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THE PERMEABILITY OF LIPID MEMBRANES TO NON-ELECTROLYTES

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SUMMARY

The permeabilities of smectic mesophases (liposomes) to polar non-electrolytes were studied by measuring solute reflection coefficients. The measurements employed a rapid reaction stopped-flow apparatus. For a given solute the permeability is a function of the area per lipid molecule in the membrane. Thus the permeability of lecithin liposomes is decreased by incorporation of cholesterol and increased by incorporation of phosphatidic acid. A comparison of different solutes shows that liposome permeability depends on solute size and lipid solubility in a manner similar to that observed for non-electrolyte permeation through synthetic polymers and some "non-porous" cell membranes. In the presence of 1 mM 2,4-dinitrophenol at pH 3.6 the permeability of lecithin liposomes to non-electrolytes decreases. A similar, but much smaller effect is observed in the presence of 5 mM Ca^{2+}.

INTRODUCTION

The results of the permeation of non-electrolytes through cell membranes have been interpreted in terms of membrane structure. Thus, the classical studies of Collander and Barland[1,2] as well as more recent work[3], have pointed to the dual nature of many cell membranes which act firstly as selective solvents allowing rapid permeation of lipid soluble solutes and secondly as molecular sieves allowing rapid permeation of small polar solutes. This molecular sieving aspect of cell membranes has lead to the concept of the "equivalent pore"[4]. Penetration through pores in cell membranes has been considered to be largely determined by steric effects. The equivalent pore theory has been criticized by Lieb and Stein[5] who suggest that cell membranes behave towards penetrating solutes as homogeneous polymer networks. In view of this controversy, attention has been directed towards permeability studies on model membrane systems whose structure and composition are defined[6]. The present study is concerned with measurement of reflection coefficients of non-electrolytes penetrating the bimolecular lipid membranes of smectic mesophases (liposomes)[7].

The reflection coefficient of a solute permeating a membrane is a measure of the permeability of the membrane to that solute relative to the water permeability.

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It is defined by the phenomenological equations relating the volume flow, J_V, across the membrane to the driving forces[8]:

$$J_V = L_p(\Delta P - \Sigma\sigma_i\Delta\pi_i) \quad (1)$$

where ΔP and $\Delta\pi_i$ are the difference in hydrostatic pressure and difference in osmotic pressure exerted by each solute across the membrane, respectively. L_P is the hydraulic conductivity of the membrane, and σ_i the reflection coefficient for solute i. Eqn 1 assumes that the solutions on either side of the membrane are ideal. For an ideal semipermeable membrane all σ_i values are unity and no solute can cross the membrane. As the membrane becomes more permeable to solutes the reflection coefficients decrease. When $\sigma_i = 0$, solute i and solvent move at equal velocities through the membrane. Negative σ_i values are also possible.

Bangham *et al.*[7] have shown that shrinking of liposomes in response to osmotic pressure gradients conforms to the van 't Hoff law. The same is true for swelling provided the osmotic pressure difference is less than 5 atm. Hence it is unlikely that a significant hydrostatic pressure difference can be maintained across the lipid membranes, and in the presence of one permeant solute, s, and one impermeant solute, imp, Eqn 1 reduces to:

$$J_V = -L_p(\Delta\pi_{imp} + \sigma_s\Delta\pi_s)$$

Under conditions of zero volume flow:

$$\sigma_s = -\left(\frac{\Delta\pi_{imp}}{\Delta\pi_s}\right)_{J_{V=0}} \quad (2)$$

Changes in liposome volume are paralleled by changes in the absorbance of the liposome dispersion[7]. Thus it is a relatively simple matter to find the conditions under which there is zero volume flow, when the "effective osmotic pressure" gradient of the permeant solute balances that of an impermeant solute. In the present case KCl was used as the impermeant solute. Efflux measurements were made to confirm that σ_{KCl} was unity under the relevant experimental conditions.

EXPERIMENTAL

Materials

Lecithin was extracted from fresh egg yolks and purified by alumina and silicic acid chromatography[9]. Phosphatidic acid was a gift from the Institute Pasteur, Paris. Both egg yolk lecithin and the phosphatidic acid were shown to be chromatographically pure by thin-layer chromatography on Merck silica gel F_{254}-coated plates and a chloroform–methanol–water (65:25:4, by vol.) solvent system. $^{42}K^+$ was obtained as ^{42}KCl from the Radiochemical Centre, Amersham, England. All other reagents were commercial and of analytical reagent grade.

Methods

Liposomes were prepared from dry lipids by using the method described by Bangham *et al.*[7]. The liposomes were prepared in either aqueous KCl solution or a solution of non-electrolyte, and were allowed to equilibrate at room temperature

for at least 1 h before use. Thin-layer chromatography was used to check for decom position of lipids during an experiment.

All permeability experiments were carried out at 25 °C. Reflection coefficients were measured by the "zero-time" method of Goldstein and Solomon[4]. In some experiments a suspension of liposomes (0.2 μmoles lipid/ml) in KCl solution (approximately pH 6) was mixed in a rapid reaction stopped-flow apparatus with an equal volume of a solution of the solute whose permeability was being studied. The stopped-flow apparatus has a dead time of 2 ms and has been described by Gibson and Milnes[10]. In other experiments a liposome suspension made in a solution of the probing non-electrolyte was mixed with KCl solutions. Any resultant swelling or shrinking of the liposomes was monitored by recording the intensity of the light (wavelength 450 nm) that passed through the observation cell of pathlength 2 cm. This transmitted light was measured using a photomultiplier whose output was amplified and displayed on a storage oscilloscope. The resultant trace was then photographed. Three different traces were recorded at six different non-electrolyte or KCl osmolarities. As a control liposomes were mixed with an equal volume of the solution in which they were prepared. Each output trace of light intensity (I) as a function of time, t, was magnified and after subtracting from this trace the average control trace, the tangent at zero time (dI/dt) was drawn by eye. These zero time tangents were plotted against the osmolarity gradient of either the probing solute or KCl depending on the experimental conditions. From the graph the osmolarity gradient corresponding to zero volume flow was found by interpolation.

$^{42}K^+$ leakage from liposomes during osmotic shrinking was measured by a method similar to that described by Bangham *et al.*[7]. A lipid dispersion (48 μmoles/ml) was formed in 0.145 M KCl containing $^{42}K^+$. After equilibration the dispersion was dialysed against tracer-free 0.145 M KCl until the diffusate was essentially free of $^{42}K.^+$ 1.5-ml aliquots of the dispersion were then transferred to flasks containing 3 ml of 0.425 M non-electrolyte in 0.145 M KCl, 0.572 M KCl, and 0.145 M KCl, respectively. These solutions gave concentration differences of 0.32 M non-electrolyte, 0.32 M KCl and zero between the solutions outside and inside the liposomes. 1.5-ml aliquots of each dispersion were then dialysed for three 30-min periods against 15 ml of tracer-free solutions of the same composition (0.32 M non-electrolyte in 0.145 M KCl, 0.465 M KCl, and 0.145 M KCl, respectively). The total radioactivity leaving each dialysis bag was measured, corrected for isotope decay, and expressed as a percentage of the total radioactivity trapped in each bag.

Osmolarities were measured to $\pm$ 2 mosM by freezing point depression using an Advanced Osmometer (Model 31LA).

RESULTS AND DISCUSSION

Electron micrographs of the lipid dispersions after negative staining with ammonium molybdate showed the liposomes to be heterodisperse, consisting of from one to eight concentric membranes.

$^{42}K^+$ leakage from liposomes and the value of σ_{KCl}

Table I shows the amount of $^{42}K^+$ leakage when liposomes (lipid composition

TABLE I

LEAKAGE OF $^{42}K^+$ FROM 18 μmoles LIPID FOLLOWING EXPOSURE TO HYPERTONIC SOLUTIONS

The liposome dispersion was made in 0.145 M KCl from a mixture of lecithin and phosphatidic acid (molar ratio 100:7.3)

Concentration of solute in 0.145 M KCl	*$^{42}K^+$ leakage expressed as a % of the total $^{42}K^+$ trapped at the beginning of each period*		
	1st 30 min	*2nd 30 min*	*3rd 30 min*
0	3.2	1.9	1.3
0.32 M methanol	3.5	2.2	1.6
0.32 M *n*-butanol	33.8	26.8	23.8
0.32 M propylene glycol	3.2	1.8	1.6
0.32 M propionamide	3.3	1.7	1.6
0.32 M urea	9.8	4.8	2.4
0.32 M KCl	3.5	2.9	1.7

7.3 moles phosphatidic acid/100 moles lecithin) were exposed to hypertonic non-electrolyte and KCl solutions. The experimental conditions were chosen to correspond to those used in reflection coefficient measurements that would give the maximum effect on K^+ leakage. That is, when measuring reflection coefficients the non-electrolyte concentration never exceeded 0.32 M, and the phosphatidic acid–lecithin molar ratio never exceeded 7.3:100. For the solutes studied there is no significant difference between the rate of $^{42}K^+$ loss in isotonic and hypertonic solutions, except when the solution is made hypertonic with *n*-butanol and urea. An increase in the permeability of lipid membranes to K^+ in the presence of *n*-butanol has been previously demonstrated[11], and is consistent with the disordering of the bimolecular lipid membrane which occurs when the alcohol is present in sufficiently high concentration in the membrane[12]. The observation that urea also causes some increase in $^{42}K^+$ leakage is unexpected. It is unlikely that urea at a concentration of 0.32 M would significantly weaken the hydrophobic interactions between the lipid hydrocarbon chains. However, adsorption of the urea dipole to the zwitterionic lecithin head group may have two effects. Firstly, a polarization distortion of the lecithin dipoles could open up a K^+ leakage pathway. Secondly, the possibility cannot be excluded that shape changes on shrinkage lead to greater membrane breakage and release of K^+ in the presence of urea. As will be discussed later reflection coefficients for urea were always indistinguishable from unity. Hence the KCl permeability of liposome membranes in the presence of urea is still small enough to put $\sigma_{KCl}=1$. This assignment will also be valid for the other non-electrolytes (except *n*-butanol) shown in Table I. In addition to these solutes the reflection coefficients of formamide, acetamide, 1,2-ethanediol, glycerol and glucose were measured. The lipid solubility of these solutes are all less than that of propionamide, and their dipole moments and H-bonding ability all less than that of urea. It is therefore also reasonable to assume that in the presence of these solutes $\sigma_{KCl}=1$.

Reflection coefficients for non-electrolytes

Figs 1a and 1b show typical output signals when liposomes containing a permeant solute were mixed with a hypertonic KCl solution, and the solution in which the liposomes were dispersed respectively. A small oscillatory component is seen at the start of both traces when the flow has stopped. This effect has been observed by other workers using rapid reaction stop flow techniques[13] and probably reflects a mechanical oscillation of the liposomes in the fluid. In some cases, particularly when liposomes dispersed in KCl were mixed with solutions containing a high concentration of non-electrolyte with a correspondingly high viscosity, this oscillation was so large as to obscure the time course of the change in intensity of transmitted light due to liposome volume changes. Such results were discarded. For the control experiment in Fig. 1 the transmitted light intensity is not a function of time after mixing. This indicates that mixing was not so vigorous as to cause liposome particles to disintegrate. When KCl solutions were mixed with non-electrolyte solutions invariant output signals were also obtained. Thus any change in the transmitted light intensity when liposome dispersions were mixed with anisotonic solutions may be attributed to liposome volume changes.

Fig. 2 shows the data obtained in an experiment in which liposomes made in an acetamide solution were mixed with anisotonic KCl solutions. The KCl concentration gradient which corresponds to zero volume flow ($dI/dt=0$) is found by interpolation. The reflection coefficient can then be evaluated from Eqn 2.

It has been shown that in contrast to shrinking when liposomes swell there is some bursting of liposomes with a significant loss of trapped K^+ (ref. 7). Since in the present case reflection coefficients are evaluated from "zero-time" tangents the effect of this K^+ leakage should be small. This is confirmed by the observation

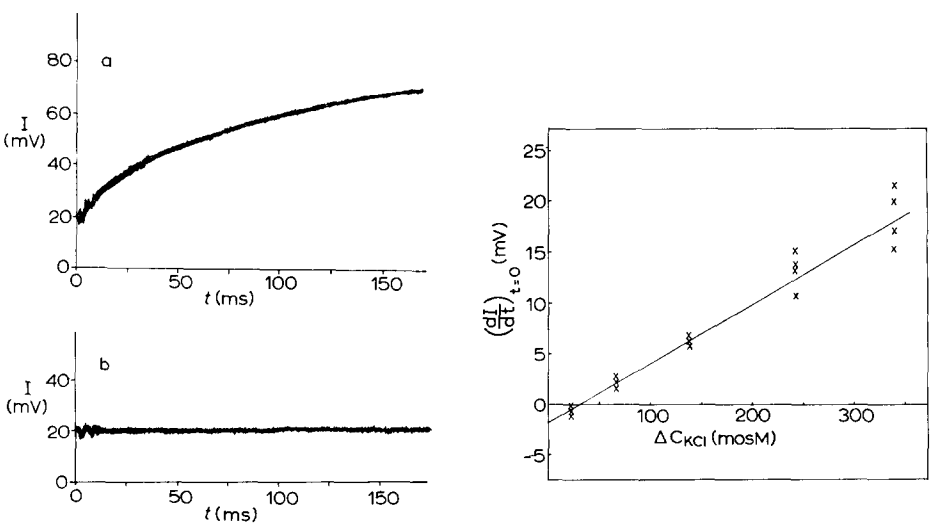

Fig. 1. Stopped-flow output traces when (a) a suspension of liposomes containing a permeant solute is mixed with hypertonic KCl solution, and (b) the same suspension is mixed with the solution in which the liposomes were formed.

Fig. 2. "Zero time" slopes, $(dI/dt)_{t=0}$ plotted as a function of KCl concentration differences, ΔC_{KCl}. The lecithin liposomes were formed in 180 mosM acetamide.

that "zero-time" tangents plotted as a function of concentration difference show no discontinuity on passing from shrinking to swelling.

Table II summarizes all the reflection coefficient results for liposomes of different composition. For each solute studied the same value for the reflection coefficient was obtained whether the solute was either initially present within the liposomes without KCl or present in the outside medium with only KCl within the liposomes. Bangham *et al.*[7] have shown that the water permeability of charged liposomes apparently depends on the KCl concentration inside the liposomes. That is, as the KCl concentration increases, and the ζ potential decreases, the water permeability decreases. Since reflection coefficients measure rate of movement of solute relative to that of water, they will depend less on KCl concentration than water permeability coefficients. However, in view of the observation (discussed later) that the value of a reflection coefficient is in part determined by solute size, some dependence of reflection coefficients on KCl concentration might be expected. One reason why this expectation is not realized may lie in the difficulty of estimating liposome surface areas, and taking into account the heterodisperse nature of liposome dispersions[14] in calculating water permeability coefficients. A second factor, namely an underestimation of the driving force for water movement when neglecting the increase in electrostatic repulsion between the charged lamellae as water moves into the liposomes, is unlikely to be of sufficient magnitude to affect the value of the water permeability coefficient.

Use of the "zero-time" method to measure reflection coefficients overcomes the problem of heterodispersity. The results are not affected by the number of concentric membranes in, or by differences in the initial volumes of the liposomes. The only limitations to the accuracy of the measurements lie in the difficulty of detecting small volume changes. For example, a series of experiments when liposomes containing urea were mixed with KCl solutions showed it was not possible, over a time scale of 200 ms, to distinguish between urea reflection coefficients of 0.95 and 1.00.

The dependence of reflection coefficients on membrane composition

Table II shows that incorporation of cholesterol into lecithin liposomes causes an increase in reflection coefficient values. Cholesterol has been shown by proton magnetic resonance[15] and electron spin resonance[16] to have a condensing effect on the lecithin hydrocarbon chains in phospholipid–water systems when the phospholipid is above the crystalline to liquid crystalline transition temperature. The decrease in fluidity leads to a decrease in the permeability of lipid membranes to ions and non-electrolytes[17,18]. In the present case this effect leads to an increase in reflection coefficient values.

In contrast to cholesterol, phosphatidic acid causes the area per lipid molecule in lecithin monolayers and bulk lipid–water systems to increase by charged head group repulsion. Thus, in general, the reflection coefficient of a given solute decreases with increase in area per molecule. This suggests that the rate limiting step for non-electrolyte permeation may be penetration of the solute from the aqueous solution into the hydrophobic barrier. Such a suggestion is consistent with the results of De Gier *et al.*[19] on permeation of polyhydroxyalcohols and those of Johnson and Bangham[11] on permeation of K^+ through lipid membranes. However, the present

TABLE II

REFLECTION COEFFICIENTS FOR NON-ELECTROLYTES PENETRATING LIPOSOMES OF DIFFERENT LIPID COMPOSITION

All values are shown as the mean ± range of values. The figure in brackets is the number of measurements. The liposome compositions are quoted in terms of molar ratios.

Solute	*Liposome Composition*			
	Lecithin	*Lecithin–cholesterol (100:10)*	*Lecithin–phosphatidic acid (100:3.6)*	*Lecithin–phosphatidic acid (100:7.3)*
Methanol	0.00 ± 0.01 (4)	—	—	—
Ethylene glycol	0.29 ± 0.02 (3)	—	0.17 ± 0.01 (3)	0.07 ± 0.01 (2)
Propylene glycol	0.45 ± 0.01 (3)	0.87 ± 0.01 (2)	0.20 ± 0.01 (3)	0.10 ± 0.02 (2)
Glycerol	0.71 ± 0.01 (4)	1.00 ± 0.01 (2)	0.34 ± 0.01 (2)	0.16 ± 0.01 (2)
Glucose	1.00 ± 0.01 (2)	—	1.00 ± 0.02 (2)	1.00 ± 0.02 (2)
Formamide	0.21 ± 0.02 (4)	—	0.10 ± 0.01 (2)	0.04 ± 0.01 (2)
Acetamide	0.31 ± 0.03 (5)	0.76 ± 0.02 (2)	0.20 ± 0.03 (9)	0.09 ± 0.01 (2)
Propionamide	0.48 ± 0.02 (2)	0.82 ± 0.02 (2)	0.23 ± 0.02 (3)	0.11 ± 0.01 (2)
Urea	1.00 ± 0.05 (4)	—	1.00 ± 0.03 (2)	1.00 ± 0.03 (2)

results do not exclude Trauble's suggestion[20] that permeability is controlled by movement of "kinks" or "free spaces" through the hydrocarbon phase of the membrane. In this context it should be pointed out that incorporation of phosphatidic acid into lecithin–water systems not only increases the area per lipid molecule, but also decreases the thickness of the lipid lamellae. This implies that the lipid hydrocarbon chains become more disordered and the concentration of kinks increases. In Trauble's[20] treatment of movement of small solutes across lipid membrane the membrane permeability is directly proportional to the kink concentration, and hence incorporation of phosphatidic acid into lecithin membranes would lead to an increase in membrane permeability.

Reflection coefficients and the nature of the permeating solute

The rates of permeation of non-electrolytes through membranes depend primarily on three variables: molecular size, solubility in the membrane, and, related to the latter, the chemical nature of the solute. Reflection coefficients are related to permeability coefficient, ω_s, by[21]

$$\sigma_s = 1 - \frac{\omega_s \bar{V}_s}{L_p} - \frac{\omega_s f_{sw} \delta_m}{\varphi_{=w}} \tag{3}$$

where $\bar{V}_s$ = partial molar volume of the solute, f_{sw} = frictional coefficient between a mole of solute and an infinite amount of water, δ_m = membrane thickness, φ_w = volume fraction of water in the membrane. The results of De Gier *et al.*[19] showing that at least some non-electrolytes permeate lipid membranes in a dehydrated state, and the low solubility of water in hydrocarbon solvents suggest that $f_{sw} = 0$. Hence

$$\frac{\omega_s}{L_p} = \frac{(1 - \sigma_s)}{\bar{V}_s} \tag{4}$$

If it is assumed that L_p is not affected by the presence of different non-electrolytes, then the values of ω_s/L_p are a measure of relative permeability coefficients. These are summarized in Table III together with values for the smallest cylindrical radius which will contain the unhydrated molecules. This radius has been used as a parameter to describe size effects, since molecules appear to permeate membranes in such a way that steric hindrance is at a minimum[3,22]. If, in the present case, steric hindrance is important in determining permeation rates, the larger the solute radius the greater will be the increase in permeability with increase in lipid area per molecule in the liposome membrane. An examination of the data in Table III shows this to be the case. However, values of ω_s/L_p for a given liposome composition show a very poor correlation with cylindrical radius alone.

TABLE III

RELATIVE PERMEABILITIES, ω_s/L_p, FOR NON-ELECTROLYTES PENETRATING LIPOSOMES

r is the radius of the narrowest cylinder fitting a molecular model of the solute; taken from Sha'afi *et al.*[3] and Soll[22]. k_s is the ether–water partition coefficient; taken from Collander and Barland[2].

Solute	r (Å)	k_s	$\omega_s/L_p \times 10^2$ (*moles·cm*$^{-3}$)*		
			Lecithin	*Lecithin–phosphatidic acid (100:3.6)*	*Lecithin–phosphatidic acid (100:7.3)*
Ethylene glycol	2.5	0.0053	1.27	1.48	1.66
Propylene glycol	2.7	0.018	0.75	1.09	1.23
Glycerol	2.8	0.00066	0.40	0.90	1.15
Glucose	3.7	$4.5 \cdot 10^{-6}$	0	0	0
Formamide	2.1	0.0014	2.09	2.39	2.54
Acetamide	2.4	0.0025	1.17	1.35	1.54
Propionamide	2.6	0.013	0.74	1.10	1.27
Urea	2.4	0.00047	0	0	0

* Calculated from $\omega_s/L_p = (1-\sigma_s)/\bar{V}_s$, where $\bar{V}_s$ is assumed to be the molecular weight divided by density of the pure compound.

A more reasonable prediction of reflection coefficient values can be made taking into account the chemical nature of the solute, through the solubility of the solute in non-polar solvents. Fig. 3 shows the data in Table III plotted in the form $\omega_s/L_p k_s$ against cylindrical radius. k_s is the ether–water partition coefficient, and is taken as a measure of membrane solubility. This is an arbitrary choice justified only by the observations of Hansch *et al.*[23] that partition coefficients between water and a wide range of organic solvents are mainly determined by aqueous solubility. Fig. 3 shows that the relative diffusion rates of urea are apparently

aberrant. However, as pointed out elsewhere[24], when $(1-\sigma_s)$ approaches zero it is not possible to satisfactorily calculate permeability coefficients. Recently Cohen and Bangham[25] have also calculated relative diffusion rates of non-electrolytes within liposome membranes. For their membranes urea is in no way aberrant and appears to diffuse as freely as glycerol.

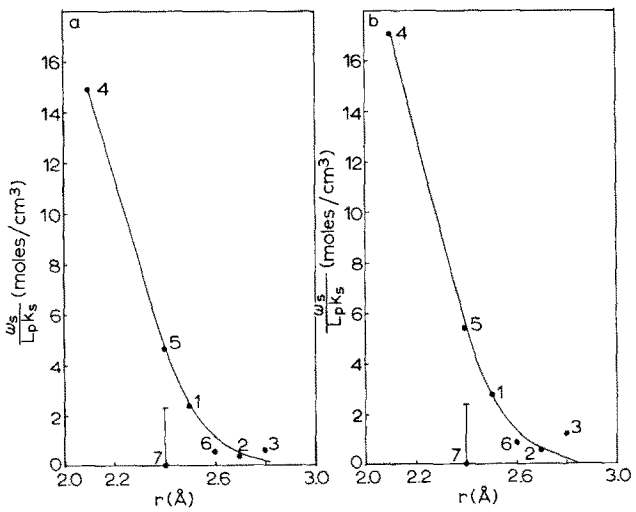

Fig. 3. Relative diffusion rates, $w_s/L_p k_s$, of non-electrolytes in liposome membranes as a function of cylindrical radius, r. (a) Lecithin liposomes, (b) lecithin–phosphatidic acid liposomes (molar ratio 100:3.6). The diffusing molecules are: 1, ethylene glycol; 2, propylene glycol; 3, glycerol; 4, formamide; 5, acetamide; 6, propionamide; 7, urea.

A comparison between non-electrolyte permeability of liposomes and cell membrane

A comprehensive set of non-electrolyte reflection coefficient data has been obtained by Solomon and co-workers[4,26] studying penetration of small hydrophobic solutes across mammalian red blood cell membranes. The data are consistent with these solutes permeating through "equivalent pores". The areas A_s and A_w for filtration of solute and water, respectively, through a porous membrane are related to reflection coefficients by[21]:

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

For polar solutes penetrating red cell membranes, $\omega_s \overline{V}_s / L_p$ values are negligibly small. Hence assuming permeation takes place only through the pores, σ_s can be directly related through the equivalent pore radius, r_p, to the radii of solute, r, and water, r_w, by Renkin's equation[27]:

$$1 - \sigma_s = \frac{A_s}{A_w} = \frac{[2(1 - r/r_p)^2 - (1 - r/r_p)^4]\,[1 - 2.104\,r/r_p + 2.09(r/r_p)^3 - 0.95(r/r_p)^5]}{[2(1 - r_w/r_p)^2 - (1 - r_w/r_p)^4]\,[1 - 2.104\,r_w/r_p + 2.09(r_w/r_p)^3 - 0.95(r_w/r_p)^5]} \tag{6}$$

Using this equation the red cell reflection coefficient data for polar solutes may be fitted by a single value for the equivalent pore radius. Since liposome membranes are extremely unlikely to contain pores, Eqns 5 and 6 would not be expected to describe the present reflection coefficient data. Fig. 4 shows that the experimental results are inconsistent with a single value for r_p. This observation suggests that permeation of polar non-electrolytes through the red cell membrane is not comparable to permeation through a lipid membrane.

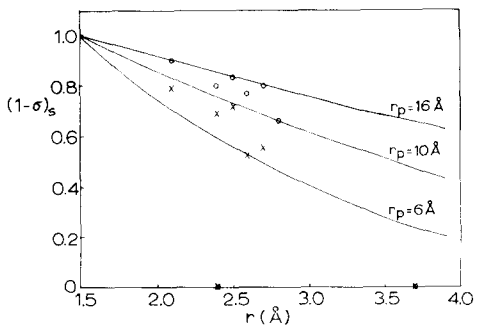

Fig. 4. $(1-\sigma_s)$ from Table II as a function of solute cylindrical radius, r. The theoretical curves for different pore radii, r_p, were drawn using 1.5 Å as the radius of a water molecule. ×, lecithin liposomes; ○, lecithin–phosphatidic acid liposomes (molar ratio 100:3.6).

However, there are many other cell membranes for which reflection coefficient data do not require the assumption of pores. Lieb and Stein[24] have pointed out that for membranes such as those of *Chara*, *Nitella*, and rabbit gall bladder permeation of both hydrophilic and hydrophobic solutes resembles diffusion in hydrophobic polymers. Thus, they show it is possible to make good predictions of relative permeabilities and reflection coefficients by correlating the ratio of the relative permeability to the lipid solubility of the solutes with a molecular size parameter, the molecular weight. For such a statistical analysis to be valid the data to be correlated should correspond to a larger number of solutes than studied here. In the present work the molecular size has been represented by the cylindrical radius. For non-electrolytes, the latter shows a high correlation with molecular weight. This provides support for Cohen and Bangham's[25] suggestion that there is a similarity between the permeability barrier of liposome membranes and "non-porous" cell membranes.

The effect of Ca^{2+} and 2,4-dinitrophenol on liposome permeability

Table IV shows the effect of firstly 5 mM $CaCl_2$ and secondly 1 mM 2,4-dinitrophenol on the reflection coefficients of some non-electrolytes.

Ca^{2+} causes a small increase in reflection coefficient values. Shah and Schulman[28,29] found that the presence of Ca^{2+} (10 mM) in the aqueous phase below lecithin monolayers raises the monolayer surface potential, and lowers the surface pressure required to condense the monolayers. It was suggested[29] that Ca^{2+} forms a bridge between two phosphate groups of adjacent lecithin molecules restricting their degrees of freedom. This is consistent with Trauble's[30] observations using acidic fluorescent and absorption indicator molecules on the effect of Ca^{2+}

on phase changes in bulk lecithin–water systems. However, it has recently been shown[31] that Ca^{2+} has no effect on pure lecithin monolayers, and it is only lecithin monolayers containing acidic impurities that show a binding affinity for Ca^{2+}. Therefore, the present observed effect of Ca^{2+} on lecithin liposome permeability may be due to traces of acidic impurities.

TABLE IV

THE EFFECT OF 5 mM Ca^{2+} AND 1 mM 2,4-DINITROPHENOL ON REFLECTION COEFFICIENTS FOR LECITHIN LIPOSOMES

Solute	*Reflection coefficients*		
	Lecithin liposomes	*Lecithin liposomes in 5 mM $CaCl_2$*	*Lecithin liposomes in 1 mM dinitrophenol*
Acetamide	0.31 ± 0.03 (5)	0.37 ± 0.02 (2)	0.61 ± 0.01 (2)
Propylene glycol	0.45 ± 0.01 (3)	0.50 ± 0.01 (2)	—
Glycerol	0.71 ± 0.01 (4)	0.82 ± 0.03 (3)	1.00 ± 0.01 (2)

A particularly interesting observation is that in the presence of 1 mM 2,4-dinitrophenol at pH 3.6 reflection coefficients also increase. It is unlikely that the change in pH can account for this result since lecithin is zwitterionic from pH 3–11[32]. At pH 3.6 2,4-dinitrophenol is 74% undissociated. It has been shown that both the dinitrophenate and dinitrophenol bind to lecithin monolayers (G. T. Rich, unpublished) and lecithin liposomes (J. Lelievre and G. T. Rich, unpublished). Binding of the acid form involves H-bonding of the OH group to the lecithin phosphate and causes a broadening of the proton magnetic resonance peak arising from the CH_2 protons on the fatty acid chains in bulk lecithin–water systems (Lelievre, J. and Rich, G. T., unpublished). Thus the presence of 2,4-dinitrophenol at low pH leads to effects similar to the incorporation of cholesterol into lecithin membranes. This is reflected by a decrease in non-electrolyte permeability.

Dinitrophenol in common with other uncouplers of oxidative phosphorylation causes an increase in the conductance of black lipid membranes. The pH–conductance curve at a fixed concentration of 2,4-dinitrophenol shows a conductance maximum, which for uncharged black lipid membranes is close to the pK of 2,4-dinitrophenol. This observation is consistent with various carrier models[33,34] describing the effect of 2,4-dinitrophenol but it has not proved possible to describe the pH–conductance curve over the whole pH range. If 2,4-dinitrophenol at low pH lowers the permeability of black lipid membranes then this will affect the pH–conductance curve. At high pH binding of the dinitrophenate will also have an effect by altering the electrostatic potential at the membrane surface.

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