

# Permeability of Lipid Bilayer Membranes to Organic Solutes

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**ABSTRACT** A sensitive fluorescence technique was used to measure transport of organic solutes through lipid bilayer membranes and to relate permeability to the functional groups of the solute, lipid composition of the membrane, and pH of the medium. Indole derivatives having ethanol, acetate, or ethylamine in the 3-position, representing neutral, acidic, and basic solutes, respectively, were the primary models. The results show: (*a*) Neutral solute permeability is not greatly affected by changes in lipid composition but presence or absence of cholesterol in the membranes could greatly alter permeability of the dissociable substrates. (*b*) Indole acetate permeability was reduced by introduction of phosphatidylserine into membranes to produce a net negative charge on the membranes. (*c*) Permeability response of dissociable solutes to variation in pH was in the direction predicted but not always of the magnitude expected from changes in the calculated concentrations of the undissociated solute in the bulk aqueous phase. Concentration gradients of amines across the membranes caused substantial diffusion potentials, suggesting that some transport of the cationic form of the amine may occur. It is suggested that factors such as interfacial charge and hydration structure, interfacial polar forces, and lipid organization and viscosity, in addition to the expected solubility-diffusion relations, may influence solute flux.

## INTRODUCTION

Several factors make the experimental lipid bilayer membrane (Mueller, Rudin, Tien, and Wescott, 1962, 1963, 1964) an attractive tool for the study of physicochemical factors affecting permeability of cellular membranes to substances of physiological interest. Both the thickness and molecular organization of the experimental membrane appear to be closely related to those proposed for the cellular membrane (Davson and Danielli, 1952). They may be readily prepared as a diffusion barrier separating two aqueous compartments, corresponding to the inner and outer cellular environment, each of which is

open and accessible for sampling or medium control. Within certain limits the composition of the membrane may also be controlled.

These membranes have been utilized extensively for the investigation of mechanisms of ion transport, but certain experimental difficulties have limited their application, so far, in the study of permeability relations of organic substrates. The few studies reported (Vreeman, 1966; Wood and Morgan, 1967) have utilized radioactive substrates, of high specific activity, to permit detection of the limited amount of substrate which may diffuse through the relatively small membrane area. In order to avoid many of the handling and artifactitious problems associated with the use of substrates of high specific activity, we have investigated the rates of diffusion of a number of fluorescent compounds through the membranes. This approach permitted the study of a group of substrates of varied structure and function while maintaining a sensitivity close to that attained with radioactive tracers. In this report we present some results concerned with the relation of permeability of organic substrates to their functional structure and degree of ionization, and to the membrane composition and charge structure.

## PROCEDURES AND MATERIALS

### *Membrane Formation*

The membrane supporting assembly, used in these studies, is essentially the same as that used in the original studies of Mueller et al. (1962, 1963). This system consists of a plastic cup with an aperture in a thinned area, held in a low glass dish so that fluid can be placed inside and outside the cup. The membranes were formed by brushing lipid solutions on the aperture. Other systems were also used, but the results reported here were obtained almost entirely with this simple assembly.

Resistance of the membranes was monitored with a sensitive ammeter and a high input impedance ( $10^{14}$  ohms) electrometer through Ag-AgCl or calomel electrodes and an agar-salt bridge. Temperature was controlled at  $30^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  with a heater, and magnetic stirring was used for both compartments except where noted. The medium used in these experiments was 0.1 M NaCl, usually buffered with 5 mM histidine or phosphate.

### *Sampling Procedures*

After membranes had thinned to the bilayer state and attained a constant resistance, the transport substrate was added to the inner (plastic cup) compartment by withdrawing 50  $\mu\text{l}$  of the medium and then adding back the same volume of a 0.1–1.0% solution of the substrate. The volume of the inner compartment was 2.0 ml; that of the outer compartment, 5.0 ml. At various intervals samples were withdrawn from the outer compartment while simultaneously adding fresh medium with an infusion pump arrangement to maintain a constant volume. Various asymmetry factors (addition of substrate to one side, imbalance in monitoring electrodes) occasionally

gave rise to potentials and currents across the membrane. In order to avoid electrophoretic and electroendosmotic effects on substrate movement, currents were maintained near zero ( $\pm 10$  picoamperes) by suitable applied potentials.

### *Materials*

Brain lipid was prepared by the procedures of Folch and Lees (1951) using the chloroform-soluble fraction after 48 hr partitioning with water. Phospholipids were freed of neutral lipids by acetone precipitation or chromatographic elution from silicic acid columns. Sphingomyelin and phosphatidylserine were obtained from Nutritional Biochemicals Corporation. The former was usually used as received but phosphatidylserine was freed of colored, oxidative impurities by acetone precipitation from chloroform and silicic acid chromatography. Cholesterol was obtained from several commercial sources. It was recrystallized twice from alcohol before using. The  $\alpha$ -tocopherol was generously supplied by the Roche Division of Hoffmann-LaRoche Laboratories.

The organic substrates used in the diffusion experiments were commercial products from Sigma Chemical Company, Mann Research Laboratories, and K&K Chemical Company. They were used, as received, in an aqueous solution with pH adjusted to correspond to the pH of the medium in the experiments.

### *Membrane Composition*

All membranes were prepared with solutions of phospholipids in 2:1 chloroform-methanol. The three basic membrane systems were: brain phospholipid (2% w/v), tocopherol (20% w/v), and cholesterol (2% w/v); sphingomyelin (6% w/v) and tocopherol (40% w/v); and sphingomyelin (3% w/v), tocopherol (20% w/v), and cholesterol (3% w/v). For one series of experiments 0.5% phosphatidylserine was incorporated into the basic sphingomyelin-tocopherol system. The final composition of the lipid bilayer cannot, of course, be predicted from these solution proportions since selective adsorption to the interface may alter the ratios in the surface layers which form the final bilayer structure.

### *Analytical Procedure*

Fluorescence measurements were made with an Aminco-Bowman spectrophotofluorimeter. Samples withdrawn from the membrane compartment were analyzed under the same conditions used for preparation of a standard curve for the substrate. The pH of the samples was adjusted to a pH appropriate to the analysis of the substrate (Udenfriend, 1962; American Instrument Co. brochure, 1963) by addition of acid, base, or buffer for analysis. A blank, control sample was obtained by withdrawing an aliquot from the membrane incubation medium just prior to addition of the substrate. Each system was checked to insure that the fluorescence of the medium did not change significantly, in the absence of substrate, over the normal period of incubation. Some brain lipid preparations, aged by storage, caused a steady increase in background fluorescence of the medium. Such preparations were avoided in the experiments reported here.

*Sensitivity and Accuracy*

Several of the indole derivatives used in these studies may be quantitatively analyzed in the range of 1 to 100 nanograms. Accordingly, sufficient flux for accurate analysis could readily be obtained for any of the substrates with permeability coefficients in the region of  $10^{-6}$  cm sec $^{-1}$  with a concentration gradient of 100  $\mu$ g/ml within a period of 1–2 hr with the membrane areas normally used. With many of the substrates having higher permeability coefficients, sufficient flux was obtained to permit analysis of samples taken at intervals of 5 min.

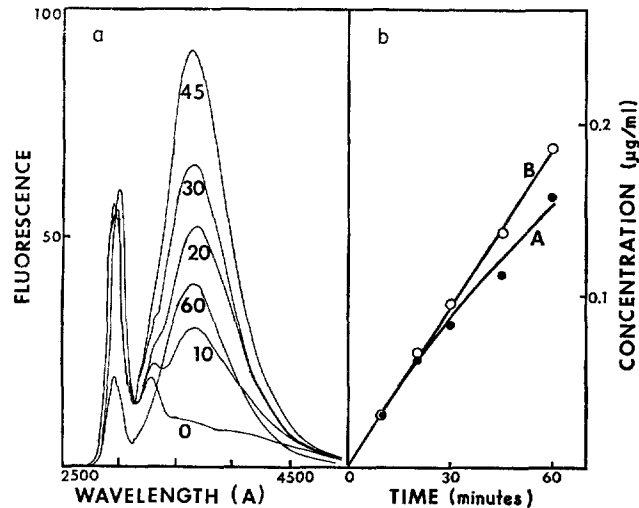


FIGURE 1. Application of fluorescence techniques to measurement of indole-3-ethanol permeability. At zero time, 50  $\mu$ l of solution containing 2 mg/ml indole-ethanol was added to the inner compartment (volume 2 ml). Inner and outer compartments were stirred continuously. At indicated intervals, a 1 ml sample was withdrawn from the external compartment (5 ml total volume) while simultaneously adding fresh buffer. Membrane: Sphingomyelin, 3%; cholesterol, 3%; tocopherol, 20%; in chloroform-methanol, 2:1. Aperture 1.0 mm diameter. Fluorescence curves were made directly on samples (pH 7) without further dilution. a. Fluorescence curve tracings. Time of sampling (minutes) indicated on each curve. The 60 minute curve is attenuated 3-fold. The peak at 290 mμ is due to scattering of the exciting light. b. Indole-ethanol concentrations: curve A, observed indole-ethanol concentrations. Curve B, indole-ethanol concentrations corrected for cumulative sampling dilution.

Excellent consistency and accuracy were normally obtained for a series of analyses at different time intervals during a permeability experiment, as illustrated in Fig. 1. Occasionally, there were problems from inclusion of light-scattering materials (excess lipid dispersed in the medium or lint) or failure to provide adequate stirring in the sampling compartment of the diffusion cell. Duplicate transport experiments, set up under apparently identical conditions, could, however, vary by as much as 25%. This difference is greater than would be expected from normal experimental vari-

ations, and must be due to differences in membrane structure or composition arising during the complex thinning process.

Neither the size of the diffusion area nor continuous stirring appeared to have significant effect upon variability of measured permeability coefficients (Table I) so long as the substrate was thoroughly mixed into the medium initially.

Hydrostatic bulging of the membranes could occur due to evaporation from the external compartment over a long period of time. Since this could greatly increase the area of the membrane and alter apparent permeability relations, it was essential to maintain the membranes in a flat configuration by occasional additions of water to the external medium. Light reflected from the membrane surface was used to gauge the shape and position of the membrane.

TABLE I  
EFFECT OF STIRRING AND MEMBRANE AREA ON APPARENT  
PERMEABILITY COEFFICIENT OF INDOLE-3-ETHANOL

Membrane area	Aperture depth*	Permeability coefficient†	
		Stirred	Unstirred
mm <sup>2</sup>	mm	cm/sec × 10 <sup>6</sup>	
0.85	0.35	320-355	290-310
1.61	0.40	290-350	270-360
5.5	0.23	270-320	280-340

\* Apertures were formed with a hot needle in a heat-thinned area of the polyethylene septum. This procedure gives a smooth, rounded torus surrounding the aperture. The aperture depth given is the maximum thickness of this torus and this exceeds the thickness of the surrounding septum. The rounded edges of these apertures tend to make the membrane center itself in the aperture rather than forming at one end of the aperture.

† Sphingomyelin-tocopherol membranes.

### Calculations

Each sample withdrawn from the external compartment resulted in dilution of the remaining solution by the replacing medium. Fluorimetric measurements were corrected for this dilution factor, usually amounting to a 20 % cumulative correction for each sample. Total flux,  $J_s$ , and apparent permeability coefficient,  $K_p$ , were calculated from the relations:

$$J_s = \frac{FV''}{F_o V_x A t} \quad \text{and} \quad K_p = \frac{J_s}{(C' - C'')}$$

where  $F$  is corrected fluorescence of the sample,  $F_o$  is the fluorescence for unit weight of substrate,  $A$  is membrane area in cm<sup>2</sup>,  $V''$  is the volume of the outer compartment,  $V_x$  is the volume of the fluorescence sample,  $C'$  and  $C''$  the substrate concentrations of the inner and outer compartments, respectively, and  $t$ , the time of diffusion. Since  $C'' = 0$  at the beginning of all experiments and remains very small relative to  $C'$

throughout the experiments, the second equation reduces to:

$$K_p = \frac{J_s}{C}$$

## EXPERIMENTAL RESULTS

### *Effects of Stagnant Aqueous Layers*

Since membranes are formed in a relatively small aperture in a septum which is very thick in relation to the thickness of the membrane, there is a possibility that stagnant, unstirred layers of water in the apertures may be sufficiently deep to become the main barrier to diffusion rather than the membrane. Accordingly, the region limiting diffusion must be reasonably defined. Most of the substrates used in these experiments will have aqueous diffusion coefficients in the range of  $5 \times 10^{-6}$  cm<sup>2</sup>/sec, or slightly higher. The maximum permeability coefficients observed were between three to four times  $10^{-4}$  cm/sec. To insure that stagnant aqueous layers were not affecting coefficients in this range, the effective depth of such layers should be less than 0.01 cm. Since aperture depths ranged from 0.023 to 0.040 cm, it appears essential to prove that the effective, unstirred depth is much smaller than the actual thickness of the septum. The data in Table I suggest very strongly that this is the case. A 3-fold variation in ratio of membrane diameter to aperture depth (1.06 mm diameter with 0.35 mm depth and 2.6 mm diameter for 0.23 mm depth) gave no significant change in the permeability coefficients for indole-3-ethanol (Table I) with or without vigorous mechanical stirring. The larger diameter should greatly increase the effective depth of stirring within the aperture, consequently decreasing the effective unstirred layers. It appears unlikely that the permeability coefficients could show such independence of the conditions if the stagnant layers were contributing significantly to the diffusional barrier. This view is strengthened by the observation that visible inclusions in the membrane surface (thickened lenses of lipid) may move rapidly around the surface in a circular motion due to mechanical stirring of the aqueous medium. Accordingly, it is probable that the membrane is the only restricting factor on the diffusion of substances showing permeability coefficients similar to or lower than those of indole-ethanol in the sphingomyelin-tocopherol membranes.

### *Effects of Functional Groups on Permeability*

Table II, column A, compares the permeability at pH 7, of a group of organic solutes, with varying functional character, in a membrane containing mixed brain phospholipids, cholesterol, and tocopherol. Unsubstituted indole, the substance most nearly approximating a simple aromatic hydro-

carbon nucleus, shows the highest permeability, followed closely by the neutral, substituted indoles, indole-3-ethanol and 5-hydroxyindole. The hydrophilic groups of these latter compounds do not appear to decrease permeability greatly, although they should favor an increased distribution into the aqueous phase.

An ionizing group, however, may introduce a change of several orders of

TABLE II  
RELATION OF PERMEABILITY IN LIPID BILAYER MEMBRANES  
TO FUNCTIONAL STRUCTURE AND MEMBRANE COMPOSITION

Substrate	Permeability coefficients*		
	A† Brain phospholipid	B‡ Sphingomyelin- cholesterol	C‡ Sphin- gomyelin
	<i>cm/sec</i> $\times 10^6$		
Indole-3-ethanol	230	250	310
Indole-3-acetic acid	0.59	1.9	21
Tryptamine	6.7	1.8	74
Indole	250	—	—
5-Hydroxyindole	185	—	—
Serotonin	1.08	—	—
Tryptophan	Not detectable	—	—
Procaine	16	—	—
<i>p</i> -Aminobenzoic acid§	11.6	—	—
Salicylic acid	10.8	—	—
Sulfanilic acid	Not detectable	—	—
Sulfanilamide	Not detectable	—	—
3,4-Dihydroxyphenylalanine	Not detectable	—	—
Pyridoxamine	6.1	—	—
Pyridoxine	3.6	—	—
Quinidine	8.2	—	—
Quinine	9.5	—	—

\* Mean values for at least three determinations, all at pH 7.0. Coefficients of variance ranged from under 10% (for higher  $K_p$  values) to greater than 20% (low values).

† See text for membrane composition.

§  $K_p$  for *p*-aminobenzoic acid increased steadily to  $60 \times 10^{-6}$  cm/sec by the end of the 3rd hr.

magnitude in permeability. Tryptamine permeability is only one-fortieth of that for indole while indole-3-acetic acid diffuses only at one-four hundredth the rate of the neutral substances. Similarly, charged substances with the benzene nucleus show relatively low permeability coefficients, although it is apparent that the ionizing group does not entirely control the permeability. Even large, cationic molecules, like quinidine and quinine, diffuse through the membrane rather rapidly.

*Influence of Membrane Lipid Composition on Permeability*

A selected group of the above substrates was tested with membranes of varying lipid composition in order to obtain a preliminary evaluation of the effect of the membrane organization on permeability. The neutral solute, indole-3-ethanol, is affected only slightly by changes in membrane composition. (Compare Table II, columns A, B, C.) This suggests that the energy of transfer from aqueous to lipid phase, and vice versa, does not differ greatly in the three systems tested. Thus, relative solubility of the solute in water and the hydrocarbon interior of the membrane could be a major factor in the rate of transfer through the membrane while structural organization and interfacial characteristics may be secondary, modifying factors.

In contrast, the ionizing substrates, indoleacetic acid and tryptamine, show tremendous changes in transport rates through different membranes. In the sphingomyelin-tocopherol membranes, the permeability coefficient of tryptamine at pH 7 approaches 25% of the coefficient for the neutral compounds. This suggests that some factor in membrane organization may be very important in governing permeability of the ionizable substances.

*Effect of pH and Degree of Ionization of the Substrate*

The large permeability coefficients for ionizing substrates, noted in certain membranes, cast some doubt upon the hypothesis that the neutral, unionized form of the substrate in the bulk phase controls the diffusion gradient and is the only species actually entering the membrane.

To examine this question further, the pH dependence of tryptamine and indoleacetic acid permeability was determined. The results in Table III show that the permeability coefficient for indoleacetic acid decreased 5–10-fold for each unit decrease in pH. This could correspond roughly to the expected 10-fold decrease in the concentration gradient of the unionized form of the acid. However, the permeability coefficient calculated for the concentration gradient of the neutral form across the sphingomyelin membrane is much larger than that for the neutral model, indole-ethanol, in the same membrane.

Tryptamine permeability also changes in the direction expected but not to the degree expected for a pH change. Extraordinarily large permeability coefficients are obtained using the concentration gradients due to the free base only.

It appears unlikely that the permeability of the neutral species of ionizable substrates could so greatly exceed the permeability of the neutral model. It is apparent that the bulk phase concentration of the neutral form cannot be the only factor involved in controlling the diffusion gradient and permeability.



TABLE III  
VARIATION IN PERMEABILITY OF  
DISSOCIABLE SOLUTES WITH pH

		Permeability coefficients*					
		A. Brain phospholipid‡		B. Sphingomyelin-cholesterol‡		C. Sphingomyelin‡	
Substrate	pH	Total solute	Undissociated solute	Total solute	Undissociated solute	Total solute	Undissociated solute
<i>cm/sec × 10<sup>6</sup></i>							
Indole-3-acetic acid	6.0	5.5	98	17	304	103	1,840
	7.0	0.59	105	2.4	430	21	3,750
	7.9	<0.1		—		—	
Tryptamine	6.0	4.3	36,000			9.5	79,000
	7.0	6.7	5,600	1.8	1,500	74	62,000
	8.0	8.2	690	7.9	660	105	8,700

\* Values represent averages of two to five experiments in each case. Permeability coefficients listed under "total solute" are calculated using total solute as the concentration providing the diffusion gradient. Coefficients under "Undissociated solute" are calculated on the basis that only the undissociated form of the solute contributes to the diffusion gradient. Bulk phase concentrations of undissociated solutes are calculated using the dissociation constants  $pK_a = 4.75$  for indole acetic acid (Merck index) and  $pK_a = 9.92$  for tryptamine (McMenamy and Seder, 1963).

† See text for membrane composition.

TABLE IV  
EFFECT OF EXCESS MEMBRANE ANION SITES  
ON ANIONIC SOLUTE PERMEABILITY

	$K_p$ for indolacetate*	
	Sphingomyelin membrane	Sphingomyelin-phosphatidylserine membrane
	<i>(cm/sec × 10<sup>6</sup>)</i>	
pH 6.0	110	8.9
pH 7.0	—	0.95

\* Average of two measurements for each value.

The complex mixture of brain phospholipids contains significant quantities of phosphatidylserine, a lipid whose dissociation might also be affected by changes in pH. To determine whether such weakly ionizing groups in the membrane might be a significant factor in mediating permeability, the effect of incorporation of phosphatidylserine into sphingomyelin membranes on the diffusion of indoleacetate was examined. The results of these tests (Table IV) show that the charged lipid does, indeed, affect the permeability of the anionic substrate. The introduction of the anionic lipid greatly reduces the

apparent permeability constant, at both pH 6 and 7, suggesting that the ionization of weakly dissociable groups in the membrane-water interface may be a considerable factor in governing permeability of ionizing substrates. One interpretation of the observed effect might be that the ionic environment in the interfacial region may be altered by the variable, fixed charge so as to alter the degree of ionization of the substrate, and therefore, the effective concentration gradient.

However, the failure of the amines to respond to variation in pH as much as expected remains difficult to explain if it is assumed that only the uncharged, neutral forms of the substrate can enter into and diffuse through the membrane. Evidence having some bearing on this problem may be found in the observation that most of the amines, and a few anionic substrates, gave rise to significant membrane potentials. As illustrated in Fig. 2, these

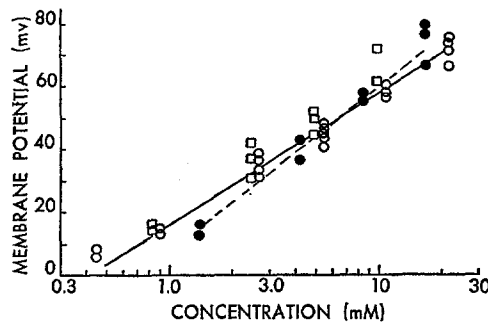


FIGURE 2. Membrane potentials due to procaine and related anesthetics. Equilibrium potentials obtained upon addition of the analgesic amines to one compartment of the membrane system. All measurements were made at pH 7 with the substrates adjusted to the same pH before addition. Each point represents an independent experiment. Open circles, procaine; filled circles, butacaine; open squares, tetracaine.

membrane potentials for the analgesic amines, procaine, butacaine, and tetracaine, are linearly related to the logarithm of the substrate concentration, when added to one side of the membrane. The potential relations illustrated are similar to those expected for diffusion potentials, arising from gradients of a membrane-permeable ion across the membrane. Although diffusion potentials normally are used to indicate specific permeability, they do not reflect, in any manner, a degree of permeability since they may arise in instances of unmeasurable transport. However, in conjunction with the changes in membrane conductance that accompany addition of the amines, there is a strong suggestion that the cationic form actually does enter into the membrane. The substrate may not actually continue to diffuse through the membrane in the charged form. Ionization may be suppressed by entry into a region of low dielectric constant, leading to formation of the neutral, diffusible species by exchange reactions in the interface.

*Other Interactions*

Certain other aspects of diffusional behavior indicate that the movement across the membrane can be a rather complex process. With most substrates tested, the rate of diffusion remained relatively constant during the 1–6 hr period of observation. However, the permeability coefficients for a few substrates were found to have a marked dependence upon the duration of the experiment. In particular, *p*-aminobenzoic acid permeability increased more than 5-fold during a 3 hr experimental period (Fig. 3). Simultaneously, the membrane conductance increased proportionately. Obviously, the *p*-aminobenzoic acid is affecting the membrane structure in some manner that facilitates its own diffusion, as well as inorganic ion diffusion, through the membrane.

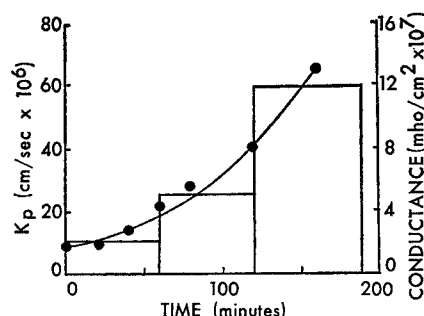


FIGURE 3. *p*-Aminobenzoic acid-induced increase in membrane permeability and conductivity. Membrane conductance was constant prior to addition of *p*-aminobenzoic acid (250  $\mu\text{g/ml}$ ) to one side of the brain lipid membrane. Measurements at pH 7.0. Bars indicate the average permeability coefficients over each period of sampling. Solid points indicate the change in membrane conductance.

An unusual, selective membrane interaction was also discovered with indoleacetic acid. At the concentrations used for the transport experiments, the indoleacetate caused a rapid, 10 to 50-fold increase in the conductance of the sphingomyelin-tocopherol-cholesterol membranes but not in either the sphingomyelin-tocopherol or the brain lipid-tocopherol-cholesterol membranes. Although a number of other substances are known to alter membrane conductances (cyclic peptides, certain cationic dyes, iodine, etc.) the indoleacetate appears unique in its specificity. In contrast with the *p*-aminobenzoate effect mentioned above, the conductance change induced by indoleacetate is completed within a few minutes and there is no apparent effect upon the substrate permeability.

## DISCUSSION

This investigation should be considered as a preliminary evaluation of the lipid bilayer membrane as a tool in the study of a complex problem. Objec-

tives were initially limited to determining whether the lipid composition of the membrane would have a significant effect upon organic solute permeability and how the ionization of a solute affected its permeability.

It is apparent that variation of lipid composition, within the limits tested, does not greatly change the permeability of neutral solutes, but it may have a profound influence upon transport of ionizable solutes. Two separate, important factors were found. Permeability could be altered both by a neutral lipid, such as cholesterol, and by an ionizable amphiphile, such as phosphatidylserine.

The reduction of tryptamine and indoleacetate permeability by cholesterol is similar to the cholesterol-induced decrease in water permeability described by Finkelstein and Cass (1967) for lipid bilayer membranes. The change in water permeability was ascribed largely to an increase in membrane "viscosity" and decrease in water solubility in the hydrocarbon layers. These conclusions were based mainly upon observed changes in bulk phase models, consisting of a paraffin hydrocarbon with added cholestane. A "condensing" effect of cholesterol on phospholipid monolayers at a gas-water interface has long been recognized (van Deenen, Houtsmuller, de Haas, and Mulder, 1962) although the basis for the area reduction may be a subject of some dispute.<sup>1</sup> A similar condensation seems to occur in fully hydrated bimolecular phospholipid lamellae (Rand and Luzzati, 1968). If such condensations were due to a closer packing or reduced thermal movement within the hydrocarbon layers, one might expect that the energy requirements for insertion of a foreign molecule and its movement through the hydrocarbon medium might increase. Although Cadenhead<sup>1</sup> points out that the surface viscosity of phospholipid monolayers is actually decreased by cholesterol, the effect could be distinctly different in the more complex lipid bilayer membrane system where incorporation of cholesterol may displace the plasticizing solvent molecule (tocopherol or paraffin hydrocarbon) and alter interface group spacings. In the sphingomyelin bilayer membranes, the effect of cholesterol on membrane rigidity can be strikingly and visually apparent. The bilayer area of the cholesterol-free membrane normally may be rapidly expanded by application of a small hydrostatic pressure to one side of the membrane to create a bulge in the membrane. Upon release of pressure, the membrane will immediately return to its minimal area, maintaining a taut appearance throughout. In contrast, a membrane containing cholesterol reacts very sluggishly to pressure changes. It may rupture, rather than expand, if pressure is applied rapidly. Once expanded by careful application of pressure, release of the pressure leaves the membranes flapping loosely, adjusting to the pressure changes by folding

<sup>1</sup> Cadenhead, D. A. 1967. Monolayer characteristics of some possible bilayer components. Personal communication delivered at Symposium on Lipid Bilayers, Buffalo.

rather than contracting. Although the transitional mechanisms during expansion and contraction may be complex, involving the thickened lipid regions as well as the bilayer, it is apparent that the sphingomyelin-cholesterol structure is much more rigid than that obtained in the absence of cholesterol. The cholesterol affects other systems entirely differently.

It appears dubious, however, that the differences due to cholesterol can be entirely ascribed to packing within the hydrocarbon region of the membranes. The striking effects should not be limited to tryptamine or indoleacetate if only the viscosity or packing of the hydrocarbon lattice were involved. It may be necessary to invoke changes in the interfacial structure, the interaction of polar groups, hydrogen bonding, degree of hydration (or "ice" structures), or fixed charge distributions, to account for the magnitude of variation.

The influence of fixed charges in the membrane was also striking. Addition of phosphatidylserine to a neutral (sphingomyelin) membrane greatly decreased indoleacetate permeability. Although there appears to be a direct relationship between concentration of undissociated indoleacetate and permeability, these results suggest that membrane surface charges are just as influential in controlling permeability of weakly ionizing substrates. Mechanisms of this nature could be responsible for some of the semispecific permeability relations found previously in cells. For instance, organic anions may penetrate the red blood cell much more rapidly than organic cations, even though the latter may have a much higher lipid/water partition coefficient and would, accordingly, be expected to diffuse more rapidly through the lipid membrane (Schanker, Johnson, and Jeffry, 1964; Schanker, Nafpliotis, and Johnson, 1961). Although the effect of cationic lipids in the bilayer membranes was not investigated here, analogy with the effect of excess anion groups in these membranes upon anion permeability suggests that the observations of Schanker et al. could be partly attributed to the charge structure of the lipid membrane.

The results and discussion above simply suggest some of the latitude available to the cell in controlling permeability at an elementary level. Even here, the transport relations may be greatly complicated by specific interactions, such as those illustrated for *p*-aminobenzoic acid which appears to act as its own catalyst for better transport. In addition, the introduction of a normal complement of proteins and polysaccharides, usually associated with cellular membranes, will cause further complications. Preliminary investigations along this line have already demonstrated that certain proteins which interact with the membrane may alter permeability greatly. A protein from *Aerobacter cloacae* which penetrates the membrane to form ion-conductive channels (Mueller et al., 1962, 1963, 1964) also increases the permeability of even such large cations as quinidine, without affecting anion permeability.

These investigations do suggest, however, that the lipid bilayer may be useful in clarifying many of the physicochemical relations in membrane transport.

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