsin	20		2	i

a. Cogan et al. [15]. Lipid dispersion, 0.02 % lipid.
b. Cogan et al. [15]. Same dispersion, lecithin–cholesterol molar ratio 1.0.
c. Devaux and McConnell [12]. Oriented bilayers of phosphatidylcholine; contain water, 30 % by weight. Viscosity computed as described in text, using 6.8 Å as radius of spin-label analog of phosphatidylcholine, computed as if probe were a sphere.
d. Sackmann and Träuble [18]. The diffusion coefficient has not been extrapolated to 20 °C because that is below the phase transition temperature for dipalmitoyllecithin. Träuble (private communication) states that the water content is greater than 30 %.
e. Present study.
f. Rigaud et al. [9]. Lipid–water lamellar phases.
g. Scandella et al. [13]. 20 °C figure obtained by Arrhenius extrapolation of author's data at higher temperatures (40–70 °C). Viscosity computed as described in text.
h. Rudy and Gitler [16]. Hemoglobin free membranes in 0.15 M NaCl, pH = 7.4 data computed by Arrhenius extrapolation using activation energy given by Rudy and Gitler.
i. Cone [14].

benzene in the lipidic core is about one order of magnitude less than the diffusion coefficient of $1.6 \cdot 10^{-5}$ cm$^2 \cdot$ s^{-1} for diphenyl in benzene at 25 °C [19].

The lipidic diffusion coefficient of valeramide at the highest water content studied, $\Phi_w = 0.30$, is $0.65 \cdot 10^{-8}$ cm$^2 \cdot$ s^{-1} about one order of magnitude greater than from the figure of $1.8 \cdot 10^{-8}$ cm$^2 \cdot$ sec^{-1} obtained by Devaux and McConnell [12] for diffusion of a spin-labelled analog of phosphatidylcholine in vesicles of about the same water content. This relatively small difference raises the possibility that valeramide, with its polar head, could diffuse along the lipid–water interface, requiring the displacement of all or part of a phosphatidylcholine chain for each valeramide diffusion jump.

The microviscosities computed by Cogan et al. [15] from fluorescence depolarization of perylene are more difficult to understand. Comparison of the first two lines of the table reveals quite clearly the effect of cholesterol addition on the microviscosity of phosphatidylcholine vesicles in accord with the electron spin resonance findings of Oldfield and Chapman [20]. However the value of 1.5 poise for the microviscosity of egg lecithin vesicles of high water content is appreciably higher than the values obtained by the other methods. Further, Rudy and Gitler [16], using the same probe, give data which show that the microviscosity of red-cell ghosts is 1.7 poise, virtually the same value as that given for lecithin vesicles, notwithstanding the very high content of cholesterol in red-cell membranes.

The apparent viscosity of 2 poise given by Cone [14] for the visual receptor membrane is in reasonable agreement with the estimate of 0.9 poise for sarcoplasmic reticulum vesicles, based on the data of Scandella et al. [13], since both these membranes are characterized by very low cholesterol contents. The increased discrimination which the human red cell makes between the isomers of valeramide, as compared to lipidic regions in phases of low water content, $\Phi_w = 0.08$, would argue that the apparent viscosity of the human red-cell membrane is appreciably greater than the 2 poise apparent viscosity of the lipidic regions, in conformity with the high cholesterol content of human red-cell membranes.

APPENDIX

Briefly the derivation of Eqn 3 is as follows. The differential equations which describe the diffusion process are:

$$\frac{\delta}{\delta t} C_1(x,t) - D_1 \frac{\delta^2}{\delta x^2} C_1 - K\left(\frac{C_2(x,t)}{A_2} - \frac{k\,C_1(x,t)}{A_1}\right) = 0 \tag{4}$$

$$\frac{\delta}{\delta t} C_2(x,t) - D_2 \frac{\delta^2}{\delta x^2} C_2 - K\left(\frac{k\,C_1(x,t)}{A_1} - \frac{C_2(x,t)}{A_2}\right) = 0 \tag{5}$$

where K determines the partition rate and k the partition coefficient. C_1 and C_2 are the linear concentrations in the two channels and A_1 and A_2 the cross-sectional areas. The subscripts 1 and 2 refer to water and lipid respectively. The equations can be solved by a spatial Fourier transformation and a temporal Laplace transformation. The transforms of $C_1(x,t)$ and $C_2(x,t)$ are equivalent to those which describe a single-diffusion mechanism with the diffusion coefficient given in Eqn 3 for

$$p^2 < \frac{K}{A_2 D_1} \quad \text{or} \quad p^2 < \frac{K}{A_2 D_2}$$

where p is the spatial Fourier transform variable. This implies that so long as measurements are made on a scale course compared to the length $\sqrt{(A_2 D_1/K)}$ this diffusion coefficient will be observed.

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