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THE INFLUENCE OF THE LIPID ON THE WATER PERMEABILITY OF ARTIFICIAL MEMBRANES

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Summary

The water permeabilities of artificial membranes formed from various monoglycerides or phospholipids in alkane solvents have been measured using an osmotic method, and it has been shown that the permeability depends upon the type of lipid used. For monoglycerides, the permeability was found to increase with the unsaturation and decrease with the length of the acyl chain. Membranes formed from either egg phosphatidylcholine or dioleyl phosphatidylcholine had an osmotic permeability coefficient of approx. 35—40 μm/s at 25°C; with sphingomyelin as the membrane lipid, the permeability was an order of magnitude lower than that for phosphatidylcholine. It is suggested that the water permeabilities of biological membranes might be partly controlled by the types of lipid present.

Introduction

The barrier to the movement of water across unmodified lipid bilayer membranes is usually considered to be the non-polar interior which is composed of the acyl chains of the lipid. Since it is believed that biological membranes contain a lipid bilayer substructure, it might be possible to account for some of the variation in water permeabilities of biological membranes in terms of their different lipid compositions. One approach to this problem is to study the water permeability of lipid bilayers as a function of the lipid composition. Two techniques are available for measuring water permeabilities [1—3]; these measure either an exchange diffusion as indicated by tritiated water transfer, or an osmotic flow produced by a concentration gradient of a solute to which the membranes are effectively impermeable. The osmotic method is preferable as it is less subject to the presence of unstirred layers adjacent to the membrane [3—5].

There have been several investigations of the water permeabilities of lipid

membranes, but little data exists for osmotic permeabilities of membranes of known composition. Even for a common lipid such as egg phosphatidylcholine, there is a substantial scatter among the values quoted, these ranging from 17 to 100 μm/s at 36°C [2,6,7]. Previous investigations have shown that the addition of cholesterol can decrease the membrane permeability [7,8] and also that there is a correlation between the lipid unsaturation and water permeability [7].

This paper describes osmotic flux measurements for some monoglyceride and phospholipid membranes, and the effect of varying a number of parameters which alter the fluidity and thickness of the hydrocarbon core. These include lipid chain length and unsaturation, and also the solvent in which the lipid is dissolved. The present study attempts to show what factors might produce the low permeabilities reported for some unfertilized eggs and epithelial cell membranes [9—11].

Materials and Methods

Water permeability measurements. The osmotic permeability was determined from the net volume flow induced by a difference in solute concentrations between aqueous solutions on each side of the membrane. One of the aqueous compartments was closed, and the adjustment to maintain its internal volume constant (and hence prevent the membrane from bulging) was used to measure the volume flow. The apparatus was similar to that described by Cass and Finkelstein [4]. The closed compartment consisted of a piece of polytetrafluoroethylene (PTFE) tubing attached at one end to a 10 μl Hamilton syringe with a micrometer drive. Membranes were formed across the other end of the PTFE tubing. The apparatus was enclosed in a thermostatted box.

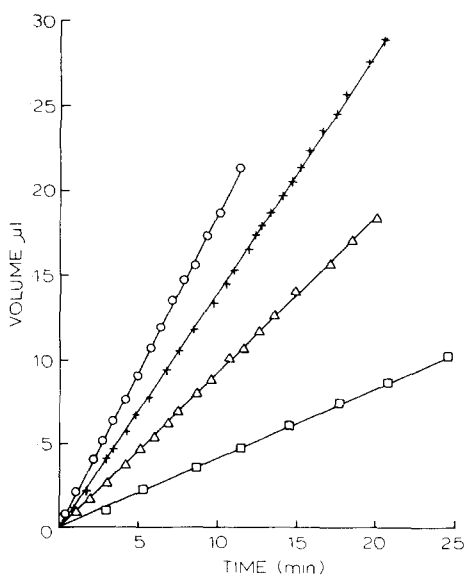


Fig. 1. *Volume flow of water across membranes formed from various lipids in decane.* ○, +, egg phosphatidylcholine; △, dioleyl phosphatidylcholine; □, glyceryl monooleate. Osmotic gradient 0.3 M NaCl. Temperature = 25° C. The differing slopes reflect different membrane areas as well as different permeabilities.

The osmotic gradient was established by addition of a known volume of concentrated NaCl solution to the outer compartment. The water flux, J_v, was determined from a linear plot of the volume flow against time and the membrane area. Examples of such plots for glyceryl monooleate/decane and phosphatidylcholine/decane membranes are shown in Fig. 1.

The electrolyte flux is negligible compared to that of the water. Thus, using the terminology of Kedem and Katchalsky [12], the filtration coefficient L_p can be related to the volume flow, J_v, by:

$$J_V = L_p \nu RT(g_2c_2 - g_1c_1) \tag{1}$$

where c_1 and c_2 are the concentrations of the aqueous solutions on either side of the membrane, g_1 and g_2 are the respective rational osmotic coefficients, and ν is equal to 2. The water permeability coefficient, P_f, is expressed in the form [13]

$$P_f = L_p \frac{RT}{\overline{V}_w} \tag{2}$$

where $\overline{V}_w$ is the partial molar volume of water. The presence of unstirred layers adjacent to the membrane could result in an over-estimate of the concentration gradient $(c_2 - c_1)$. It has been argued that, for vertical membranes, convective stirring caused by density gradients would help to dissipate stagnant layers during osmosis [14].

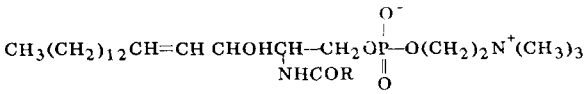
Capacitance measurements. The specific capacitances of the different types of membrane were determined in a separate experiment using techniques described previously [15]. The capacitance of the system was measured at 550 Hz with an a.c. bridge technique and at this frequency was effectively that of the membrane. The hydrocarbon core contains both solvent and alkyl chains of the lipid. Values for the dielectric constants and densities of the components were interpolated from those for bulk hydrocarbon. Dielectric constants for the unsaturated alkyl chains were derived [16] from measurements on various long chain unsaturated hydrocarbons. Interfacial tensions of glyceryl monooleate in hexadecane against 0.1 M NaCl were determined by the drop volume technique [17]. Membrane compositions and thicknesses were estimated from the specific capacitances as described previously [18].

Pure phosphatidylcholine bilayers were formed using a technique similar to that described by Montal and Mueller [19] and Takagi et al. [20] by lowering a PTFE partition through an air-water interface on which the phospholipid had been spread as a monolayer. The apparatus consisted of a cylindrical PTFE boat with a 1 cm diameter hole on one side over which a piece of 12.5-μm thick PTFE film was sealed with vaseline. The film contained a 100—200 μm diameter hole, and was lowered through the interface by slow rotation of the boat. The air/0.1 M NaCl interface was cleaned carefully before spreading the phospholipid from a pentane suspension. Capacitance measurements were carried out at room temperature (22°C). For all apparatus, the usual cleaning procedures were employed throughout using chromic acid, and rinsing in singly and doubly distilled water.

TABLE I

CHAIN COMPOSITIONS OF PHOSPHOLIPIDS

Mean chain composition: 17.8 carbons long, 1.2 double bonds per chain (A); and 20.9 carbons long, 0.4 double bonds per chain (B). The table gives the composition of the variable chain, R, in sphingomyelin, whose formula is:



Chain	Percentage total fatty acid (w/w)	Chain	Percentage total fatty acid (w/w)
(A) Egg phosphatidylcholine			
16 : 0	26	22 : 6	4
16 : 1	2	18 : 4	3
18 : 0	15	20 : 2	
18 : 1	32	20 : 5	
18 : 2	12	22 : 5	
20 : 4	6		
(B) Beef brain sphingomyelin			
16 : 0	2	24 : 0	6
18 : 0	35	24 : 1	39
20 : 0	3	20 : 1	3
22 : 0	10	22 : 1	
23 : 0	2	23 : 1	

Materials. The monoglycerides were purchased from Nu Chek Prep Inc. (Minnesota, U.S.A.) and were ⩾99% pure with respect to their fatty acid residue. A sample of synthetic dioleyl phosphatidylcholine was kindly provided by Dr. J.C. Metcalfe. This was 99% pure in its chain composition, as determined by gas-liquid chromatography. Two samples of pure egg yolk phosphatidylcholine were used. One was generously supplied by Mr. N. Miller (Agricultural Research Council Institute of Animal Physiology, Babraham); the other was purchased from Lipid Products (Redhill, Surrey, U.K.). Each sample gave a single spot on thin-layer chromatography using either acid or alkaline solvent systems, and both had similar fatty acid chain compositions. A representative chain composition determined by gas-liquid chromatography is shown in Table I. Sphingomyelin extracted from beef brain was also obtained from Lipid Products, and gave a single spot on thin-layer chromatography. Its fatty acid composition was determined and is shown in Table I. The cholesterol was a B.D.H. biochemical standard. The hydrocarbons and hexadecyl bromide were Koch-Light Puriss grade and were further purified by passage through an alumina column. Unless otherwise stated, membrane-forming solutions were made up to give a lipid concentration of approx. 7—10 mM.

The NaCl was analytical reagent and was roasted at 700°C to remove organic impurities. Aqueous solutions were made up with 'quartz-distilled' water, and had a pH of approx. 5.0.

Results

Water permeabilities of phospholipid membranes

The osmotic permeabilities of some phospholipid membranes are given in Table II. For membranes formed from either sample of egg phosphatidylcholine or from dioleyl phosphatidylcholine dispersed in decane, similar permeabilities were obtained. These were about 35—40 μm/s at 25°C. The permeability

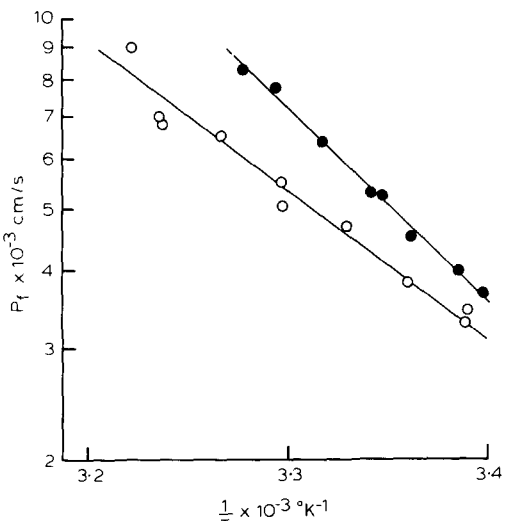


Fig. 2. Arrhenius plot for the osmotic permeability as a function of temperature. Membranes formed from: ○, egg phosphatidylcholine/decane; ●, glyceryl monooleate/hexadecane. Lines are least-square fits to the points.

TABLE II

OSMOTIC WATER PERMEABILITIES OF SOME PHOSPHOLIPID MEMBRANES IN DECANE

P_f = osmotic permeability coefficient (mean ± S.E.), C_m = the specific capacitance, and d = the inferred thickness of the non-polar region. All measurements made at 25°C. PC = phosphatidylcholine; SM = sphingomyelin; chol = cholesterol.

Lipid	P_f (μm/s)	C_m (μF/cm^2)	d (A)
Egg PC (A)	37.4 ± 2.3 (3)	0.387 ± 0.005	48.6
Egg PC (B)	36.9 ± 1.5 (5)		
Dioleyl PC	35.3 ± 0.3 (4)	0.397 ± 0.010	47.4
Egg PC + cholesterol			
Chol = 14 mM	30.3 (1)	0.490 ± 0.010	
Chol = 22 mM	17.2 ± 2.1 (2)		
Chol = 28 mM	14.4 ± 4.5 (2)	0.600 ± 0.010	33.6 **
SM + cholesterol			
Chol = 21 mM	12.3 ± 0.6 (3) *		
	7.5 ± 1.1 (3)		
Chol = 14 mM	3.7 ± 0.4 (3) *		

* Temperature = 31°C.

** Thickness calculated assuming equimolar phospholipid and cholesterol in the membrane.

for egg phosphatidylcholine was also studied as a function of temperature in the range 20—40°C. The results are presented as an Arrhenius plot (Fig. 2), from which the activation energy was estimated to be 10.8 ± 0.8 kcal/mol. The permeabilities obtained here lie within the range of those reported by other people for the same system [2,6,7]. A depressant effect on the permeability was observed on addition of cholesterol to the phosphatidylcholine (see Table II), and this is in agreement with the findings of Cass and Finkelstein [8]. The value of 14.4 μm/s (T = 25°C) with 28 mM cholesterol was the minimum permeability that could be achieved by addition of the sterol. Substitution of sphingomyelin as the phospholipid was also found to be effective in reducing the permeability. Thus for one sphingomyelin/cholesterol mixture in decane, a permeability of 7.5 ± 1.1 μm/s (T = 25°C) was measured. If the amount of cholesterol was reduced, the permeability was further diminished to 3.7 μm/s (T = 31°C). For the latter mixture, measurements could not be made at 25°C as the membranes were very fragile and viscous. A correction using the temperature coefficient observed for the higher cholesterol concentration would predict a permeability at the lower cholesterol concentration of 2.3 μm/s at 25°C.

Water permeabilities of monoglyceride membranes

Membranes formed using decane as the solvent are known to retain a substantial fraction of this solvent in the non-polar interior, but this fraction may be reduced by the use of solvents of longer chain length [18]. Membranes formed from glyceryl monooleate dissolved in either decane or hexadecane had similar permeabilities of about 51 μm/s at 25°C (Table III). The hexadecane membranes retain only about 24% by volume of solvent as compared to 49% for the decane membrane. The effect of temperature on the permeability for

TABLE III

OSMOTIC WATER PERMEABILITIES OF SOME MONOGLYCERIDE MEMBRANES

P_f = osmotic permeability coefficient (mean ± S.E.); C_m = specific capacitance, d = inferred thickness, and ϕ = volume fraction of lipid chains in the hydrocarbon core. The dielectric constant used for chains of 18 : 1, 22 : 1 and 24 : 1 was 2.20 and for 18 : 2 and 18 : 3 were 2.28 and 2.38, respectively. All measurements made at 25°C. Monoglycerides were dissolved in decane, except the ones indicated by **. Monoolein/mononervonin mixture contained 4 mM of each glyceride. Monoolein/monostearin mixture contained 7.1 mg/ml of the former and 2.9 mg/ml of the latter. For both mixtures, it was necessary to warm the solution to dissolve glycerides.

Lipid	P_f (μm/s)	C_m (μF/cm^2)	d (Å)	ϕ
Monoolein (18 : 1)	51.2 ± 0.3 (7)	0.380 ± 0.003	48.9	0.51
Monoolein (18 : 1) *,**	52.7 ± 0.4 (2)	0.584 ± 0.003	32.8	0.76
Linolein (18 : 2) **	73.2 ± 1.2 (3)	0.667 ± 0.006	29.7	0.82
Linolenin (18 : 3) **	—	0.803 ± 0.009	25.9	0.92
Monoerucin (22 : 1)	41.0 ± 1.6 (2)	0.326 ± 0.005	57.0	0.52
Monoolein (18 : 1) * Mononervonin (24 : 1)	33.7 ± 0.9 (4)	0.320 ± 0.008	58.1	0.50
Monoolein (18 : 1) * Monostearin (18 : 0)	39.3 ± 0.4 (3)	—	—	—

* Temperature = 26°C.

** Monoglycerides dissolved in hexadecane.

the hexadecane system was also measured and from the Arrhenius plot (Fig. 2) an activation energy of 14.2 ± 1.0 kcal/mol was inferred.

It is difficult to interpret the results of using the different hydrocarbon solvents as the substitution may result in several factors changing simultaneously, each of which may influence the permeability. On the simple idea that water permeation is limited by solution and diffusion of the water in the non-polar interior of the membrane, changes in thickness and fluidity will both influence the permeability. Thus while membranes formed using decane as the solvent are thicker they also contain a considerable amount of lower viscosity solvent in which water might more easily dissolve and diffuse. A similar difficulty ensues when attempting to interpret the effects of variation in other lipid parameters. Thus if the thickness is varied by altering the lipid chain length, it is likely that the fluidity will also alter, for it is known that the viscosity of hydrocarbons is a strong function of chain length [21]. Membranes formed from monoglycerides of longer chain length were thicker and had lower permeabilities (Table III), but the change in thickness was not the only factor affecting the permeability. Thus with monoerucin (22 : 1) as the lipid, the mean permeability was 41.0 ± 1.6 μm/s, but if the same membrane thickness was achieved with an equimolar mixture of monoolein (18 : 1) and mononervonin (24 : 1), the permeability was 33.7 ± 0.9 μm/s.

Altering the degree of unsaturation of the monoglyceride was also found to influence the permeability. With the lipid as monolinolein (18 : 2) which contains two double bonds per chain, the permeability increased by about 50% as compared to monoolein. Dilution of monoolein (18 : 1) with the saturated monostearin (18 : 0) reduced the permeability by 20% (Table III). Membranes formed from the mixture were estimated to contain about 30% monostearin on the basis of the compositions measured by Pagano et al. [22].

While examining the polyunsaturated glycerides, it was found that the specific capacitances of the membranes increased with the degree of unsatura-

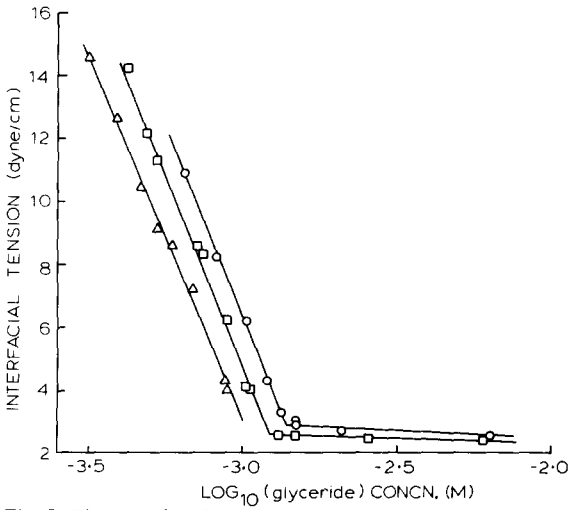


Fig. 3. The interfacial tension of monoglycerides at the *n*-hexadecane/0.1 M NaCl interface at 20°C as a function of the glyceride concentration. ○, glyceryl monooleate (18 : 1); □, glyceryl monolinoleate (18 : 2); △, glyceryl linolenate (18 : 3).

TABLE IV

SPECIFIC CAPACITANCES AND HYDROCARBON THICKNESS OF SOME MEMBRANES MADE FROM EGG PHOSPHATIDYLCHOLINE

Values represent means ± S.E. PC = phosphatidylcholine.

System	C_m (μF/cm^2)	d (Å)
PC/hexadecane		
0.1 M NaCl	0.603 ± 0.010	32.3
5.92 M NaCl	0.655 ± 0.009	29.7
PC/hexadecyl bromide		
0.1 M NaCl	0.713 ± 0.014	27.3
5.92 M NaCl	0.720 ± 0.033	27.1
PC/no solvent		
0.1 M NaCl	0.760 ± 0.040	25.6

tion. This effect was not totally explained by an increase in the dielectric constant of the lipid chains or a change in the adsorption of the glyceride. Fig. 3 shows the results of the adsorption measurements. These were interpreted in terms of the Gibbs adsorption equation [23] in order to obtain the limiting areas per molecule which for monoolein (18 : 1), monolinolein (18 : 2), and monolinolenin (18 : 3) were found to be 39 Å^2, 38 Å^2 and 40 Å^2, respectively. Inferred thicknesses and compositions for membranes formed from these monoglycerides in hexadecane are given in Table III. The changes in thickness result from a progressive exclusion of solvent from the membrane in going from monoolein to monolinolenin. It should be noted that, on the bulk solution and diffusion assumption, the decrease in thickness is not enough to explain the observed increase in water permeability for the polyunsaturated monoglyceride, and it is concluded that this is due to the increase in double bond content.

The bulk hydrocarbon assumption

It has been suggested, on the basis of specific capacitance measurements for membranes containing no solvent that there might be substantial water penetration between the lipid headgroups into the non-polar core [19]. This would considerably alter the properties of the non-polar core, including its dielectric constant and the solubility of water in it. It would also cast doubt on the assumption that it behaves like a layer of bulk hydrocarbon whose thickness may be obtained from capacitance measurements. These problems affect the interpretation of the water permeabilities. Thus measurements of the specific capacitances of solventless membranes made from egg phosphatidylcholine were performed. Values in the range 0.66—0.81 μF/cm^2 were obtained with a mean of 0.76 ± 0.04 μF/cm^2. This value is lower than the values of 0.9—1 μF/cm^2 originally reported [19], but is similar to that of 0.72 μF/cm^2 found by Benz et al. [24] for synthetic dioleyl phosphatidylcholine. If a value of 2.20 is assumed for the dielectric constant of the non-polar core, the measured capacitance corresponds to a thickness of 25.6 Å. By comparison, the thickness, as deduced from X-ray diffraction data, of the non-polar region of the fully

hydrated lamellar phase of egg phosphatidylcholine is approx. 26 Å [25].

Another set of experiments were performed to compare the specific capacitances of membranes in 0.1 M and saturated NaCl solutions. Conventional membranes, formed by suspending phosphatidylcholine in long chain solvents, were used, with hexadecane or hexadecyl bromide as the solvent. For both systems, it is believed that only a small fraction of the solvent is retained in the membrane [18]. It was found that the specific capacitances in saturated salt were similar to or slightly higher than in 0.1 M salt (Table IV). The water content of the membranes might be expected to be lower in saturated salt as its water activity is only about 0.76. The fact that this does not significantly reduce the measured specific capacitance is consistent with the idea that water penetration is not a serious problem in interpreting the capacitance data. Thus the membranes can be regarded as two layers of polar headgroups sandwiching a non-polar interior whose thickness can be reasonably estimated from specific capacitance measurements.

Discussion

The use of planar lipid bilayers allows the formation of membranes of known compositions but suffers from the disadvantage of the retention of solvent in the bilayer. This may be avoided by using liposomes but with this technique it is not usually possible to obtain an absolute value for the osmotic permeability [26]. The present results demonstrate a reproducible permeability for phosphatidylcholine membranes, and the activation energy for permeation in this system is comparable to that obtained by other people for egg phosphatidylcholine bilayers [7] and liposomes [27,28]. Some of the scatter in reported water permeabilities [2] may have arisen from lipid impurities. This can be avoided by using synthetic monoglycerides.

The small effect on the water permeability of using hexadecane rather than decane as a solvent for monoolein leads one to suppose that the same might hold for phosphatidylcholine, as changes in solvent content and thickness are likely to be similar for the two types of lipid [18]. This allows a comparison with the permeability values obtained for phospholipid vesicles. Reeves and Dowben [28] estimated the osmotic permeability of egg phosphatidylcholine vesicles from turbidity measurements, and although the interpretation of these measurements is more difficult, their inferred permeabilities of 40.6—44.8 μm/s ($T = 25^{\circ}$C) are in reasonable agreement with the present value for the phospholipid in decane of about 37 μm/s.

It has been shown here that the osmotic permeability can be reduced by almost an order of magnitude by using sphingomyelin as the phospholipid. A reduction was also observed by Finkelstein [29], who measured the permeability of sphingomyelin/cholesterol membranes to tritiated water.

Some of this reduction in permeability might be accounted for by the high content of long and saturated acyl chains in the sphingomyelin (Table I), as these factors have been shown to reduce the permeability of monoglyceride membranes. It has been suggested [30,31] that there could be substantial headgroup interaction in sphingomyelin, occurring by hydrogen bonding between the 3-hydroxy goups of the sphingosines. This would restrict the chain move-

ment and might also lower the water diffusion coefficient as compared to phosphatidylcholine. These various factors also presumably manifest themselves in the high transition temperature for sphingomyelin, which is in the region of 20—45°C [32,33]. The lowest permeabilities measured in the present work are still several times larger than the value of approx. 1 μm/s which has been obtained for the osmotic permeability of membranes of unfertilised eggs and some epithelial cells [9—11]. Nevertheless the present results raise the possibility that cells may be able to control the water permeability of their surface membrane over a fairly wide range by altering the types of lipid present.

Acknowledgements

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