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THE PERMEATION OF ORGANIC ACIDS THROUGH LECITHIN BILAYERS RESEMBLANCE TO DIFFUSION IN POLYMERS*

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SUMMARY

1. The fluxes of aliphatic acids and their derivatives through black lipid membranes made of egg lecithin in decane were measured by means of a proton titration method.

2. Permeability coefficients were calculated and these were divided by the partition coefficient of the diffusing solute in different solvent systems: *n*-decane, olive oil, ether and octanol. The logarithms of the diffusion coefficients thus obtained were plotted against the logarithm of the molecular weight. The data could not be fitted to a single regression line in any solvent system.

3. When the logarithm of the diffusion coefficients were correlated to the logarithm of the molecular volume (= molecular weight/ specific gravity) all the diffusants could be fitted to the same regression line, indicating that the molecular volume is a better index of molecular size and shape than the molecular weight.

4. Analysis of the experimental results assuming a model of diffusion through soft polymers (Lieb, W. R. and Stein, W. D. (1971) Current Topics in Membranes and Transport, vol. 2, pp. 1–39. Academic Press, New York) showed that decane and olive oil are not adequate model solvents for planar lecithin membranes but ether and octanol are good models.

5. The differential mass selectivity coefficient was found to be similar to that for soft polymers and biological membranes, i. e. greater than 3.0.

6. Water could be fitted by the same regression line, thus emphasizing the generality of passive transfer and implying that water crosses lipid membranes as single molecules.

INTRODUCTION

The mechanism of passive permeation of non-electrolytes through biological membranes is a subject of great importance for understanding the primary function

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of the membrane: separating the intracellular medium from external environment. For those permeants which are in equilibrium at the membrane/water interfaces the overall transfer is the result of two processes: partitioning of the solute inside and outside the membrane and diffusion within the membrane. These processes depend upon certain physical and chemical properties of the permeating molecule and the membranes, such as lipid solubility of the permeant, its molecular size and shape, its hydrogen bonding ability in water, the liquidity of the membrane and the charge density at the membrane-water interface. Such a complexity of influencing factors is probably the reason for controversies regarding the rate-limiting step in the permeation process. Aqueous pores across the membrane have been suggested in order to account for some observed permeability patterns among members of homologous series of non-electrolytes [1, 2].

Recently Lieb and Stein [3] proposed a unifying theory for the selectivity and diffusion of non-electrolytic solutes of all sizes across biological membranes, their theory not requiring the postulate of porous parallel pathways. They showed that diffusion of non-electrolytes closely resembles diffusion in soft polymers with respect to diffusant size and shape, temperature dependence and the presence of low-molecular weight additives. They thus provided an insight into the behavior of biological membranes by linking this behavior to that of polymers, for which the diffusion process and the factors influencing it are much better understood. We have attempted in the present work to use planar phospholipid membranes to test the Lieb and Stein theory [3] with respect to one group of permeants, namely, organic acids. The theory has been tested experimentally previously using smectic mesophases [4–6]. Flux measurements of non-electrolytes in planar bilayers were recently summarized by Goldup et al. [7]. The advantage of planar bilayers lies in the possibility of direct flux measurements across membranes of known area and thickness. Important also is the possibility of determining the effect of unstirred layers without resorting to deriving the relevant parameters from phenomenological coefficients. The determination of absolute fluxes enables direct calculation of the diffusion coefficients.

MATERIALS AND METHODS

All aqueous solutions were prepared with triple-distilled water. Chemicals were purchased from the following suppliers: From Hopkins and Williams: formic, 2,4-dihydroxybenzoic, salicylic, phenylacetic, propionic, gallic acid. From B.D.H.: iodoacetic, butyric, isobutyric, valeric, isovaleric, lactic acid. From Eastman Organic Chemicals: *p*-hydroxybenzoic acid. From Fluka, A. G.: tiglic, vanillic, chloroacetic, bromoacetic, pivalic acid. From Frutarom: acetic acid. From Sigma Chemical Co.: *n*-decane, α-hydroxybutyric, α-hydroxyvaleric and α-hydroxycaproic acid. All chemicals were of analytical grade. Radioactive materials were purchased from the Radiochemical Centre, Amersham.

Phosphatidylcholine was prepared from fresh egg yolks according to Lea et al. [8] and Hanahan et al. [9]. The final compound appears as a single spot following thin-layer chromatography using a solvent mixture of chloroform/methanol/water (65 : 25 : 4, v/v).

Black lipid membranes were formed from a solution of 1–2 % (w/v) phosphatidylcholine in *n*-decane. The mixture was spread across a hole of 3 mm diameter

drilled in a teflon partition separating two chambers of a rectangular cuvette. The cuvette was positioned in a hollow brass mantle which was connected to a thermostatic bath so as to maintain a constant temperature of 25 ± 0.2 °C within the cuvette. Thinning of the membrane was observed optically.

Measurement of fluxes

Membranes were formed in 10 mM KCl solution free of CO_2. The pH of one of the chambers (the back chamber) was adjusted to slightly above 8.2 by adding a minute amount of KOH. The solute is added to the front chamber. Since all the solutes tested were weak acids, they remained in their undissociated form in the front chamber but once they crossed the membrane to the back chamber they dissociated due to the large difference between their pK and the pH prevailing there. The change of pH in the back chamber was monitored continuously by means of an expanded scale pH meter (Radiometer Model 26) and recorder (Yokogawa Model 3077). Thus the amount of protons entering the back chamber solution per unit time could be calculated. The background acidification due to CO_2 dissolution was subtracted from this value.

Measurement of partition coefficients

Partition coefficients were determined for most of the carboxylic acids used as follows: 10 ml of 10 mM aqueous solution were mixed an equal volume of *n*-decane for 1–5 min on a vortex mixer. Phases were then allowed to separate for 2 h in a 25 °C bath. Aliquots were then taken from both phases and assayed by means of one of the following methods: radioactive, spectrophotometric or by titration. In the first method labelled acid was added to the aqueous phase to obtain a specific activity of 1–3 mCi/mol. Samples from both phases were added to 15 ml of scintillation liquor (2,5-diphenyloxazole, 5.5 g · l^{-1}; 2,2-*p*-phenyl bis (phenyloxazole), 0.1 g · l^{-1}; Triton X-100, 33 % (v/v) in toluene) and counted in a liquid scintillation spectrometer (Packard Instruments 3385). Counts were corrected for quenching and the partition coefficient was calculated from the ratio of cpm in the two phases. When using the spectrophotometric method we first determined the molar extinction coefficient (ε) and the wavelength for maximum absorbance in water and decane, respectively, using a Gilford 240 spectrophotometer. The partition coefficient K was calculated using the following equation:

$$K_{\text{decane}} = \frac{A_{\text{decane}}}{A_{\text{H}_2\text{O}}} \cdot \frac{\varepsilon_{\text{H}_2\text{O}}}{\varepsilon_{\text{decane}}} \tag{1}$$

where ε is the molar extinction coefficient in the proper solvent. In both methods pH was adjusted to partitioning in order to keep the acid in its undissociated form. In the titration procedure, the phases were separated and then titrated with 10 mM NaOH. The partition coefficient is given by:

$$K_{\text{decane}} = \frac{\text{ml NaOH}_{\text{decane}}}{\text{ml NaOH}_{\text{H}_2\text{O}}} \tag{2}$$

This procedure was used also for measuring the partition coefficients for the phenyl derivatives in octanol. Values for ether and olive oil were taken from Seidell [10]

and for octanol from Collander [11], or derived from Collander's empirical equation:

$$\log K_{oct} = 1.24 \log K_{but} - 0.42 \quad (3)$$

where K_{oct} and K_{but} are the partition coefficients of a given solute in octanol and butanol, respectively. Values for K_{but} were taken from Collander [12]. Whenever possible, values were taken for the case of aqueous solution containing 10 mM solute and at 25 °C.

In some cases, as detailed in Table I, estimates of partition coefficients were derived from those of closely related solutes according to accepted rules relating the influence of additional functional groups to the solute on the partition coefficient [13].

RESULTS

Measurement of fluxes

The method used for flux measurements is convenient but limited to weak acids having relatively high permeabilities. This limitation is due to the background titration caused by CO_2 dissolution in the back chamber. The low buffer capacity of the back chamber allows a rapid decrease in the pH and the flux deviates from linearity after a relatively short time. Each pH unit is equivalent to $8 \cdot 10^{-8}$ mol of protons in our experimental set-up. Thus the flux of acid is given by:

$$J_{acid} = (a - b) \cdot 8 \cdot 10^{-8} \text{ mol} \cdot \text{s}^{-1} \quad (4)$$

where a and b are the rates of titration of the acid and of the CO_2, respectively, in pH units per s. The apparent permeability coefficient is given by:

$$P_{app} = J_{acid} / C \cdot A \quad (5)$$

where C is the concentration of the undissociated acid in the front chamber and A is the membrane area.

Correction for unstirred layers

In order to obtain the actual membrane permeability coefficient (P_{mem}) one should correct for unstirred layer effects [14]. This can be done as follows: it has been established by Gutknecht and Tosteson [15] that the permeability coefficient of salicylic acid in black lipid membranes is much larger than that in the adjacent aqueous unstirred layers. Therefore the thickness of the unstirred layer in any experimental set-up can be derived from P_{app} for this acid using the following equation:

$$\delta = D_{sal}/P_{sal} \quad (6)$$

where δ is the thickness of the unstirred layer and D_{sal} is the diffusion coefficient of salicilic acid in water. The value derived for our system was $8 \cdot 10^{-3}$ cm. Thus P_{mem} was calculated by the following equation:

$$\frac{1}{P_{mem}} = \frac{1}{P_{app}} - \frac{\delta}{D} \quad (7)$$

where D is the diffusion coefficient in water.

Influence of the pH in the front chamber on the flux

Since flux measurements are carried out by establishing a pH gradient across the membrane and the different acids tested have different pK values, one should test the influence of the pH in the front chamber on the flux. This has been done for isobutyric acid and the results shown in Fig. 1 indicate a linear relationship between the flux and the concentration of undissociated acid.

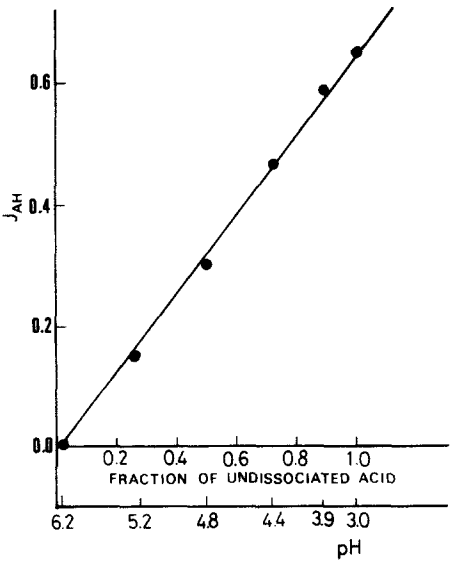


Fig. 1. Flux of isobutyric acid as a function of the pH in the front chamber. Flux is given in units of pH per min, as the difference between the slope of the rate of titration of the back chamber in the presence of acid in the front chamber, minus the slope of the rate of titration by atmospheric CO_2; 25 °C.

Parenthetically, such linearity also rules out an effect of solvent drag due to osmotic volume flow created by establishing an osmotic gradient across the membrane. This is due to a reflection coefficient of unity and/or a mean solute concentration in the membrane ($C_m = (C_f - C_f)/\ln(C_f/C_b)$) which is very low initially.

The effect of low pH(2.5), when the phosphate group of the phospholipid starts to protonate, has been tested with most acids. No effect could be detected beyond the effect on the undissociated form. Thus, lowering the pH so as to charge the front chamber interface has no measurable effect on the acid fluxes.

Diffusion coefficients and mass selectivity

Diffusion coefficients within the membrane were calculated according to the eqn [3]:

$$D_x/l = P_{mem}/K_x \tag{8}$$

where K_x is the partition coefficient for the solute between organic solvent x and water, and l is the thickness of the membrane. Thus D_x is the diffusion coefficient calculated with respect to a specific solvent x. Lieb and Stein [3] suggested that the

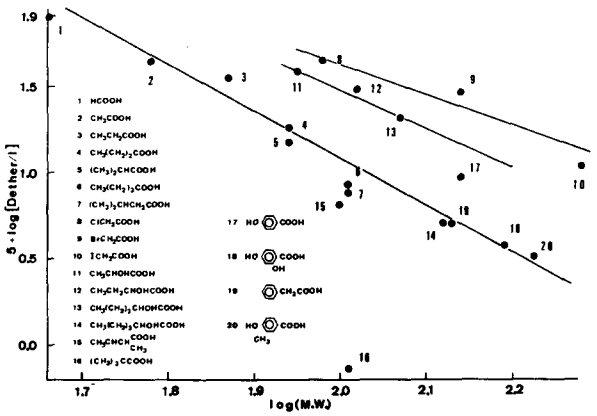


Fig. 2. Logarithm of D_{ether}/l obtained from Eqn 8 and using the partition coefficients ether/water vs logarithm of the molecular weight of the diffusant. Lines were obtained from regression analysis. Lower line was computed from solutes 2, 3, 4, 5, 6, 7, 14, 15, 18, 19 and 20, and yielded a slope of −2.68 and a correlation coefficient of 0.96. Addition of solutes 1 and 17 reduced the slope to −0.782 and the correlation coefficient to 0.565. Numbering of solutes corresponds to that of Table I.

following empirical equation should link the diffusion coefficient D to the Molecular weight M:

$$D = A \cdot M^{-sm} \tag{9}$$

A and sm are constants characteristic of a given membrane at a given temperature. A plot of log (D_x/l) vs log (molecular weight) should give a straight line with a slope sm which is defined as the differential mass selectivity coefficient. Figs 2–5 show such plots.

The general shape of $\log(D/l)$ vs $\log(M)$ lines is consistent with the empirical equation of Lieb and Stein [3], at least for the aliphatic acids. The regression slopes obtained with all solvent systems used for the determination of the partition coefficients (K_x) were much more negative than those obtained for diffusion in liquids [16] and closer to the differential mass selectivity coefficient (sm) for soft polymers [3].

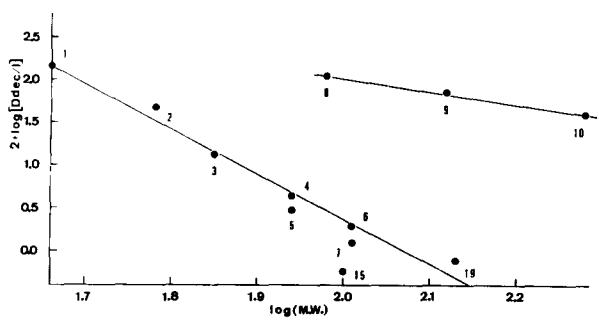


Fig. 3. Logarithm of D_{decane}/l obtained from Eqn 8 and using the partition coefficients decane/water vs logarithm of the molecular weight of the diffusant. Lines were obtained from regression analysis. Lower line was computed for solutes 2, 3, 4, 5, 6, 7 and 19 yielding a slope of −3.22 and a correlation coefficient of 0.94. Adding the phenolic derivatives (17, 18 and 20, not shown here) gave a slope of +0.726 and $r = 0.25$. Numbering of solutes corresponds to that of Table I.

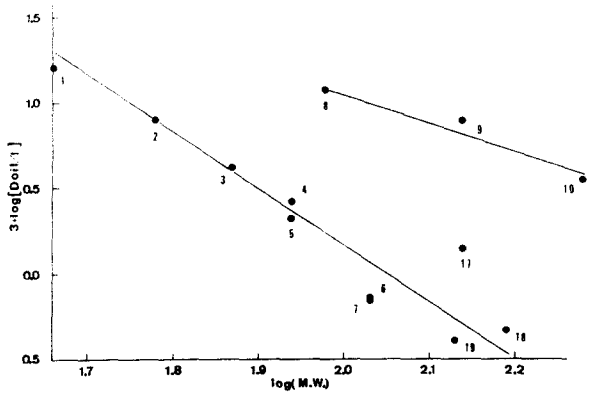


Fig. 4. Logarithm of D_{oil}/l obtained from Eqn 8 and using the partition coefficients olive oil/water vs logarithm of the molecular weight of the diffusant. Lines were obtained from regression analysis. Lower line was computed for solutes 2, 3, 4, 5, 6, 7 and 19 yielding a slope of -3.22 and $r = 0.94$. Adding solutes 1 and 17 gave a statistical analysis of slope -0.76 and $r = 0.50$. Numbering of solutes corresponds to that of Table I.

The use of different solvents for the determination of K_x yielded different values of *sm* but always over 2.5. This analysis has been carried out on the assumption that the organic solvent used is a good representative of membrane solvation. This assumption will be discussed critically later on.

Steric hindrance

Diffusion coefficients are very sensitive to molecular shape and steric hindrance: the three isomers of valeric acid together with the closely related tiglic acid serve as a good example. Such behavior is well known and widely documented for biological membranes [2, 16]. Branching in an isomer generally lowers the partition coefficient due to a decrease in the molecular surface area for van der Waals interactions with the lipid phase. The decrease in P, however, in passing from valeric to isovaleric, for example, is larger than the decrease in K (except for K_{oil}). This is an

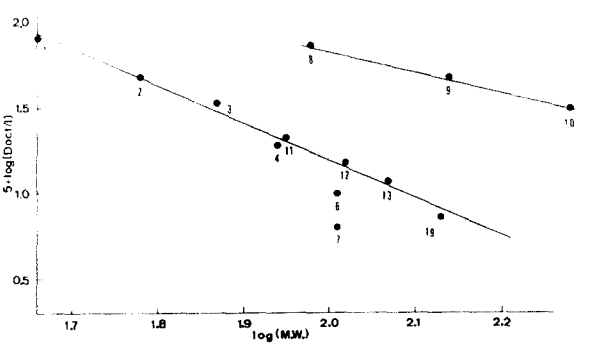


Fig. 5. Logarithm of D_{oct}/l obtained from Eqn 8 and using the partition coefficients octanol/water vs logarithm of the molecular weight of diffusants. Lines were obtained from regression analysis. Lower line was computed for solutes 1, 2, 3, 4, 5, 6, 7 and 19, and yielded a slope of -2.55 and $r = 0.96$. Numbering of solutes corresponds to that of Table I.

indication that branching affects the diffusion process per se. The behavior of pivalic acid is an interesting case in this respect, since its values of K are all larger than those for its straight chain isomer, while its permeability is much smaller. The extreme influence of steric hindrance in this case is indicative of the ordered character of the lipid barrier, so that the long hydrocarbon chains of the phospholipids accommodate preferentially elongated diffusants rather than spherical ones.

The effect of hydroxyl group

Comparison of aliphatic chain acids with their α-hydroxy derivatives which interact more strongly with water due to two additional hydrogen bonds shows that the decrease in P upon the addition of an -OH group is equal to or smalller than the decrease in K. Thus the increase in the ability of hydrogen bonding influences the free energy of partition much more than the free activation energy of the diffusion step.

This idea can be discussed also by comparing the relative effects of $-CH_2-$and -OH groups on P and K_{ether}, for example. While K_{ether} increases 3.1 times for each $-CH_2-$ group, it is decreased 15 times by one -OH group. Thus one -OH group balances 2.4 $-CH_2-$ groups ($(15)^{1/2.4} = 3.1$). However, in terms of permeability coefficients in the present system one -OH group balances the effect of 6 $-CH_2-$ groups, very similarly to the relations found for alcohols in the rabbit gall bladder [17]. The conclusion reached there concerning alcohols is applicable to carboxylic acids in the present system, namely, that the solute crosses the membrane through a hydrocarbon core free of hydrogen-bonding sites.

DISCUSSION

The aims of the present work were to get a deeper insight into the mechanism of membrane selectivity. In discussing possible mechanisms one must consider the controversy between the proponents of a membrane which acts as a molecular sieve and consists of a mosaic of lipophilic areas and aqueous pores [1, 2] and those who suggest that permeation is governed by the partition coefficient and by the diffusion coefficient for movement inside the membrane which closely resembles diffusion in soft poymers [3]. It may be that biological membranes do indeed contain aqueous pores. However, the use of model membranes where the presence of aqueous pores is unlikely seemed desirable as a further step in the elucidation of the permeation mechanism of non-electrolytes.

Representation of the molecular dimensions by molecular weight and molecular volume

The validity of Lieb and Stein's [3] empirical equation has been tested only for solutes having specific gravity close to unity. For such solutes the molecular weight can be assumed to be a good representative of the molecular volume which is the correct parameter to be used for a model resembling diffusion through polymers. When we used solutes with specific gravity significantly larger than one we observed considerable departures from the main regression line for the aliphatic acids. This is exemplified by the halogen derivatives of acetic acid which show similar deviations in all solvent systems. Such deviations must, therefore, be due to the intrinsic molecular properties of these solutes and not to the character of the solvent system used.

TABLE I

Permeability coefficients (p) of organic acids in black lipid membranes formed from 1 to 2 % egg lecithin in *n*-decane; partition coefficients (organic solvent/water) for *n*-decane (K_{dec}), ether (K_{ether}), olive oil (K_{oil}) and octanol (K_{oct}); molecular volume and number of hydrogen bonds formed in aqueous solutions. The numbering of solutes is identical to that of the figures. N_H, assumed number of hydrogen bonds.

No. Acid	$10^4 \times P$ (cm/s)	K_{decane}	K_{ether}	K_{oil}	$K_{octanol}$	Molecular weight / Specific gravity ($cm^3 \cdot mol^{-1}$)	N_H
1 Formic acid	2.34	1.7	0.30	0.015	0.29	41	3
2 Acetic acid	2.38	5.25	0.52	0.03	0.49	57	3
3 Propionic acid	6.1	43.0	1.7	0.15	1.8	75	3
4 Butyric acid	11.5	250.0	6.1	0.44	6.2	91.5	3
5 Isobutyric acid	9.25	280.0	6.0	0.44		90.5	3
6 Valeric acid	18.0	1070.0	21.0	2.6	18.1	108	3
7 Isovaleric acid	13.3	900.0	17.0	1.92	21.2	108	3
8 Chloroacetic acid	11.6	10.0	2.5	0.1	1.3	68	3
9 Bromoacetic acid	12.3	17.0	4.2	0.16	2.6	72	3
10 Iodocaetic acid	10.9	28.0	7.2	0.32	4.5	90	3
11 Lactic acid	0.5		0.13		0.24	75	5
12 α-Hydroxybutyric acid	1.24		0.40		0.83	92.5	5
13 α-Hydroxyvaleric acid	2.95		1.4**		2.5†††	110	5
14 α-Hydroxycaproic acid	2.14		4.2***			127	5
15 Tiglic acid	13.5	2200.0	24.0†			104	3
16 Pivallic acid	2.4	3000.0	32.0			113	3
17 *p*-Hydroxybenzoic acid	8.25	3.8	9.0	0.6	21.0	109.5	5
18 2, 4-Dihydroxybenzoic acid	4.7	2.0	12.0	1.0	26.0	122	5 (?)
19 Phenylacetic acid	8.05	790.0	16.0	2.0	15.0	125	3
20 Vanillic acid	2.62	25.0	10.0††		19.0	134	4–5
21 Gallic acid	—*		0.45	0.025		170	8
22 Water	20	$5 \cdot 10^{-5}$	0.013	0.0014	0.04	18	3

* Below detection limits.

** Value estimated from values of butyric, valeric and α-hydroxybutyric acid.

*** K_{ether} of α-hydroxy valeric acid was multiplied by 3, the mean increase in K_{ether} upon addition of $-CH_2-$ group.

† K_{ether} for crotonic acid multiplied by 3.

†† K_{ether} for vanillin multiplied by factor relating partition of aldehydes to that of acids.

††† Value estimated from K_{oct} values of butyric, valeric and α-hydroxy butyric acid.

Validity of the parameter used to estimate size could be improved by using instead of the molecular weight the molecular volume: the molecular weight divided by the specific gravity.

An example which justifies the use of molecular volume ($\bar{M}$) is the case of the halogen derivatives of acetic acid. From Table I it can be seen that the molecular volumes of these derivatives cluster around $\bar{M}$ of propionic acid. Indeed, it is well known from pharmacological studies that the molecular volume of a $-CH_3$ group is equivalent to that of bromide (isosterism), larger than the volume of chloride and smaller than that of iodide [18]. As for the phenolic derivatives, it would be incorrect to use their specific gravity for the calculation of molecular volume since these phenols possess an extra hydrogen which considerably increases their specific gravity. For these compounds it is more correct to use the specific gravity of benzoic acid to which they bear geometrical resemblance. The $\bar{M}$ for α-OH derivatives has been calculated without taking into account their extra hydrogen-bond. Hence the molecular volume may have been underestimated. When the data of log (D/l) are replotted against log ($\bar{M}$), all solutes group around one single regression line (Fig. 6).

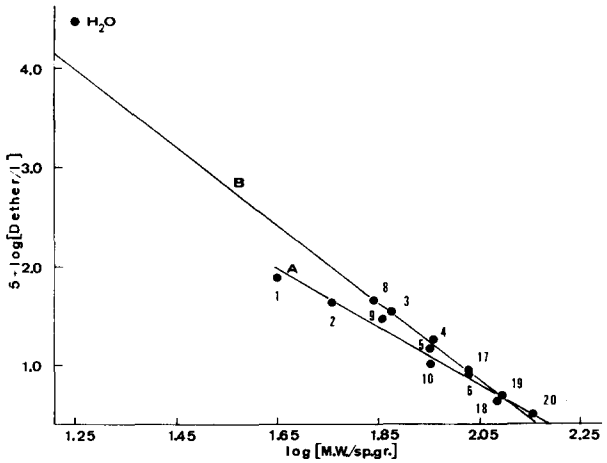


Fig. 6. Logarithm of D_{ether}/l vs logarithm of the molecular volume (molecular weight/specific gravity) of the diffusant. (A) The regression line for all diffusants except α-OH derivatives; slope = −3.611; r = 0.891. (B) The regression line except for α-OH derivatives but including water; slope = −3.88; r = 0.731. Numbering of solutes corresponds to that of Table I.

Choice of the most representative solvent system

We are now in a position to evaluate the extent to which the partition data in the different solvent systems used are adequate for the phosphatidylcholine planar membrane. The following test can be carried out [3]. The permeability coefficient is given by

$$\log P = \log P_0 + sk \log K - sm \log \bar{M} \qquad (10)$$

where sk is a coefficient which measures the extent of the deviation of K from the real partition coefficient; sk will equal 1 when the hydrophobicity of the solvent system is the same as that of the membrane. sm is the differential mass coefficient for diffusion

TABLE II

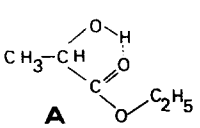
RESULTS OF DOUBLE LINEAR REGRESSION ANALYSIS OF LOG(P) VS LOG($\bar{M}$) FOR ALL THE SOLUTES AND THE VARIOUS SOLVENTS

r is the correlation coefficient.

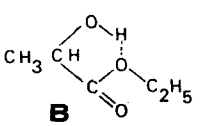
Solvent	*sk*	*sm*	*r*	Remarks
n-Decane	0.155	−0.245	0.581	
Olive oil	0.559	−2.203	0.645	
Ether	0.790	−2.770	0.950	
Ether	0.979	−3.611	0.891	without α-OH derivatives
Octanol	0.980	−4.245	0.860	

across the membrane. The values of *sk* and *sm* are obtained by means of a double regression analysis. Results of such an analysis are given in Table II. For both decane and olive *sk* values are much below unity and thus cannot serve as models for phosphatidylcholine membranes on pure statistical grounds. The value of *sk* for olive oil is very similar to that found for phosphatidylcholine liposomes by Cohen [4]. Lieb and Stein [3] found olive oil to be a good model for Collander's results on *Chara* membranes and to be more hydrophilic than *Nitella* or erythrocytes membranes. Since licithin membranes are best represented by the even more hydrophilic solvents ether and octanol, one may conclude that lecithin membranes are more hydrophilic than the biological membranes considered by Lieb and Stein.
This may be due to the high degree of unsaturation of egg lecithin [19]. Ether can serve as a good model when the α-OH derivatives are omitted. In contrast in octanol it is the phenolic compounds and the halogen derivatives of acetic acid which increase the statistical error of the fit.

The departure of the α -OH derivatives in the ether system can be explained as follows: It is known from infrared spectra that ethyl esters of lactic acid adopt two conformational states, A and B, due to intramolecular hydrogen bonding [20]. Conformation A is preferred over conformation B at a ratio of 6:1.



A



B

Thus the ester forms its own lipophilic environment. It is most likely that lactic acid as well as other α -OH acids would adopt the A configuration once dissolved in a lipophilic medium. Thus the relatively high diffusion coefficients of these derivatives may be explained by the reduced interaction with the medium, and hence the reduced fit when the diffusion coefficient of these compounds are included in the regression analysis. In the case of octanol which is more hydrophilic than ether and is able to form the hydrogen bonds with the solute, this departure is not observed, as expected. An extreme case of the influence of intramolecular hydrogen bond on the diffusion coefficient is found with salicylic acid which has a strong and permanent intramolecular hydrogen bond. The diffusion coefficient of this acid calculated from the permeability coefficient in black membranes [15] and from known K [12] is 30-fold larger than that of *p*-hydroxybenzoic acid found in the present work.

The halogen derivatives exhibit a general tendency for high diffusion coefficients compared to acetic acid in spite of their larger molecular volume. This can be related to a reduced dipole moment of the carbonyl link as a result of the inductive effect of the halogen. This effect can explain a larger diffusibility of the halogen derivatives due to reduced electronic interactions with membrane medium [1]. Dipole-dipole interactions can influence the diffusion rate by creating energetic barriers for the permeating molecule when the moving dipole is passing over a fixed dipole (permanent or induced). Weaker interaction with the medium can result even from a localized reduction of the dipole moment while the total one remains unchanged.

One must conclude from the foregoing discussion that while octanol is probably an adequate representative of phosphatidylcholine membranes with respect to hydrophilicity, by using less hydrophilic ether as a solvent system it is possible to reduce the scatter of experimental data considerably. One should try therefore other solvents, such as simple esters, to get a better fit.

Attempts to fit water into the general curve

The mechanism of water permeation through a lipid barrier is still obscure. The demonstration that the water permeability coefficient in lipid bilayer membranes is within the range of biological membranes was rather unexpected [19, 21–23], since the high permeability of biological membranes to water was formerly attributed to aqueous pores. We tried therefore, as an obvious test, to calculate the permeability coefficient of water (P_w) from the data obtained using the regression lines obtained using solutes other than water for the octanol system. We obtained a P_w of $7 \cdot 10^{-3}$ $cm \cdot s^{-1}$. This is to be compared with the $1.0 \cdot 10^{-3}$–$1.8 \cdot 10^{-3}$ $cm \cdot s^{-1}$ obtained by Andreoli et al. [25] using red blood cell membrane lipids or with the $3.7 \cdot 10^{-3}$ $cm \cdot s^{-1}$ reported by Cass and Finkelstein [22] for egg lecithin in tetradecane or with the $2 \cdot 10^{-3}$ $cm \cdot s^{-1}$ (extrapolated to 25 °C) reported by Graziain and Livne [19] for egg lecithin in decane. The fact that the calculated value is only some 2–3-fold larger than the expected values allows us to conclude that water probably permeates through the lipid membrane by a process of partitioning into and diffusion across the membrane.

Some comparisons between phosphatidylcholine bilayers and biological membranes

The fluxes of aliphatic acids (from propionic to octanoic) have been measured in toad bladder [24] and were found to increase with chain length for the first three acids and remain at the same values thereafter. It may well be that unstrired layers affect the more lipophilic solutes. When an approximate correction is made for unstirred layers, the ratio of the permeability coefficients is found to obey the same mass selectivity as phosphatidylcholine model membranes, though the bladder membrane is far more rigid (Table III).

Another comparison has been attempted between relative permeabilities derived from reflection coefficients measured in rabbit gall bladder [16] and the present data (Table IV). Here also we observe a rather good agreement in the selectivity pattern of both types of membranes. The important point in this comparison is that halogenic substitution enhances the permeability considerably more than methylic substitution, although the changes in the partition coefficients and molecular volumes

TABLE III

COMPARISON OF PERMEABILITY COEFFICIENTS, P ($cm \cdot s^{-1}$) OF TOAD BLADDER AND PHOSPHATIDYLCHOLINE MEMBRANES

Correction for unstirred layers in the toad bladder was done assuming $\delta = 3 \cdot 10^{-2}$ cm, obtained from the flux of octanoic acid. $10^4 \times P(cm \cdot s^{-1})$.

Acid	Toad bladder		Phosphatidylcholine bilayer
	Observed	Corrected for unstirred layers	
Propionic acid	0.67	0.9	6.1
Butyric acid	1.13	1.8	11.5
Valeric acid	1.44	3.2	18.0
Hexanoic acid	2.45	?	–
Heptanoic acid	2.35	?	–
Octanoic acid	2.38	?	–

from those of acetic acid or acetamide are very similar in both substitutions. This observation supports our conclusion concerning the effect of halogenic substitution on the diffusion coefficient.

In conclusion we can state that since the experimental values for the differential mass selectivity coefficient derived in the present work are very significantly different from $-1/2$ or $-1/3$. It is therefore most unlikely that passive transfer of organic acids occurs through aqueous pores. Lieb and Stein's [3] model of diffusion through soft polymers is preferable in this context. In analyzing permeation processes both partition and diffusion coefficients must be considered since both influence membrane selectivity. A separate analysis of these two parameters enables the derivation of *sk* which is a measure of the hydrophilicity of the membrane and *sm* which reflects the aggregative and fluidity properties of the membrane. The explicit relationship of *sm* to the molecular characteristics of the membrane requires further theoretical and experimental investigations in which the planar black membrane could serve as a very useful tool.

TABLE IV

COMPARISON OF PATTERNS OF PERMEABILITY COEFFICIENTS OF AMIDES IN RABBIT GALL BLADDER MEMBRANE [16] WITH THE PATTERNS OF PERMEABILITY COEFFICIENTS IN PHOSPHATIDYLCHOLINE BILAYERS

The permeability coefficients for the rabbit gall bladder were obtained from the relationship $(1-\sigma)\,\bar{V}_s = \omega_s/L_p$ assuming that solutes cross the membranes do not interact with water.

Solute	Rabbit gall bladder ($10^2 \times \omega_s/L_p$ ($mol \cdot cm^{-3}$))	Phosphatidylcholine bilayer ($10^4 \times P$ ($cm \cdot s^{-1}$))
Chloroacetamide	1.03	
Chloroacetic acid		11.6
Iodoacetamide	0.76	
Iodoacetic acid		10.9
Propionamide	0.49	
Propionic acid		6.1

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