

Diffusion within Egg Lecithin Bilayers Resembles That within Soft Polymers

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ABSTRACT An analysis is presented of how the permeability coefficient/octanol:water partition coefficient ratio for 33 different chemical substances crossing egg lecithin bilayers depends on the molecular volume of the substances. From this analysis we conclude that bilayers made from egg lecithin behave as soft polymers in their discrimination between permeants of different sizes and shapes.

INTRODUCTION

In a recent study (Wolosin and Ginsburg, 1975) two of us, using a proton titration method, measured the fluxes of aliphatic acids and their derivatives through black lipid membranes made of egg lecithin in decane. Relative diffusion coefficients within the membranes were estimated by dividing the measured permeabilities by the partition coefficients obtained from model solvent systems. The dependence of these relative diffusion coefficients on the size of the permeant molecules was very steep. It was concluded that diffusion within these lipid bilayers resembles diffusion in soft polymers (p-type diffusion) but not diffusion in simple liquids (s-type diffusion).

Finkelstein (1976) has now criticized the experimental basis of the Wolosin-Ginsburg study and, using his new data, has reached an essentially opposite conclusion. We wish to show that his criticism is not justified, that these data are consistent with those of three other studies in the literature and that a consideration of all the published data, taken together, leads to the conclusion that diffusion within egg lecithin bilayers displays a steep mass dependence typical of diffusion in soft polymers.

Finkelstein's only objection to our experimental data lay in his belief that the measured fluxes were not adequately corrected for unstirred layers of water. Using values for decane:water partition coefficients published in Table I of our paper, he concluded that the rate of penetration of the acids through the bilayers must be exceedingly fast, so that the measured fluxes were determined mainly by unstirred layers. Unfortunately, a typographical error¹ in this Table

¹ A factor of 10^{-4} for the partition of the acids between *n*-decane and water was missed. We have already sent an erratum note to *Biochimica et Biophysica Acta*, but for clarity we will state here that the partition coefficient of, e.g., acetic acid is 5.25×10^{-4} and not 5.25 as published. The erroneous values appeared only in Table I, and the correct values were indeed used throughout the remainder of the paper.

TABLE I
PERMEABILITY COEFFICIENTS OF NONELECTROLYTES IN EGG LECITHIN
LIPID BILAYER MEMBRANES AND THEIR OCTANOL-WATER PARTITION
COEFFICIENTS

No.	Compound	$P \times 10^4$	Ref.	K_{oct}	Ref.
		<i>cm/s</i>			
0.	Water	20-22	1, 2	0.04	I
1.	Formic acid	2.34	1	0.29	I
2.	Acetic acid	2.38	1	0.49	I
3.	Propionic acid	6.1	1	1.8	I
4.	Butyric acid	11.5	1	6.2	I
5.	isoButyric acid	9.25	1	6.0	II
6.	Valeric acid	18.0	1	18.1	I
7.	isoValeric acid	13.3	1	21.2	I
8.	Chloroacetic acid	11.6	1	1.3	I
9.	Bromoacetic acid	12.3	1	2.6	I
10.	Iodoacetic acid	10.9	1	4.5	I
11.	Lactic acid	0.5	1	0.24	I
12.	α -Hydroxybutyric acid	1.24	1	0.83	I
13.	α -Hydroxyvaleric acid	2.95	1	2.8	II
14.	α -Hydroxycaproic acid	2.14	1	6.4	II
15.	Pivallic acid	2.4	1	29	I
16.	α -Hydroxybenzoic acid	8.25	1	21	I
17.	2,4-Dihydroxybenzoic acid	4.7	1	26	I
18.	Phenylacetic acid	8.05	1	15	I
19.	Vanillic acid	2.60	1	19	I
20.	Formamide	1.03	2	0.062	II
20.	Formamide	0.78	3	0.062	II
20.	Formamide	1.6	4	0.062	II
21.	Acetamide	1.6	2	0.089	II
21.	Acetamide	0.24	3	0.089	II
21.	Acetamide	1.43	4	0.089	II
22.	Propionamide	0.61	3	0.24	I
23.	Valeramide	1.80	3	2.40	III
24.	Ethylene glycol	0.18	4	0.086	II
25.	Propylene glycol	0.04	4	0.13	II
26.	Glycerol	0.057	4	0.011	II
26.	Glycerol	0.046	5	0.011	II
27.	Erythritol	0.0075	5	0.0063	I
28.	Urea	0.036	4	0.026	II
28.	Urea	0.042	4	0.026	II
28.	Urea	0.041	3	0.026	II
29.	<i>N</i> -methyl urea	0.39	4	0.072	I
30.	<i>N,N</i> -Dimethyl formamide	1.13	4	0.56	II
31.	1,4-Butanediol	2.6	2	0.12	IV
32.	Thiourea	0.046	4	0.1	II
				<u>K hexadecane</u>	
33.	Pentanol			0.40	V
34.	Hexanol			1.28	V
35.	1,6-Hexanediol			5.4×10^{-4}	VI

1, Wolosin and Ginsburg, 1975; 2, Finkelstein, 1976; 3, Poznansky et al., 1976; 4, Gallucci et al., 1971; 5, Vreeman, 1966.

I, Already published data derived either from direct octanol-water measurements (Collander, 1951; Wolosin and Ginsburg, 1975) or from isobutanol-water measurements (Collander, 1950), by using Collander's formula (Collander, 1951).

II, New data; present study; direct measurements.

III, Computed by using the ratio between the known partition coefficient for propionic and valeric acids and the value for propionamide.

IV, This is the partition coefficient for 2,3-butanediol (Collander, 1951).

V, Aveyard and Mitchell, 1969.

VI, Finkelstein, 1976.

made these decane:water partition coefficients appear to be 10,000 times larger than they actually are, thus understandably leading Finkelstein to his incorrect conclusion. In fact, all permeability coefficients were carefully corrected for unstirred layers, which were measured by use of a highly permeant molecule (Gutknecht and Tosteson, 1973).

To test whether our data were a good measure of the permeability of egg lecithin bilayers, we decided to construct a composite plot of data taken from all published studies on black films of egg lecithin that we were able to locate. These data consist of studies of the permeabilities of a wide variety of different chemical substances. In order to put all these together on a single plot, it is necessary to relate each permeability measurement to the partition coefficient for that substance by using one single representative solvent for the entire range of permeants. For reasons that will become clear later, we decided to take octanol as the reference solvent. Thus for each measurement we needed the ratio of the permeability coefficient to the octanol-water partition coefficient (i.e. the relative diffusion coefficient). Since these were not available in the literature, we had to measure some of these octanol-water partition coefficients.

These partition coefficients are reported in the present paper. An analysis is presented of how the permeability coefficient/partition coefficient ratio depends on molecular volume for 33 different chemical substances crossing egg lecithin bilayers.

MATERIALS AND METHODS

Aqueous solutions were prepared with double-distilled water. Chemicals were obtained from the following suppliers: from Sigma Chemical Co. (St. Louis, Mo.), α -hydroxy valeric acid and α -hydroxy caproic acid; from Riedel De Haën (Seelze-Hannover, West Germany), formamide, acetamide, ethylene glycol, and *n*-octanol; from Frutarom (Haifa, Israel), propylene glycol and glycerol; from Hopkins and Williams (Chadwell Heath, Essex, England), urea and thiourea; from Merck AG (Darmstadt, W. Germany), isobutyric acid and *N,N*-dimethyl formamide. Radioactive materials were purchased from the Radiochemical Centre, Amersham, England.

Measurement of Partition Coefficients

Partition measurements were performed with water-saturated *n*-octanol and *n*-octanol-saturated water.

For the acids, a 20 mM aqueous solution was partitioned with an equal volume of octanol and after phase separation (2 h, 25°C) the organic phase was re-extracted with the same volume of water. The undissociated acid content in both aqueous solutions was assayed by titration with a 10 mM NaOH solution and the partition coefficient calculated from the relation

$$K_p = \frac{\text{NaOH}_r}{\text{NaOH}_o - \text{NaOH}_r},$$

where NaOH_o is the volume of base needed for the titration of the original solution and NaOH_r is the volume of base needed for the titration of the recycling solution.

For measuring the partition coefficients of amides and polyalcohols, a 0.5% (wt/vol) octanol solution of the substances was extracted with water using 0.1 or 0.2 of the volume of the octanol solution. After phase separation the water was evaporated (40°C,

20 mm Hg) and the residue weighed. From this weight, the percent of solute in both phases, and hence the partition coefficient, was derived. There was no systematic difference between the partition coefficients obtained using the 0.1 or 0.2 relative water volumes. The recorded value represents the mean between the two values obtained.

For formamide and acetamide the concentration in the octanol phase was confirmed also by quantitative infrared spectroscopy ($\nu = \text{o}$ stretch band). The organic phase concentration was determined by matching with octanol solutions of known concentration of the studied substance.

For urea and thiourea, radioactive tracer experiments were performed. A 100 mM aqueous solution was labeled to about $0.1 \mu\text{Ci/ml}$. After partition and phase separation, samples were taken from both phases and counted in a liquid scintillation spectrophotometer in scintillation liquor.

Partition coefficients were calculated from the counts per minute/milliliter ratio in each phase after correction for quenching.

RESULTS

In Table I we record the new partition coefficients which we have measured, together with those that have been previously reported in the literature. Table I also records the permeability coefficients for the substances listed. In Fig. 1, for each substance, the ratio of permeability coefficient to octanol-water partition coefficient is plotted against the ratio of the molecular weight to the specific gravity of the pure substance (i.e. the molecular volume). Taken together, it can be seen from Fig. 1 that most of the published data are fairly consistent with one another and indicate a very steep size dependence for diffusion within these black films. The aberrant behaviour of permeants 25, 28, 31, and 32 will be discussed later. When one considers that the data come from five different laboratories using permeants which include alcohols, amides, fatty acids, and aromatic compounds, the coherence of the data is remarkably good.

DISCUSSION

It is perhaps necessary first to justify our choice of octanol as a model solvent, especially in view of Finkelstein's conviction that on a priori grounds the model solvent should be a hydrocarbon (e.g., hexadecane). Simple considerations suggest that hydrocarbons are not good model solvents (cf. Table I where the necessary data are recorded). The addition of a CH_2OH terminal group to a molecule (compare pentanol and hexanediol) reduces its hexadecane:water partition coefficient by a factor of ~ 700 , but reduces its permeability coefficient by a factor of < 10 (compare ethylene glycol and glycerol, or glycerol and erythritol); any size dependence of diffusion coefficients would act so as to increase even further this disparity. The analogous figures for the addition of an OH group are 2,000 and < 10 for the hexadecane:water partition coefficients (compare hexanol and hexanediol) and the bilayer permeability coefficient (compare propylene glycol and glycerol or aliphatic and α -hydroxy acids), respectively. On the other hand, the octanol:water partition coefficients (Collander, 1951) decrease in roughly the same fashion as black film permeability coefficients upon the addition of these groups.

A more rigorous approach put forward a number of years ago (Lieb and

Stein, 1969, 1971 *a, b*) is to perform a multivariate regression analysis of permeability coefficients on partition coefficients and size. One of the outputs of such an analysis is the validity index s_k , which is a measure of the appropriateness of the organic solvent as a model for the permeation process. For a perfect match, the validity index is unity. Wolosin and Ginsburg (1975) found that for octanol $s_k = 0.98$, whereas for decane $s_k = 0.16$. This confirms the

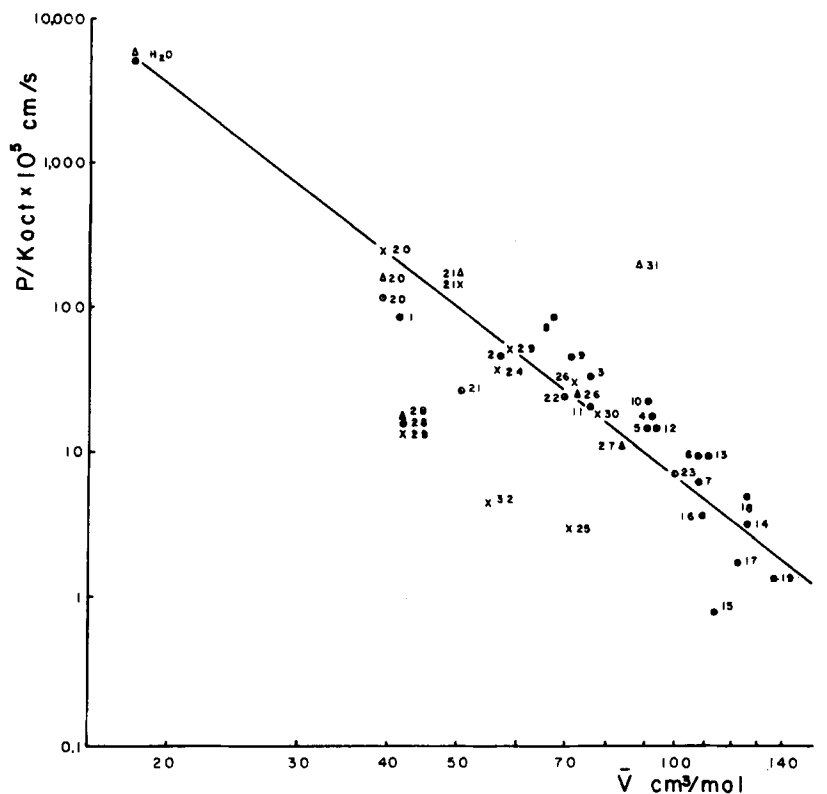


FIGURE 1. Composite plot of all published data on permeability of nonelectrolytes across black films of egg lecithin. Ordinate, permeability coefficient ($\text{cm} \cdot \text{s}^{-1}$) divided by relevant octanol-water partition coefficient. Abscissa, molecular weight of permeant divided by the specific gravity. Numbering of points as in Table I. ●, Wolosin and Ginsburg, 1975; Δ, Finkelstein, 1976; ○, Poznansky et al., 1976; ×, Galluci et al., 1971; ▲, Vreeman, 1966.

result of the simple analysis mentioned above, that octanol is a good model solvent whereas hydrocarbons are not. Of four solvents treated in this fashion by Wolosin and Ginsburg (1975), octanol was shown to be the best model solvent.

It may seem strange that octanol proves to be a good model solvent for the permeation process in lipid bilayers, as Finkelstein rightly points out, since such bilayers have an interior composed of hydrocarbon chains. However, the permeation experiments probe those parts of the membrane which provide the

greatest resistance to passage of solutes (Diamond and Katz, 1974; Poznansky et al., 1976). There is no reason to assume that the hydrocarbon-like interior is the rate-limiting barrier. Indeed, it is well known that the fluidity of the bilayer interior is much greater than that near the glycerol backbone, and it may well be that this glycerol backbone region is the rate-limiting barrier for permeation. If so, then the relevant partition coefficient is that for partitioning into this region, not for partitioning into the hydrocarbon-like interior. The fact that it is octanol and not hexadecane that mimics the permeation pattern supports this interpretation. On the other hand, if one calculates from the data in Table I absolute diffusion coefficients assuming 30 Å thickness for the rate-limiting diffusion barrier, one obtains values ranging from 1.5×10^{-8} for water to around 2×10^{-12} for the bigger solutes. No direct measurement is available of the diffusion coefficient of any solute in the direction perpendicular to the membrane plane, but diffusion coefficients for phospholipids or hydrophobic probes in the plane of the membrane fall in the 10^{-8} range (Lee et al., 1974). Thus the values we obtain using Table I are rather low. There is, however, no reason to assume that the relevant partition coefficients for the lecithin bilayer have the same absolute values as those for the water:octanol system. It may be that octanol correctly represents the discriminatory power of the rate-limiting barrier but the absolute partition values between that region and the bulk water may well be lower. It is interesting to note that the partition coefficients of solutes in saturated lecithin liposomes (Katz and Diamond, 1974) are indeed close to the values for the octanol:water system. This suggests, if the above arguments are correct, that these workers determined partitioning into that region which is rate-limiting for permeation, namely the region near the glycerol backbones. It should be pointed out that the rate-limiting barrier may be different for different types of permeants (e.g., large vs. small, hydrophilic vs. hydrophobic) and that more than one barrier may be rate-determining (Diamond and Katz, 1974).

Urea, thiourea, 1,4-butanediol, and propylene glycol deviate markedly from the main pattern of points in Fig. 1. The permeability of urea has been independently determined three times with very consistent results, so that the low value of its relative diffusion coefficient must be real. It may be that the shape and rigidity of the urea molecule confer upon it a low diffusion coefficient, or else that a different region of the membrane is the rate-limiting barrier for urea permeation. The latter possibility is supported by the anomalously low values of enthalpy and entropy of partitioning into lecithin liposomes compared to other hydrophilic molecules (Katz and Diamond, 1974). Similar considerations may well apply to thiourea. The deviation of propylene glycol and 1,4-butanediol from the main pattern needs further substantiation. Propylene glycol seems to have an abnormal measured permeability in comparison with the value for glycerol, which has been measured twice and does fit the main pattern of Fig. 1, since propylene glycol is smaller than glycerol by a single hydroxyl group, so that on any current model its permeability should be substantially higher, whereas in fact it is lower.

The data in Fig. 1 refer to black films made of egg lecithin only, since most of the available data is on this system. Finkelstein (1976) also measured

permeability coefficients for membranes formed from initial mixtures of about 4:1 molar ratios of cholesterol to phospholipids. Such large quantities of cholesterol have not been reported in natural membranes and exceed the limiting concentrations that can be associated with lecithin in liposomes (Demel and de Kruffy, 1976). Indeed, membranes containing such high levels of cholesterol would be expected to have separate islands of crystalline cholesterol (Ladbroke et al., 1968; Lecuyer and Dervichian, 1969), providing additional pathways for solute permeation. The molar ratio of cholesterol in the black film need not be the same as that in the initial lipid mixtures (Cook et al., 1968), but in view of these complications we refrain from analyzing these data further.

A serious problem in the interpretation of Finkelstein's data is his choice of permeants and the small number used. His probe molecules were almost all extended chain molecules. To the extent that it is the least cross-sectional area of a permeant that determines its diffusion coefficient (Lieb and Stein, 1971 *a*, *b*), it is not surprising that little size dependence of diffusion coefficients was observed. In the Wolosin-Ginsburg study, valeric acid isomers of extreme shapes were deliberately chosen and the permeability coefficients given in

TABLE I
PERMEABILITY AND PARTITION COEFFICIENTS OF THE THREE VALERIC
ACID ISOMERS

	$P \times 10^4$ <i>cm/s</i>	$K_{\text{dec}} \times 10^4$	K_{ether}	K_{oct}
Valeric acid	18.0	1,070	21	18.1
Isovaleric acid	13.3	900	17	21.2
<i>t</i> -Valeric acid (pivallic)	2.4	3,000	32	29.2

Table II were obtained. In going from the straight chain *n*-valeric acid to the more globular *t*-valeric acid, the partition coefficient actually increases, yet the permeability coefficient decreases as expected for a soft polymer membrane. An actual increase in diffusion coefficient would be predicted for simple diffusion.

Our conclusion is that bilayers made from egg lecithin behave as soft polymers in their discrimination between permeants of different sizes and shapes. If this analysis were to be extended to the study of other membranes, we would recommend a study in which a large number of molecules of different sizes, shapes, hydrophilicities, and chemical character were carefully chosen and compared.

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REFERENCES

- AVEYARD, R., and R. W. MITCHELL. 1969. Distribution of *n*-alkanols between water and *n*-alkanes. *Trans. Faraday Soc.* **65**:2645-2653.
- COLLANDER, R. 1950. The distribution of organic compounds between isobutanol and water. *Acta Chem. Scand.* **4**:1085-1089.

- COLLANDER, R. 1951. The partition of organic acids between higher alcohols and water. *Acta Chem. Scand.* **5**:774-780.
- COOK, G. M. W., W. R. REDWOOD, A. R. TAYLOR, and D. A. HAYDON. 1968. The molecular composition of black hydrocarbon films in aqueous solutions. *Kolloid Zeit.* **227**:28-36.
- DEMEL, R. A., and B. DE KRUYFF. 1976. The function of sterols in membranes. *Biochim. Biophys. Acta.* **457**:109-132.
- DIAMOND, J. M., and Y. KATZ. 1974. Interpretation of nonelectrolyte partition coefficients between dimyristoyl lecithin and water. *J. Membr. Biol.* **17**:121-154.
- FINKELSTEIN, A. 1976. Water and nonelectrolyte permeability of lipid bilayer membranes. *J. Gen. Physiol.* **67**:45-66.
- GALLUCCI, E., S. MICELLI, and C. LIPPE. 1971. Non-electrolyte permeability across thin lipid membranes. *Arch. Int. Physiol. Biochim.* **78**:881-887.
- GUTKNECHT, J., and D. C. TOSTESON. 1973. Diffusion of weak acids across lipid bilayer membranes: effects of chemical reactions in the unstirred layer. *Science (Wash. D. C.)*. **182**:1258-1261.
- KATZ, Y., and J. M. DIAMOND. 1974. Thermodynamic constants for nonelectrolyte partition between dimyristoyl lecithin and water. *J. Membr. Biol.* **17**:101-120.
- LADBROOKE, B. D., R. M. WILLIAMS, and D. CHAPMAN. 1968. Studies on lecithin-cholesterol-water interactions by differential scanning calorimetry and X-ray diffraction. *Biochim. Biophys. Acta.* **150**:333-340.
- LECUYER, H., and D. C. DERVICHIAN. 1969. Structure of aqueous mixtures of lecithin and cholesterol. *J. Mol. Biol.* **45**:39-57.
- LEE, A. G., N. J. M. BIRDSALL, and J. F. METCALFE. 1974. Nuclear magnetic relaxation and the biological membrane. In *Methods in Membrane Biology*. E. D. Korn, editor. Plenum Press, New York.
- LIEB, W. R., and W. D. STEIN. 1969. Biological membranes behave as non-porous polymeric sheet with respect to the diffusion of non-electrolytes. *Nature (Lond.)*. **224**:240-243.
- LIEB, W. R., and W. D. STEIN. 1971a. The molecular basis of simple diffusion within biological membranes. In *Current Topics in Membranes and Transport*. Vol. 2. F. Bonner and A. Kleinzeller, editors. Academic Press, Inc., New York. 1-39.
- LIEB, W. R., and W. D. STEIN. 1971b. Implications of two different types of diffusion for biological membranes. *Nat. New Biol.* **234**:220-222.
- POZNANSKY, M., S. TONG, P. C. WHITE, J. M. MILGRAM, and A. K. SOLOMON. 1976. Nonelectrolyte diffusion across lipid bilayer systems. *J. Gen. Physiol.* **67**:45-66.
- VREEMAN, H. J. 1966. Permeability of thin phospholipid films. III. *Koninkl. Ned. Acad. Wet. Sec. B.* **69**:564-577.
- WOLOSIN, J. M., and H. GINSBURG. 1975. The permeation of organic acids through lecithin bilayers. Resemblance to diffusion in polymers. *Biochim. Biophys. Acta.* **389**:20-33.