Biochimica et Biophysica Acta 905 (1987) 320–328
Elsevier

BBA 73784

Determination of reflection coefficients of liposomes for some non-electrolytes by osmotic pressure measurement

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(Received 4 June 1987)

Key words: Liposome; Reflection coefficient; Membrane permeability; Membrane pore; Osmotic pressure

The reflection coefficients of bilayer lipid vesicles (liposomes) of various compositions have been determined for a number of non-electrolytes. The solutes were the same and the method of measurement was essentially the same as those which have been used to estimate an equivalent pore radius for erythrocytes. The method involves matching the osmotic pressure of solutions of a permeant test solute with that of a known inpermeant solute. Reflection coefficients for cholesterol-containing liposomes and those of erythrocytes are, when account is taken of those solutes known to permeate the erythrocyte by specialized pathways, not greatly different. Lipid bilayers can thus account for most of the permeability characteristics of the cell originally interpreted as due to aqueous pores. Reflection coefficients are significantly higher for egg phosphatidylcholine membranes that contain cholesterol than those which do not. There is a strong correlation between relative permeabilities derived from reflection coefficients and oil-water partition coefficients. There is also good agreement betwen these permeabilities and permeabilities measured by others, which exhibit an inverse dependence on molecular size. It is suggested that this tendency of membranes to pass small molecules more readily than large molecules, other properties being equal, is a consequence of the surface pressure of the constituent monolayers of the membrane.

Introduction

The question of whether biological membranes possess pores has been a matter of controversy since the first decade of this century when Overton [1] and Ruhland [2] took opposite sides. Since then, much of the effort directed toward unequivocal resolution of this question has continued understandably to utilize permeability data. As a result, a great wealth of data has accumulated to be interpreted, in large part, either positively or negatively according to the bias of the investigator. An important reason for this continuing uncertainty has been the reliance upon measurements of transport kinetics. The power of kinetic analysis lies in its ability to eliminate incorrect mechanisms, however, its correct application requires the knowledge of the forces that drive the fluxes. This approach has thus not always been definitive because, among other reasons, concentration gradients have not been located with sufficient accuracy, solute-solute or solute-solvent coupling has been ignored, or because the choice of a correct theoretical analysis has required more detailed knowledge of the membrane matrix than was available.

We applied to simple membranes in the form of lipid bilayer-bounded vesicles (liposomes) a technique that has produced evidence in favor of

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pores in erythrocyte membranes. The results suggest that these supposedly 'poreless' membranes have reflection coefficients for a number of solutes that do not differ greatly from those of the same solutes in red cell membranes [3–5]. This finding raises the question as to whether such experiments on red cell membranes do in fact support the classical concept of a pore.

Relative permeabilities derived from reflection coefficients are in agreement with relative permeabilities of liposomes measured by another method [6] which themselves are consistent [7,8] with the hypothesis that the permeability barrier of many membranes depends upon molecular size as well as oil solubility [9]. It is evident that lipid bilayers do indeed have the 'sieve' properties proposed a number of years ago for cell membranes [10].

Materials

Phosphatidylcholine was obtained from egg yolks by extraction with chloroform-methanol, precipitation with acetone and chromatography on silica gel, according to standard techniques [11]. The product contained somewhat under a percent (according to microelectrophoretic data) of negatively charged lipid. This charge was sufficient to permit imbibition by the liposomes of enough of a non-electrolyte solution that they exhibited swelling and shrinking. Gangliosides and cholesterol were obtained from Nutritional Biochemical Co., and dicetyl phosphate and distearylamine from K and K Laboratories, Inc. These substances were used without further purification. Phospholipids with or without additives were dried down from chloroform solutions and dispersed in 0.1 or 0.3 M sucrose solution with very brief sonication. Sucrose was reagent grade and test compounds were Eastman Chemical products.

Method and Apparatus

Theoretical

The object of these experiments is to determine the concentration of a test solute that produces an osmotic pressure identical to that of a given concentration of a solute to which the membrane under investigation is known to be impermeable. From this concentration it is possible, given certain assumptions, to calculate a reflection coefficient for the experimental solute, as well as a corresponding 'equivalent' pore radius for the membrane. Our technique is very similar to that which has been applied to erythrocytes [3], the major differences being that we have employed stopped flow, rather than continuous flow, rapid mixing, and monitored transmitted, rather than scattered, light.

The method is based upon principles of non-equilibrium thermodynamics [12], in particular, those which generate an expression for the net volume flow across a membrane (vol/time-area), namely

$$J_v = L_p(\Delta P - \Sigma\sigma_j RT\Delta C_j) \tag{1}$$

where J_v is the volume flow, L_p the hydraulic conductivity, ΔP the hydrostatic pressure difference across the membrane, σ_j Staverman's reflection coefficient, ΔC the concentration difference across the membrane of the solute identified by the running index j, and R and T have their usual meanings. When there are only two solutes, one permeant and one impermeant, and if the cell or vesicle membrane under consideration is unable to support an appreciable hydrostatic pressure difference, this equation reduces to

$$J_v = -L_p RT\Delta C_i - L_p\sigma_s RT\Delta C_s \tag{2}$$

where s and i indicate the permeant and impermeant molecules, respectively, and where σ_i has been replaced with unity, the appropriate value for an impermeant molecule. Setting the left side of this equation equal to zero gives the condition for zero net volume flow across the membrane,

$$\Delta C_i = -\sigma_s \Delta C_s^o \tag{3}$$

the value of the concentration difference for the permeant species that satisfies this condition having been indicated by a superscript o. Since the gradients of i and s are opposed, ΔC_i and ΔC_s have opposite signs.

Sidel, Goldstein and Solomon [3] and Sidel and Solomon [13] developed an ingenious method for measuring values of σ for a variety of non-electrolytes in the erythrocyte [3,14]. Utilizing the fact

that, over a certain range of volume changes, the light scattered by a population of red cells is proportional to their volume, they rapidly mixed red cells which had been equilibrated with sucrose, (an essentially impermeant molecule) into test non-electrolyte solutions of several different concentrations. Since the permeant test molecule is not initially present inside the cell, the value of C_s for that particular molecule is that concentration at which there is no change in light scattering, i.e., the cells neither swell nor shrink. Rapid mixing techniques are essential, since the flow of water coupled to the entry of such molecules will eventually always cause swelling.

Our procedure was to hydrate lipids in 0.3 M sucrose to produce lipid bilayer vesicles or liposomes bathed within and without by this concentration of sucrose. One volume of this dispersion was rapidly mixed with 6.25 volumes of one of several different concentrations of the same nine non-electrolytes that were used in the red cell experiments [3]. The average slope of the oscilloscope trace of optical density against time was then plotted against the test solute concentration to obtain the value of σ. Liposomes were then mixed with the graphically determined isotonic concentration to ascertain that this concentration did indeed permit no initial volume change of the liposomes. This was invariably the case.

ΔC_i and ΔC_s must, of course, be calculated from the concentrations prior to mixing and the mutual dilution factors. When this is done, the reflection coefficient for the case where the liposomes are equilibrated with the impermeant molecule at a concentration of 0.3 M becomes

$$\sigma_s = \frac{C_i}{C_s} \tag{4}$$

where C_s is the test solution concentration (before mixing) of the permeant species for the zero volume flow condition. Molar osmotic coefficients for the test solutes are very close to unity [3] but the osmolality of a 0.3 M sucrose solution is 0.314. In calculating values of σ we have therefore replaced the former with the latter in Eqn. 4. Since $\sigma = 1$ is the condition of absolute solute impermeance, the σ values so determined must fall between zero and unity.

If the solute and water both penetrate the membrane via the same pathway, then the reflection coefficient can be expressed as [15],

$$1 - \sigma_s = \frac{\omega_s \bar{V}_s}{L_p} + \frac{A_s}{A_w} \tag{5}$$

where ω_s is the solute permeability, $\bar{V}_s$ the partial molar volume of the solute at the concentration of interest, and A_s and A_w represent essentially the pore area seen by solute and water molecules, respectively. When water and solute traverse the membrane by partitioning and diffusing separately across the bilayer, the pore area term drops and Eqn. 5 becomes

$$1 - \sigma_s = \frac{\omega_s \bar{V}_s}{L_p} = \frac{P_s \bar{V}_s}{P_w \bar{V}_w} \tag{6}$$

The second equality is obtained from the first according to $P_s = \omega_s RT$ and $P_w = L_p RT / V_w$, where P_w and P_s represent the water and solute permeability coefficients, and $\bar{V}_w$ the partial molar volume of water.

In all cases the reflection coefficient may be less than unity. The existence of zero volume flow when $\Delta C_s \neq -\Delta C_i$ does not in itself, therefore, mean that pores exist; it only means that if the net volume flow is zero and if the water volume flow is not zero, then the latter must be compensated by an equal and opposite solute volume flow.

Experimental

The rapid mixing apparatus consisted of two (1 ml and 5 ml) vertically mounted, weight-driven syringes and an integral mixing chamber-flow cell. The 5 ml and 1 ml syringes were filled through valve connections with stock solutions of the test compound and liposome dispersion, respectively. The syringes were loaded by raising their plungers against a seven-pound weight that was initially locked above them on a vertical runway. For each trial, the valves were set to direct the contents of the syringes to the mixing chamber (tangential input, axial output type) and flow cell were constructed of a plexiglas block of appropriate size to replace the cuvette carrier of a Gilford model 2400 spectrophotometer. From the path length from chamber to cell and from the overall flow rate, it

was calculated that 70 ms was the minimum time elapsing between mixing and observation. The syringe barrel diameters were such that the mixture in the flow cell consisted of 6.25 parts of test solute solution and 1 part of liposome dispersion. The spectrophotometer was set at 400 nm and its output was recorded with a storage oscilloscope, triggered to sweep by electrical contact made at the lower limit of the falling weight or with an oscillographic recorder started before the weight was released. The observation period was from 110 to between 200 and 400 ms after mixing. All experiments were done at room temperature. Although the mixing chamber-flow cell was not thermostatted, runs in which identical solutions were mixed revealed no thermal artifacts.

To illustrate the time dependencies of the processes involved in the reflection coefficient measurements, we have recorded responses for one set of liposomes using an oscillographic recorder. For economic reasons these liposomes were composed of soy lipids (Sigma, type II-S) and for convenience the lipids were swollen in 0.1 M sucrose. These liposomes were mixed with several different concentrations of urea and the records of the turbidity as a function of time shown in Fig. 1 were obtained. As may be seen, there is a mixing artifact that obscures the initial part of the record, but even with this relatively low reflection coefficient, 0.78, there is little change in the slope over the first several hundred milliseconds.

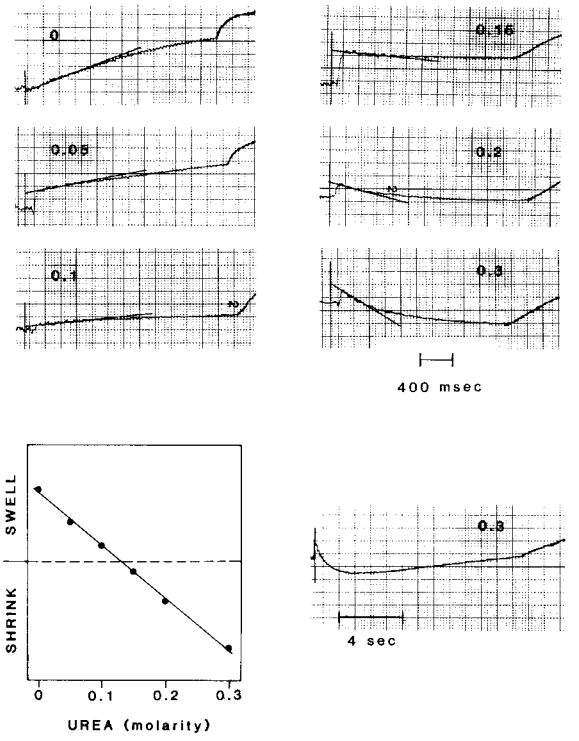

Fig. 1. Dependence of rate of volume change of liposomes containing sucrose on the concentration of urea in the external phase. Soy phospholipids were formed in 0.1 m sucrose and rapidly mixed with urea solutions of the concentrations marked on the tracings in the upper half of the figure. The liposomes swell in urea solutions with concentrations below 0.1 M and initially shrink in more concentrated urea solutions. The initial shrinkage followed by swelling is shown on a longer time scale in the lower right for liposomes mixed with 0.3 M urea. The change in slope seen at the right of all the traces is due to a reduction in chart speed. In the lower left, the initial slopes of the volume changes are plotted against the urea concentrations.

Results

Egg phosphatidylcholine (PC) liposomes were characterized with respect to their reflection coefficients for six of the nine solutes studied. These were thiourea, malonamide, glycerol, methyl urea, urea and acetamide. The results of experiments with these solutes are presented in Fig. 2 as a plot of the rate of change of absorbance (dA/dt) subsequent to mixing the solute concentrations shown on the abscissa with liposomes containing 0.3 M sucrose. Both swelling and shrinking rates were obtained for all solutes plotted in Fig. 2 except acetamide. Liposomes mixed with solutions of acetamide, ethylene glycol, propylene glycol and propionamide exhibited minimum volumes at too short a time to permit accurate measurement of shrinkage rates, but the extrapolation from swelling rates for acetamide was sufficiently short to provide a reasonable estimate of σ for this solute. As Levitt [16] has emphasized, reflection coefficients can be underestimated if the minimum volume occurs within the time used for calculating the rate of volume change.

Each slope in Fig. 2 represents an average of five or six determinations. Although there is some scatter in the data, the resultant uncertainty is less than ± 0.02 M except in the case of acetamide where it is about ± 0.05 M. The lines have slopes compatible with a common origin at 0 M, i.e., at

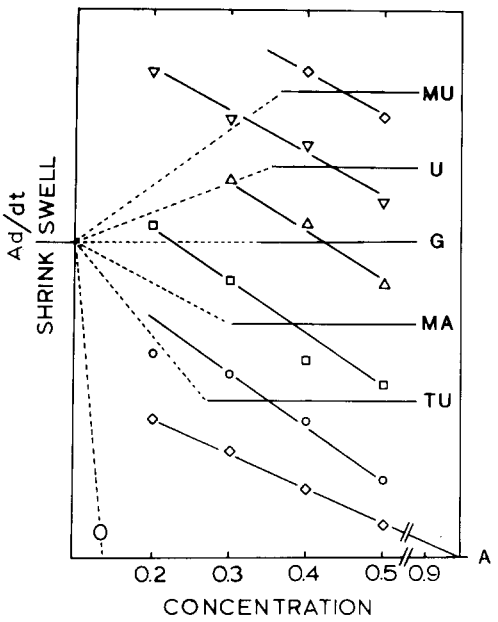

Fig. 2. Graphical determination of test solute isosmotic concentrations for liposomes. Initial rates of swelling or shrinking (dA/dt, in arbitrary units) of PC liposomes. Liposomes were equilibrated with 0.3 M sucrose and rapidly mixed with the concentration of test solutes shown on the abscissa. The short horizontal line bounded by a dashed line on the left and the abbreviation of the test solute on the right represent zero initial volume change for the corresponding solute. These reference lines have been fanned out along the vertical axis to avoid overlap of symbols. The isotonic concentration is the point of intersection of these references lines with the lines drawn through the corresponding experimental points.

the point where swelling would be determined only by the sucrose concentration. Isotonic concentrations are: thiourea, 0.35; malonamide, 0.38; glycerol, 0.43; urea, 0.44; methyl urea, 0.45; acetamide, 0.95, all in molarity. Reflection coefficients derived from these results are given in Table I. The scatter of the points of Fig. 2 results in an uncertainty of the values in the table of about ± 0.04. According to relative rates of swelling at single concentrations, reflection coefficients for the remaining three solutes decrease in the order propionamide > ethylene glycol > propylene glycol.

Liposomes consisting of equimolar egg PC and cholesterol were sufficiently less permeant with respect to water that swelling and shrinking rates could be determined for all but propionamide and propylene glycol. The data were plotted in the same way as those of Fig. 2 and isotonic concentrations for these liposomes were found to be: malonamide, 0.33; thiourea, 0.34; glycerol, 0.34; urea, 0.34; methyl urea, 0.37; ethylene glycol, 0.51; acetamide, 0.54, all in molarity. Corresponding reflection coefficients are given in Table I. These data were obtained from 4 to 6 determinations for each of 3–5 different solute concentrations. Plots of the rate of change of volume at different concentrations were similar to those of Fig. 2 and the uncertainty in the resultant reflection coefficients is likewise estimated to be about ± 0.04.

TABLE I

REFLECTION COEFFICIENTS OF LIPOSOMES AND ERYTHROCYTES

Permeant	Reflection coefficient					
	Liposomes [a]		Liposomes [b]		Liposomes [c]	Erythrocytes [d]
	PC	PC-cholesterol (1:1)	PC	PC-cholesterol (10:1)	PC	
Malonamide	0.83	0.95	–	–	–	0.92 ± 0.16
Thiourea	0.89	0.93	–	–	0.54	0.88 ± 0.04
Glycerol	0.73	0.92	0.71	1.00	0.78	0.88 ± 0.11
Urea	0.71	0.92	1.00	–	0.74	0.68 ± 0.16
Methylurea	0.68	0.85	–	–	–	0.83
Ethylene glycol	–	0.61	0.29	–	0.08	0.85 ± 0.14
Acetamide	0.33	0.59	0.31	0.76	–	0.72 ± 0.15
Propylene glycol	–	–	0.45	0.87	−0.4	0.85
Propionamide	–	–	0.48	0.82	–	0.81 ± 0.15

[a] This work.
[b] Ref. 22.
[c] Ref. 24.
[d] Refs. 3–5.

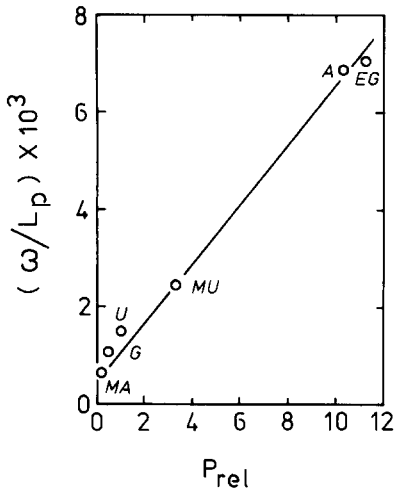

Fig. 3. Ratios of solute permeability to hydraulic conductivity obtained from Eqn. 6 are plotted against relative permeabilities obtained by Cohen [8] from the maximum swelling rate method [6]. Reflection coefficients were obtained from liposomes containing 50 mol% cholesterol and relative permeabilities were obtained from liposomes containing 44 mol% cholesterol.

The effect of additional negative charge was investigated with liposomes consisting of, by weight, 98% egg PC and 2% dicetyl phosphate. Only three solutes were tested. Reflection coefficients for malonamide, glycerol and urea were found to be 0.95, 0.85, and 0.85, respectively.

To determine whether the sign of the surface charge on liposomes had any influence on permeabilities, swelling rates were determined at a single solute concentration of solutes for liposomes containing 5–10% of either stearylamine or whole brain gangliosides. The rank order of swelling rates was much the same in the two case, the only difference being that the amides stood one step higher on the permeability rank for the ganglioside-containing liposomes than for the stearylamine-containing liposomes. It appears therefore that electrostatic effects play relatively minor roles in determining the permeability of these simple lipid membranes.

Discussion

Effect of multiple membranes on rates of osmotically-driven volume change of liposomes

It is evident from the traces shown in Fig. 1 that although the response of liposomes is quite rapid, the turbidity change is sufficiently slow that, for all but highly permeant solutes, a rather unsophisticated mixing device is quite adequate to establish rates of volume change. As may be seen, even after several hundred milliseconds, the slopes have not departed greatly from their initial values. These slow changes are in contrast to the response of erythrocytes which, when mixed with a hyperosmotic solution of a moderately permeant solute, go through the minimum volume and then swell in a time shorter than we require for a slope measurement [17,18]. The difference between the erythrocyte and the liposome is evidently the number of membranes. Thus it is clearly not the outermost bilayer, but the entire liposome that gives rise to the measured change in turbidity. Indeed, this is the conclusion Mishima [19] has come to for scattering changes through lipid phase transitions and that Yoshikawa et al. [20] came to in the specific case of osmotic swelling and shrinkage. The latter carried out a theoretical analysis of volume changes which, in its essential features, was supported by experiment. Although the numbers of layers of a multilamellar preparation is variable, the overall rate of turbidity change should be reduced essentially in proportion to the number of bilayers, i.e., a 10-layered vesicle should respond nearly an order of magnitude slower than a unilamellar vesicle of the same internal volume. Experiments such as those described here would clearly fail if one attempted to use typical preparations of unilamellar vesicles. This is one case where multilamellar vesicles are clearly superior to unilamellar vesicles.

It should be understood, however, that a complete theoretical analysis of the entire process of osmotic volume change, involving a series of membranes having finite and significant permeabilities to both water and solute, has not been carried out. This is a formidable theoretical undertaking and until it is done, prudence would counsel against dogmatic interpretation of reflection coefficients obtained on multilamellar vesicles.

The typical liposome preparation is composed of vesicles of different sizes. For absolute permeability measurements, such heterogeneity creates serious technical problems in that the area across which flux is occurring must be known. In contrast, the reflection coefficient measurement de-

pends upon the ratio of two permeabilities, water and solute, and is thus independent of liposome area.

Comparison with other reflection coefficient measurements

Two other laboratories have measured reflection coefficients of liposomes to solutes similar to those such as we have examined. Their results are included in Table I. Lelievre and Rich [21] used a commercial stop-flow apparatus, but otherwise their procedure was essentially the same as ours. Of the three solutes tested on PC liposomes by them and by ourselves, acetamide and glycerol gave reflection coefficients that are, to within experimental error, identical. The values for urea are very much different, but Lelievre and Rich [21] point out that, relative to their other data, urea gave an anomalously high reflection coefficient. For cholesterol-containing liposomes, there are only two values in common. Ours are both lower. This suggests that the solute permeabilities are similar for the two membranes since L_p is higher for the liposomes with the smaller proportion of cholesterol [22].

Lelievre and Rich [21] have compared their measurements with those of Goldstein and Solomon [3]. They concluded that transport pathways in liposomes and red cells membranes must be different because when plotted against the predictions of the Renkin equation, their lecithin liposome data corespond to an equivalent pore radius of greater than 10 Å, an apparently impossible number. In view of the fact that red cell membranes contain appreciable amounts of cholesterol, it is curious that they did not plot their data for cholesterol-containing liposomes. Although limited, their data for these liposomes fall quite close to the curve for a 4 Å equivalent pore radius.

A comprehensive procedure which is capable of generating solute permeability coefficients in addition to reflection coefficients has been published by Van Zoelen et al. [23,24]. For reflection coefficients, this procdure is similar to that of Goldstein and Solomon [3] except that it utilizes swelling rates only. This method has been applied to five of the compounds we examined, and, as may be seen from Table I, of those cases that can be compared, the agreement for glycerol and urea is excellent and that for thiourea, poor. As noted below, thiourea, in our experiments, exhibited a permeability much lower than expected from its oil-water partition coefficient. Although we did not obtain an accurate value for the reflection coefficients of ethylene glycol and propylene glycol, there is agreement with Van Zoelen et al. [24] to the extent that the former is low and the latter is very low.

The erythrocyte data included in Table I are from measurements on human, canine, and bovine cells. They are included as average values to illustrate the fact that the range of values does not exceed the range of values for simple lipid membranes, for example, the PC-cholesterol liposomes. It is thus evident that lipid bilayers can provide the range of reflection coefficients that are represented by erythrocytes and it is hence unnecessary to postulate pores in erythrocyte membranes. This comparison does not, of course, rule out specialized transport pathways in erythrocyte membranes, and there is, in fact, a well-documented pathway for urea transport [25]. As would be expected if the lipid accounted for the reflection coefficients of most of the test compounds in erythrocytes, a parallel pathway for urea would mean that its reflection coefficient for the cells would be low relative to the liposome data, as is seen to be the case. In addition, too much significance cannot be attached to the absolute values of reflection coefficients of red cells, given the fact that their measurement is susceptible to large errors from small uncertainties in the time course of the volume change [17,18,26].

Comparison with liposome permeabilities

Cohen [8] has determined the permeabilities of liposomes of a variety of different compositions to many of the non-electrolytes commonly used as probes of membrane transport. His data, which were obtained as relative permeabilities, were normalized to $P_{urea} = 1.0$. According to Eqn. 6, reflection coefficients can be related to the ratio of the solute permeability coefficient to the hydraulic conductivity by $(1 - \sigma)/\bar{V} = \omega/L_p$. Since L_p should be a constant for a particular lipid composition, a plot of our values of $(1 - \sigma)\bar{V}$ against the P_{rel} of Cohen [8] should yield a straight line. This has been done in Fig. 3 for our data on egg

PC-cholesterol (50 : 50) liposomes and Cohen's data on those liposomes with a composition closest to ours, namely, egg PC-cholesterol-phosphatidic acid (48 : 48 : 4). The correspondence of these data indicates that the two methods supply equivalent information on relative permeabilities. Molecular volumes given by Cohen [8] were used for the $\overline{V}$ values. We have measured $\overline{V}$ for several of these solutes and found them to be essentially identical to molecular volumes.

Origin of the size dependence of bilayer permeability

It has become apparent that the porosity of cell membranes for which Goldstein and Solomon [3] originally obtained evidence is, at least in part, a real phenomenon, the basis of which has to do with the organization of lipid molecules in bilayers and the way exogenous molecules permeate such ordered arrays. This conclusion is now supported by the finding that small non-electrolytes traverse bilayers relatively readily compared with water molecules in addition to previous extensive data showing a considerable degree of size selectivity in permeability coefficients [7,8]. Lieb and Stein, who first called attention to the fact that the simple diffusion of non-electrolytes across cell membranes is influenced by molecular size in addition to oil-water partition coefficients, have suggested that the bilayer behaves like a porous polymer and diffusion is more hindered, the larger the molecule [9,27]. Although analogies to other material structures may be useful, we would suggest that the origin of the size effect on membrane permeability may be more accurately identified by a direct analysis of the bilayer structure itself. A pronounced size selectivity is, in fact, expected of lipid bilayers because of the surface pressure inherent in the constituent monolayers. The complete analysis of surface pressure cannot be given here, but it is appropriate to indicated how such influences come about and what, approximately, is their magnitude.

The surface pressure of bilayers of dimyristoylphosphatidylcholine has recently been found to be nearly 50 dyn/cm per monolayer [28]. The fact that this represents a bulk pressure of about 220 atmospheres is a clue that this force is likely to have significant consequences. The effect of surface pressure on permeability comes into play both when the permeant enters one side of a bilayer and when it leaves the other. Its effect in the former instance is to reduce the membrane-water partition coefficient relative to the corresponding oil-water partition coefficient by the factor $1/\exp(\pi A/kT)$, where π is the surface pressure, A is partial molecular area, and k and T have their usual meanings. The oil-water partition coefficient is larger than the membrane-water partition coefficient because bulk hexadecane, olive oil, etc., are not organized fluids, so they cannot discriminate against larger molecules on the basis of size. Hence, the oil-water partition coefficients usually used to model solubility of non-electrolytes in membranes give overestimates, the inaccuracy of which increases with increasing molecular size.

An alternative view of the effect of surface pressure on the entry of a permeant into a membrane is that the activation energy is raised for the molecule entering the middle of the membrane (between the methyl groups) where the surface pressure is not operative and the partition coefficient is like that of a bulk oil. According to this viewpoint, the activation energy for traversing the first monolayer is raised by an amount $\exp(\pi A/kT)$. This kind of analysis is appropriate to use for the exit of the permeant from the bilayer; the second monolayer must also be crossed and thus, the surface pressure will introduce an additional factor of $\exp(\pi A/kT)$ into the activation energy. The effects of the two monolayers will be additive. Although the complete discussion of these effects requires more background than would be appropriate to include here, we can give a specific example to illustrate the magnitude of the surface pressure effects. Consider a molecule with a molecular weight of 100 and a density of 1 g/cm^3. If the molecule is taken to be spherical, the combined effects of surface pressure correspond to a size-dependent reduction of the permeability coefficient of almost two orders of magnitude. This is quite close to the molecular weight dependence of relative permeabilities reported by Cohen and Bangham [7]. Although we predict a variation of log permeabilities with the $-2/3$ power of the molecular weight rather than the -1 power, there seems to be enough scatter in the data to accommodate either relationship.

The ultimate source of the resistance of the alkyl chain region of a bilayer to incursion by foreign molecules is their tendency to maximize their configurational entropy within the contraints of the hydrophobic effect that maintains bilayer integrity. That resistance can be quantified as surface pressure and it is evident that surface pressure is of such a magnitude that simple diffusion through a bilayer is expected to diminish rapidly with increasing molecular size.

Acknowledgement

This research was supported by NIH grant NS 20814. We thank Ruby MacDonald for assistance in numerous aspects of this research.

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