

LETTERS TO THE EDITOR

A Comment on the Water Permeability through Planar Lipid Bilayers

Dear Sir:

In a recent article, Finkelstein (1976) has shown that the measured water permeability through a lecithin-decane planar bilayer membrane is similar to the calculated permeability through a sheet of *n*-hexadecane 50 Å thick. That is, the measured water permeability, P_d , was found equal to $DK_{hc}/\Delta X$, where D is the diffusion constant of water in water, K_{hc} is the water-*n*-hexadecane partition coefficient, and ΔX is the thickness of the hydrocarbon "membrane." We suggest that there are a number of internal factors in the above calculation that may compensate for one another so as to give agreement with experiment. At the heart of the problem is the question: can the interior of the bilayer be treated as an isotropic, three-dimensional liquid? There is mounting evidence that it cannot (White, 1976; Evans and Simon, 1975).

In his calculations, Finkelstein has used the diffusion constant of water and nonelectrolytes in water as a model for the diffusion constant of these molecules in the membrane interior. As the diffusion constant is inversely proportional to the viscosity, from the Stokes-Einstein equation one would presume the bilayer interior to be about 1cP.

The viscosities of water and *n*-hexadecane at 20°C are 1.002 and 3.34 cP, respectively (Handbook of Chemistry and Physics, 55th edition), whereas the microviscosity of lipid bilayers and plasma membranes, as determined by fluorescent probes (Azzi, 1975) and spin labels (Edidin, 1974), is the order of magnitude of 1P. In particular, Vanderkooi and Callis (1974) have found that at 20°C egg lecithin bilayers have a microviscosity of 57.2 cP.

Thus if the permeability through a hexadecane "membrane" and a planar lipid bilayer are the same, and the viscosity of the bilayer is two orders of magnitude higher than that of *n*-hexadecane, then the diffusion constant of water in a bilayer should be about two orders of magnitude lower in a bilayer than *n*-hexadecane. Should this be the case, then either the partition coefficient for water in planar lipid bilayers is much higher than for *n*-hexadecane, or the presence of the hydrocarbon solvent, *n*-decane, in the membrane reduced the viscosity of the bilayer. We suggest that both these effects may be important.

If the membrane thickness is 50 Å and the diffusion constant is reduced by, at most, a factor of 100, then the partition coefficient of water in the bilayer must be 100 times greater than that of an organic liquid to maintain the same permeability.

This factor may be accounted for in a number of ways. First, the partition coefficient between the bilayer interior and water for molecules with either a net

charge or a permanent dipole moment depends directly on the energy barrier for transfer of a molecule ingoing from water (dielectric constant = 80) to the bilayer interior (dielectric constant ≈ 2). This energy barrier is lower for the transfer into the bilayer than the peak Born energy of transfer into bulk hydrocarbon. Consequently, we expect the partition coefficient to be higher. The reasons for the lower energy barrier has been discussed in the literature (Parsegian, 1969; Haydon and Hladky, 1972; Andersen and Fuchs, 1975). A reduction in the barrier height of 2.8 kcal could account for the observed change.

Second, the presence of double bonds could contribute to the larger partition coefficient of water in egg lecithin than *n*-hexadecane. Their presence in bilayers has been shown to increase water permeability and, in organic liquids, water solubility (DeGier et al., 1968; Graham and Lea, 1972; Hildebrand and Scott, 1964).

Finally, we would like to point out that there is a significant difference in activation energy for water permeation through egg lecithin vesicles $\Delta E_a = 8.25$ – 8.6 kcal/mol, (Cohen, 1975) and *n*-hexadecane $\Delta E_a = 11$ – 12 kcal/mol (Haydon, 1969), implying that there may be differences between vesicles, planar bilayers with organic solvents, and organic liquids regarding water permeability.

The author cheerfully acknowledges many stimulating conversations with Dr. E. Evans. This work is supported by National Institutes of Health Grant HL-12157 and Office of Naval Research contract N00014-67-0251-0022.

S. A. SIMON
Department of Physiology and Anesthesiology
Duke University Medical Center
Durham, North Carolina 27710

Received for publication 6 December 1976.

REFERENCES

- ANDERSEN, O. S., and M. FUCHS. 1975. Potential energy barriers to ion transport within lipid bilayers. *Biophys. J.* **15**:795–830.
- AZZI, A. 1975. The application of fluorescent probes in membrane studies. *Q. Rev. Biophys.* **8**:237–316.
- COHEN, B. E. 1975. The permeability of liposomes to nonelectrolytes. I. Activation energies for permeation. *J. Membr. Biol.* **20**:205–234.
- DEGIER, J., J. G. MANDERSLOOT, and L. L. M. VAN DEENEN. 1968. Lipid composition and permeability of liposomes. *Biochim. Biophys. Acta.* **150**:666–675.
- EDIDIN, M. 1974. Transport at the cellular level. *Symp. Soc. Exp. Biol.* **28**:1–14.
- EVANS, E. A., and S. SIMON. 1975. Mechanics of electrocompression of lipid bilayer membranes. *Biophys. J.* **15**:850–852.
- FINKELSTEIN, A. 1976. Water and nonelectrolyte permeability of lipid bilayer membranes. *J. Gen. Physiol.* **68**:127–135.
- GRAHAM, D. E., and E. J. A. LEA. 1972. The effect of surface charge on the water permeability of phospholipid bilayers. *Biochim. Biophys. Acta.* **274**:286–293.
- HAYDON, D. A. 1969. Some recent developments in the study of bimolecular lipid films. *In* The Molecular Basis of Membrane Function. D. C. Tosteson, editor. Prentice-Hall, Inc., Englewood Cliffs, N. J. 111–131.

- HAYDON, D. A., and S. B. HLADKY. 1972. Ion transport across thin membranes: a critical discussion of mechanism in selected systems. *Q. Rev. Biophys.* **5**:187–282.
- HILDEBRAND, J. H., and R. L. SCOTT. 1964. The solubility of nonelectrolytes. Dover Publications, Inc., New York. 266.
- PARSESIAN, A. 1969. Energy of an ion crossing a low dielectric membranes: solutions to four relevant electrostatic problems. *Nature (Lond.)*. **221**:884–846.
- WHITE, S. H. 1976. The lipid bilayer as a “solvent” for small hydrophobic molecules. *Nature (Lond.)*. **262**:421–422.
- VANDERKOOI, J. M., and J. B. CALLIS. 1974. Pyrene: a probe of lateral diffusion in the hydrophobic region of membranes. *Biochemistry*. **13**:4000–4006.

Reply to A Comment on the Water Permeability through Planar Lipid Bilayers

Dear Sir:

Simon (1977) notes from a recent paper of mine (Finkelstein, 1976) that insofar as water permeability is concerned, an egg lecithin bilayer is equivalent to a 50-Å thick sheet of bulk hydrocarbon.¹ This he finds disturbing. Specifically, Simon points out that the microviscosity of egg lecithin bilayers, as measured by fluorescent probes, is considerably higher than that of bulk hydrocarbon (hexadecane), and he therefore feels that the diffusion constant of H₂O in the bilayer interior should be correspondingly reduced. The agreement between P_d , the water permeability of an egg lecithin bilayer membrane, and $DK_{hc}/\Delta X$ is thus in Simon's view, fortuitous—the result of the compensation of “a number of internal factors”.²

Simon considers several possible compensating factors. Rather than taking these up, however, I wish to draw attention to what, I feel, is a fallacy in his basic premise. Namely, I believe, that the so-called microviscosity of bilayers as measured by fluorescent probes (e.g., pyrene) is not relevant to the viscosity perceived by the much smaller water molecule as it traverses the bilayer. Indeed, the diffusion constant of oxygen in lecithin membranes is about two orders of magnitude larger than that of pyrene (Fischkoff and Vanderkooi, 1975). Thus, the diffusion constant of H₂O in a bilayer of viscosity ≈ 50 cP need not be substantially less than the diffusion constant of H₂O in water.

We see this clearly in Table 1. Note that although *n*-hexadecane is three times more viscous than water, the diffusion constant of H₂O in hexadecane is actually larger than in water. Even more pertinent to this discussion are the data on

¹ No particular point was made of this in the paper as it is an old observation (Hanai and Haydon, 1966; Finkelstein and Cass, 1968).

² K_{hc} is the hexadecane:water partition coefficient of H₂O, D is the diffusion constant of H₂O in water, and ΔX is the membrane thickness.

TABLE I
DIFFUSION CONSTANT (D_{H_2O}) OF WATER IN DIFFERENT MEDIA

Medium	Viscosity	D_{H_2O}
	<i>cP</i>	$cm^2 s^{-1}$
Water	1.0	2.4
<i>n</i> -Hexadecane	3.3	4.2
Perhydrosqualene	37	1.7

Viscosity is at 20°C; D_{H_2O} is at 25°C. The viscosity of water and *n*-hexadecane are from the Handbook of Chemistry and Physics, 57th edition; the viscosity of perhydrosqualene (squalane) is from the Merck Index, 8th edition. D_{H_2O} in water is from Wang (1953); D_{H_2O} in *n*-hexadecane and perhydrosqualene (squalane) is from Schatzberg (1965).

perhydrosqualene (popularly known as 2,6,10,15,19,23-hexamethyltetracosane). Although its viscosity is 37 cP (which is comparable to the 57 cP value obtained by Vanderkooi and Callis (1974) for the microviscosity of egg lecithin bilayers), D_{H_2O} in perhydrosqualene is virtually equal to that in water.

I think that the explanation for these data is that an H_2O molecule sees methyl groups as it diffuses through hydrocarbon, and their "viscosity" is not a strong function of hydrocarbon chain length and structure. (A fluorescent probe, on the other hand, sees much more of the hydrocarbon molecule, and therefore senses a viscosity more comparable to macroscopic viscosity measurements. Indeed, these probes are calibrated against bulk hydrocarbon viscosity values.) Regardless of the explanation for the data, it is clear from them that it is not unreasonable to assume that D_{H_2O} in the interior of a lecithin bilayer is comparable to D_{H_2O} in water, despite the obvious intricacy and subtlety of the bilayer's structure.

ALAN FINKELSTEIN
Department of Physiology
Albert Einstein College of Medicine
Bronx, New York 10461

Received for publication 1 March 1977.

REFERENCES

- FINKELSTEIN, A. 1976. Water and nonelectrolyte permeability of lipid bilayer membranes. *J. Gen. Physiol.* **68**:127-135.
- FINKELSTEIN, A., and A. CASS. 1968. Permeability and electrical properties of thin lipid membranes. *J. Gen. Physiol.* **52**:145s-172s.
- FISCHKOFF, S., and J. M. VANDERKOOI. 1975. Oxygen diffusion in biological and artificial membranes determined by the fluorochrome pyrene. *J. Gen. Physiol.* **65**:663-676.
- HANAI, T., and D. A. HAYDON. 1966. The permeability to water of bimolecular lipid membranes. *J. Theor. Biol.* **11**:370-382.
- SCHATZBERG, P. 1965. Diffusion of water through hydrocarbon liquids. *J. Polym. Sci.* **10** (Pt. C):87-92.

- SIMON, S. A. 1977. A comment on the water permeability through planar lipid bilayers. *J. Gen. Physiol.* **70**:123–125.
- VANDERKOOI, J. M., and J. B. CALLIS. 1974. Pyrene: a probe of lateral diffusion in the hydrophobic region of membranes. *Biochemistry.* **13**:4000–4006.
- WANG, J. H., C. V. ROBINSON, and I. S. EDELMAN. 1953. Self-diffusion and structure of liquid water. III. Measurement of the self-diffusion of liquid water with H², H³, and O¹⁸ as tracers. *J. Am. Chem. Soc.* **75**:466–470.