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MEASUREMENT OF THE GLUCOSE PERMEATION RATE ACROSS PHOSPHOLIPID BILAYERS USING SMALL UNILAMELLAR VESICLES

EFFECT OF MEMBRANE COMPOSITION AND TEMPERATURE

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Small unilamellar vesicles were used to measure the permeability of saturated phosphatidylcholine bilayers to glucose. The presented method circumvents most of the common restrictions of classical permeability experiments. Increasing the fatty acid chain length of the lipids reduced the permeation rate significantly. Raising the temperature above that of the lipid phase transition drastically increased membrane permeability. Arrhenius plots demonstrated the activation energy to be independent of membrane composition and the phase-state of the lipids. The permeation process is discussed in terms of a constant energy to disrupt all hydrogen bonds between permeant and aqueous solvent prior to penetrating the membrane. The magnitude of the permeability coefficient is partly determined by a unfavourable change in entropy of activation on crossing the water/lipid interface. All results indicate that the penetration of the dehydrated permeant into the hydrophobic barrier is the rate-limiting step in the permeation of glucose.

Introduction

The ability of lipid bilayers to act as an effective barrier to free solute diffusion has been assessed in different ways [1–11]. Liposomes have proved to be quite successful for this purpose [8,12]. Most studies on the permeability of vesicles have used the outward flow of entrapped material. To estimate this, a separation of the vesicles from all free solute is desirable. Current procedures use a variety of techniques such as dialysis [1], gel filtration [9,13], ion exchange chromatography [14] and membrane filtration [15]. Although many of these methods have been used successfully, they have some serious drawbacks. Encapsulation of the diffusant has often involved its co-sonication with the membrane lipids used and the diffusant may be liable to be damaged by the resulting cavitation. Moreover, as the encapsulation efficiency is low, high concentrations of substance are needed. Sonication can also generate aerosols from solutions which may contain hazardous labelled or carcinogenic chemicals which have been added to the lipid suspension. During the subsequent removal of non-encapsulated materials, vesicles are often subjected to column chromatography [2,3,9]. This results in a dilution of the liposome preparation. Removing the free solute from the vesicle preparation creates a concentration gradient across the membrane which causes the liposomes to leak prior to the diffusion experiment [16]. In measuring the outflux of the diffusion material, most workers have applied dialysis. As this technique is rather slow, it is only applicable to charged and ionic diffusants [1]. Other methods to measure permeability are based on

osmotic swelling or shrinking of vesicles induced by a hypo- or hyper-tonic solution, respectively [6]. Those methods are normally confined to fast diffusing substances. There is therefore a need for a reliable method to estimate quantitatively vesicle permeability to polar non-electrolytes such as sugars and amino acids.

This paper describes an improved method to study the permeability of lipid bilayers to polar substances, using small unilamellar vesicles and circumventing most of the above limitations. A second objective of this study was to evaluate the effect of membrane composition and phase phenomena on the permeability for glucose and to suggest some basic principles of this poorly understood process.

Materials

L-α-Dipalmitoylphosphatidylcholine (DPPC), L-α-dimyristoylphosphatidylcholine (DMPC) and dicetylphosphate (DCP) were obtained from Sigma Chemical Co., U.S.A. The purity of the phospholipids was regularly checked by thin-layer chromatography [17,18]. Both lipids proved to be more than 99% pure. Traces of lysophospholipids and fatty acids were detected. Analysing the lipids by GLC after methylating the fatty acids revealed a chain purity better than 99.5%.

L-α-[1-^{14}C]Dipalmitoylphosphatidylcholine (^{14}C-DPPC) with a specific activity of 20 mCi/mmol was purchased from Applied Science Laboratories, U.S.A. and D-[1-^{3}H]glucose (spec. act. 3.8 Ci/mmol) from Amersham International, U.K. The scintillation cocktails Instagel and Universal 299 were from Packard Instrument Co., U.S.A.

Sepharose CL-4B and Sephadex G-50 Medium were obtained from Pharmacia, Sweden. Triton X-100 was purchased from Fluka, Switzerland. All inorganic chemicals were analytical grade or better.

Methods

Preparation of the vesicles

20 mg of DMPC were spiked with $1.5 \cdot 10^{-3}$ μCi of ^{14}C-DPPC and spread as a thin film on the bottom of a thick-walled glass container (internal diameter 28 mm) by evaporating the chloroform/methanol (2:1, v/v) solvent by a stream of dry nitrogen gas. When charged liposomes were to be prepared, 5 mol% of DCP were included. The film was dried in vacuo for at least one hour to remove all traces of organic solvent. 5 ml of a 0.01 M sodium phosphate buffer (pH 7.2) containing also 0.01 M sodium chloride and 0.02% sodium azide were used to hydrate the lipid film. Hydration was allowed to procede for one hour at a temperature of 28°C, which is well above the phase transition temperature of DMPC.

Sonication was performed by a probe sonicator (Labsonic 1510, Braun, F.R.G.) equipped with a standard titanium probe (108 mm long and 19 mm diameter). The power was set at 100 W. The vessel containing the hydrated lipids was immersed in a circulation water bath set at 7.5°C. During sonication, the temperature of the sonicated solution quickly reached an equilibrium value of approx. 28°C. The sonicated sample was subsequently equilibrated for 15 min at 28°C and then centrifuged (JS-21, Beckman, U.S.A.) at the same temperature for 25 min at $30000 \times g$ in order to remove all titanium particles. The clear vesicle suspension was kept at 28°C for further use.

When DPPC or a mixture of DMPC and DPPC was used, the preparation conditions were essentially the same except that hydration, equilibration and centrifugation were performed at 48°C. The thermostat of the cooling circuit was set at 28°C.

Gel filtration on Sepharose CL-4B

1 ml of clear vesicle suspension, labelled with 0.1 μCi ^{14}C-DPPC prior to preparation, was subjected to molecular sieve chromatography at room temperature on a Sepharose CL-4B column (500 × 9 mm). The void volume was 14.25 ml as determined with Blue Dextran 2000 (5 mg/ml). Elution was performed by a peristaltic pump at 13 ml/h and 1.25 ml fractions were collected. The activity was measured by mixing 1 ml of each fraction with 5 ml scintillation cocktail (Universal 299) and counting the samples with a liquid scintillation counter (Beckman, LS9000).

Electron microscopy

Vesicles were routinely checked by electron microscopy. For this a Formvar 1595E (Merck, F.R.G.) coated 400 mesh copper grid (Balzers Union, Liechtenstein), strengthened with carbon, was laid on top of a drop of vesicle suspension for

90 s. The wet grid was subsequently transferred to a drop of 1% (w/v) uranyl acetate in water for 45 s. The excess staining solution was removed by a wick of filter paper. The samples were examined with a Philips EM 201 operating at 80 kV. Photomicrographs were used to deduce size distributions.

Permeability measurements

50 μl of labelled glucose (6–8 μCi) were mixed rapidly with 1.5 ml of vesicle suspension, which were previously equilibrated to the temperature of the experiment. At regular intervals an aliquot of 200 μl was applied to a column (320 × 10 mm) packed with 25 ml hydrated Sephadex G-50 Medium. The sample was eluted at approx. 1 ml/min. The first 7 ml of the eluate were discarded and a second fraction of 5.5 ml containing the vesicles, eluted in the void volume of the column, was collected. Using a battery of identical columns, a sequence of samples at different diffusion times could be handled simultaneously. The columns were consecutively rinsed with 100 ml buffer and 40 ml of the same buffer containing 0.5% Triton X-100 detergent. After equilibrating overnight, all detergent was removed by a further 100 ml of detergent-free buffer to regenerate the columns. All gel filtration experiments were performed in a cold room at 14°C.

To determine the amount of glucose entrapped in the vesicles, 5 ml of the second fraction were mixed with 7 ml of Instagel. Using a dual-label calculation procedure, the amount of lipids (^{14}C-labelled) and glucose (^{3}H-labelled) could be resolved. This was especially important as a small fraction (< 10%) of the vesicles was retained on the columns.

The ratio of ^{3}H to ^{14}C at a time, t, was proportional to the influx of glucose and by multiplying it by the ratio of the total ^{14}C to the total ^{3}H in the incubation medium, the fraction of glucose enclosed within the vesicles, M_t, could be calculated. Assuming that the permeation process follows first-order kinetics, the permeability coefficient, P, can be calculated according to

$$-\ln(1 - M_t/M_\infty) = \frac{A}{V_i} Pt \qquad (1)$$

where $M_\infty = M_t$ as $t \to \infty$ and where A represents the outer area of a vesicle (see Discussion)

$$A = \pi d^2 \qquad (2)$$

with d, the vesicle diameter determined by electron microscopy. V_i, the inner vesicle volume is given by

$$V_i = \frac{\pi}{6}(d - 2\delta)^3 \qquad (3)$$

δ being the lipid bilayer thickness. Combining Eqns. 2 and 3:

$$\frac{V_i}{A} = \frac{1}{6}\frac{(d-2\delta)^3}{d^2} \qquad (4)$$

which is used to calculate P from Eqn. 1.

Thermal analysis

Thermotropic phase changes of the pure and mixed phospholipids were analysed using a DSC-2C scanning calorimeter (Perkin Elmer, U.S.A.). All lipids were dissolved in a mixture of chloroform and methanol (2 : 1, v/v) and transferred to a sample pan. The solvent was completely removed by a gentle stream of nitrogen and the sample was stored overnight under vacuum over P_2O_5. All samples (2–6 mg lipids) were hydrated by adding about 40 μl of buffer and equilibrating the sealed pans well above the transition temperature of the lipids prior to scanning. Both heating and cooling scans were taken at 5 deg.C/min scanning speed.

Results

Probe sonication has often been indicated as a powerful means of dispersing phospholipids [19–21]. The efficiency of reducing multilamellar structures to small unilamellar vesicles is demonstrated by chromatography on Sepharose CL-4B (Fig. 1). Subjecting lipids to a short sonication of 2.5 min results in two distinct liposome populations. The activity excluded in the void volume of the column probably represents large lipid aggregates, although sonication was performed until an optically clear dispersion was obtained. The main peak consists of small unilamellar vesicles. Previous workers and the present authors find that

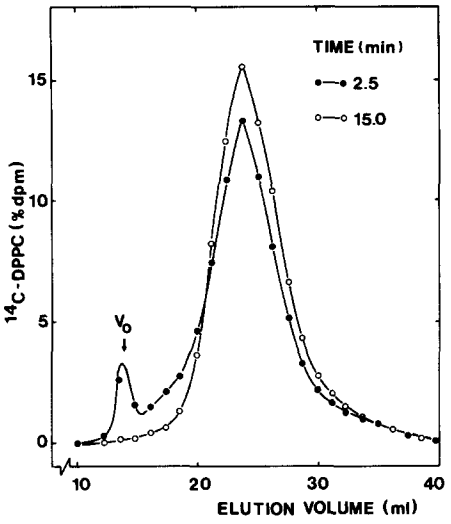

Fig. 1. Elution pattern of dimyristoylphosphatidylcholine (DMPC) labelled with [^{14}C]dipalmitoylphosphatidylcholine (^{14}C-DPPC) and sonicated for 2.5 and 15.0 minutes. V_0 represents the void volume of the Sepharose CL-4B column used.

extended sonication improves the homogeneity of the single-shelled vesicles, without altering their average size. A sonication time of 15 min was sufficient to convert all lipid structures to small unilamellar vesicles. All other vesicle preparations were consequently sonicated for 15 min and checked for multilamellar aggregates by gel filtration. In all cases only one elution peak could be detected and no lipids were eluted in the void volume. Analytical gel filtration on Sepharose CL-4B also showed the vesicles to be stable for at least 3 days when stored well above the phase transition

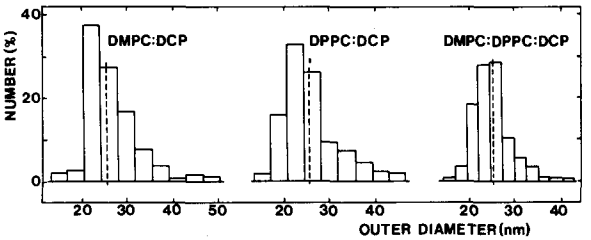

Fig. 2. Size distribution histograms of different phospholipid vesicle preparations as calculated from electron microscopic data using a negative staining technique. The broken lines represent the mean diameter of 25.5, 25.4 and 25.0 nm for DMPC/DCP, DPPC/DCP and DMPC/DPPC/DCP, respectively.

temperature of their constituent lipids. No detectable amounts of large aggregates were traced. All vesicles, however, were prepared daily and used immediately.

Unlike the results of Hauser [22], all lipids were found to be intact after sonication and gel filtration as was demonstrated by thin-layer chromatography using established solvents [17,18,22,23].

Electron microscopic observations of the sonicated dispersions showed a homogeneous population of small unilamellar vesicles. Size distributions, calculated from photomicrographs, were narrow enough to justify the use of a mean radius to represent the liposome preparation (Fig. 2). Using DMPC, DPPC or a mixture of DMPC and DPPC to produce vesicles, revealed that changing the short C_{14} saturated fatty acids to longer C_{16}-ones did not alter the mean vesicle radius (number average) significantly. For all preparations a mean radius of 12.65 nm was taken. This estimate corresponds closely with an earlier report of Huang [24], who obtained vesicles of 12.5 nm mean radius by sonication of egg phosphatidylcholine. Sonication of phospholipids by Johnson et al. [25] resulted in spherical vesicles with a Stokes radius of 12.0 nm. More recent data confirm these early results [26,27]. The fact that the vesicle radius does

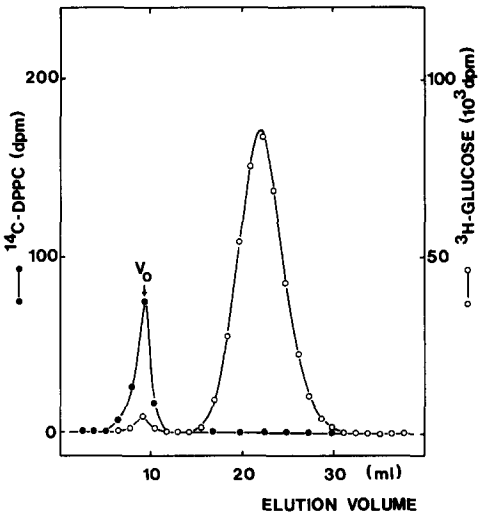

Fig. 3. Elution pattern of a mixture of [^{14}C]dipalmitoylphosphatidylcholine (^{14}C-DPPC)-labelled vesicles and ^{3}H-labelled glucose. V_0 represents the void volume of the Sephadex G-50 column used.

not change on changing the fatty acid composition indicates that the vesicle size is predominantly determined by polar headgroup interactions (on the vesicle interior). Those groups were the same for each of the lipids studied here. This view is supported by a recent observation by Cornell and co-workers [28], who reported the minimum radius of phosphatidylcholine vesicles to be independent of hydrocarbon chain length over the range of 12 to 18 carbon atoms per saturated hydrocarbon chain.

In measuring vesicle permeability, a small sample of vesicles was separated from the diffusion medium by subjecting it to analytical gel filtration chromatography on Sephadex G-50. A typical elution profile is shown in Fig. 3. A small fraction of the labelled permeant, glucose, is eluted in the void volume of the column together with the vesicles. As this represents the amount of sugar which has penetrated, this activity is used to measure the permeability of the different membranes. The remainder of the glucose is eluted in a second peak well separated from the former by at least 2 ml. No leakage from the vesicles during gel filtration occurs as tritium activity falls down again to the background activity after the first peak. This may be partly due to the relatively low permeability of the vesicle bilayer to glucose, but also to the reduced temperature at which all chromatographic column separations were performed. Examination of the vesicle content by paper chromatography and liquid scintillation counting revealed glucose as the only labelled compound present inside the vesicles [29]. Consequently, this permeability study does not suffer from doubts about the identity of the labelled penetrant as was the case with Wood et al. [30]. All labelled glucose, however, was lyophilized prior to diffusion measurements in order to remove any volatile contaminant present which could not be detected by paper chromatography after the paper had been heated at 105°C for 10 min.

The analyses of the glucose influx at intervals (M_t) applied to Eqn. 1 results in a straight line with a slope proportional to the permeability coefficient with V_i/A as the coefficient of proportionality (Fig. 4). Eqn. 4 is used to calculate this factor using the data obtained by the electron microscope and 3.2, 3.7 and 3.45 nm as bilayer thickness for

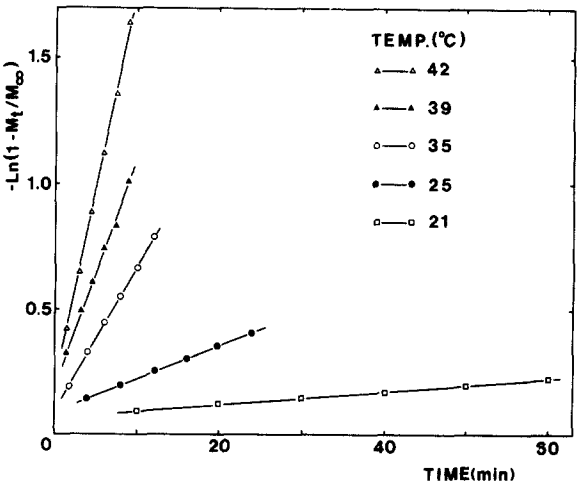

Fig. 4. Glucose permeability of DMPC/DCP (95:5 mol%) vesicles at different temperatures. M_t and M_∞ are the amount of glucose trapped inside the vesicles at time t and at equilibrium ($t \rightarrow \infty$) respectively.

DMPC, DPPC and mixed DMPC/DPPC membranes, respectively [31]. Effects of concentration of glucose and of modification of the bilayer composition are shown in Table I. Increasing the concentration gradient across the membrane has no significant effect on the permeation rate. Neither can a change in permeability be detected on incorporating 5 mol% dicetylphosphate into a membrane of pure DMPC as has been stated by Demel et al. [4]. Introducing this anionic amphiphile, however, increased the bilayer stability considerably, especially at temperatures below that of the lipid phase transition. Changing the lipid composition of the vesicles has a noticeable effect on the permeation rate, both above and below the lipid phase transition temperatures. Table I shows that the permeability decreases significantly on increasing the hydrocarbon moiety of the membrane.

Glucose penetration into vesicles of different lipid composition was measured over a wide temperature range (Fig. 5). All liposomes show an abrupt change in permeability on passing through the phase transition temperature of the constituent lipids as determined by DSC (Fig. 6). The onset of the break in the Arrhenius plots coincides fairly well with the phase transition determined on multilamellar phospholipid dispersions. A mixture of DMPC/dicetylphosphate (95 : 5 mol%) exhibited a

TABLE I

EFFECT OF THE CONCENTRATION GRADIENT, MEMBRANE COMPOSITION AND TEMPERATURE ON THE GLUCOSE PERMEABILITY OF SATURATED PHOSPHOLIPID VESICLES

Membrane composition (mol%)	Permeability coefficient [a] (95% confidence limits) (10^{-12} cm/s)		Glucose concn. (M)
DMPC	146.6	(112.9–180.3) [b]	10^{-2}
DMPC	136.5	(108.8–174.0) [b]	10^{-3}
DMPC	142.5	(135.0–150.0) [b]	10^{-6}
DMPC/DCP (95:5)	132.0	(119.7–145.6) [c]	10^{-6}
DMPC/DPPC/DCP (49:46:5)	74.6	(67.1– 83.0) [c]	10^{-6}
DPPC/DCP (95:5)	5.0	(4.6– 5.4) [c]	10^{-6}
Above the lipid phase transition temperature (44°C)			
DMPC/DCP (95:5)	508.6	(424.2–609.8) [c]	10^{-6}
DMPC/DPPC/DCP (49:46:5)	366.5	(327.1–410.6) [c]	10^{-6}
DPPC/DCP (95:5)	231.7	(217.6–246.6) [c]	10^{-6}
Below the lipid phase transition temperature (20°C)			
DMPC/DCP (95:5)	3.2	(2.8– 3.5) [b]	10^{-6}
DMPC/DPPC/DCP (49:46:5)	1.5	(1.2– 1.9) [b]	10^{-6}
DPPC/DCP (95:5)	0.7	(0.6– 0.8) [c]	10^{-6}

[a] All experiments were performed at 34°C unless otherwise stated.
[b] Estimated from single experiments either because no temperature-dependent measurements were performed or no linear Arrhenius plots were obtained.
[c] Estimated from the Arrhenius plots.

main transition at 24.3°C and a pronounced pretransition at 13.9°C on heating and only one exothermal peak at 24.0°C on cooling. All peaks broadened considerably as compared to pure DMPC (T_c at 23.8°C). Similar observations were made with DPPC. Upon heating, DPPC/dicetylphosphate (95 : 5 mol%) revealed a weak pretransition at 36.0°C and a main transition at 41.1°C again accompanied by substantial peak broadening as compared to DPPC (T_c at 41.9°C). Cooling scans showed one exothermal transition at 42.1°C. Heating a mixture of DMPC/DPPC/dicetylphosphate (49 : 46 : 5 mol%) resulted in an extended endothermal transition with its onset at 33.1°C and a pretransition at 23.8°C. Upon cooling, this sample showed a very broad exothermal peak the set being estimated to be at 35.3°C.

The limits of a phase transition are sometimes ill-defined. The incorporation of 5 mol% dicetylphosphate results in a significant broadening of the phase transition as compared to the pure lipid dispersions. Moreover, the additional packing constraints on highly-curved bilayers as in small single-bilayer vesicles can cause the phase transition to be of considerable width [32]. Due to these effects, the experimental permeability coefficients

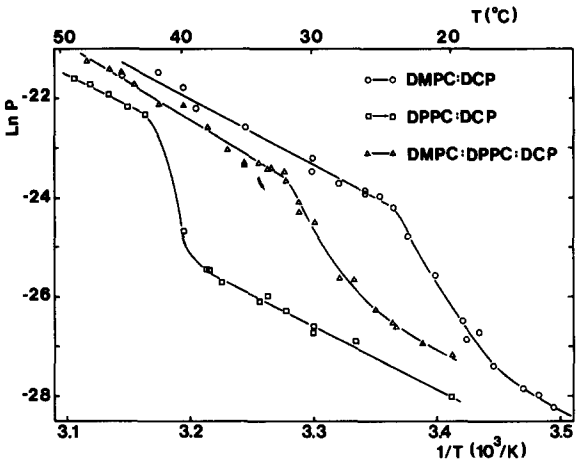

Fig. 5. Permeation rate P for D-glucose through bilayers of vesicles made of dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC). Vesicles contained 5 mol% dicetylphosphate (DCP) and the lipid mixture consisted of 49, 46 and 5 mol% DMPC, DPPC and DCP, respectively.

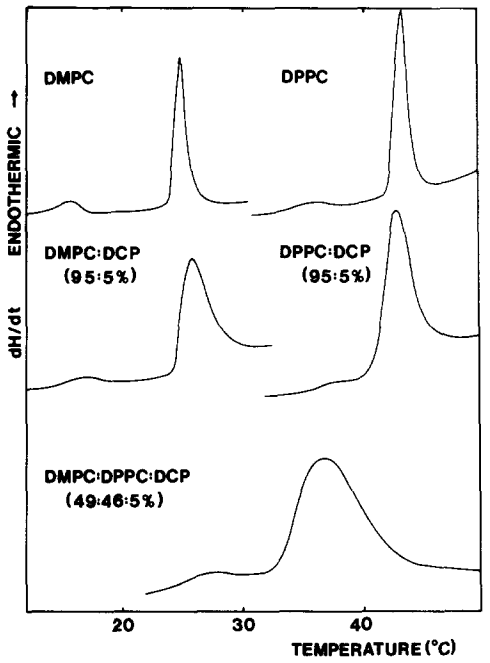

Fig. 6. Instantaneous differential heating rate, dH/dt, for some hydrated lipids and lipid mixtures as registered by differential scanning calorimetry. All ratios represent mol%.

of DMPC/dicetylphosphate (95 : 5 mol%) and DMPC/DPPC/dicetylphosphate (49 : 46 : 5 mol%) below the phase transition temperature could not be fitted by a straight line and reliable activation energies could not be calculated. The activation energy for the influx of glucose through the different membranes examined shows no significant difference although the permeability of a mixed bilayer tends to be more temperature-dependent (Table II).

TABLE II

ARRHENIUS ACTIVATION ENERGY OF THE PERMEABILITY OF PHOSPHOLIPID VESICLES FOR GLUCOSE

Membrane composition (mol%)	Temperature range (°C)	Activation energy ±95% confidence (kJ/mol)
DMPC/DCP (95 : 5)	25–45	109.2 ± 11.9
DPPC/DCP (95 : 5)	20–38	105.9 ± 10.3
	43–49	103.5 ± 20.1
DMPC/DPPC/DCP (49 : 46 : 5)	32–47	128.7 ± 12.5

Discussion

Comparing the results of these permeability measurements with available literature data is not easy as experimental conditions are sometimes omitted. Wood et al. [30] using human erythrocyte phospholipids in a 'black' lipid membrane, estimated the upper limit of the glucose permeability coefficient to be 10^{-10} cm/s. Jung [29], who used essentially the same model membrane, stated the permeability of D-glucose to be $2.3 \cdot 10^{-10}$ cm/s at 25°C. This value is about 6-times greater than our estimate of $3.8 \cdot 10^{-11}$ cm/s for DMPC/dicetylphosphate (95 : 5 mol%) vesicles. This discrepancy may be partly attributed to the different lipid composition used, but possibly also to the technique used. It is well known that in generating lipid bilayers from a membrane-forming solution of phospholipids in organic solvents, traces of these solvents may remain in the membrane. These organic substances often exercise a significant influence on the permeability of such membranes. A considerably smaller value of $6.8 \cdot 10^{-12}$ cm/s was obtained by Lossen [33] at 25°C. The author used similar small vesicles of soybean phosphatidylcholine in a sequential dialysis device. Papahadjopoulos et al. [2] reported for phosphatidylserine a permeability coefficient of $4.13 \cdot 10^{-11}$ cm/s at 36°C whereas at this temperature DMPC/dicetylphosphate (95 : 5 mol%) membranes in the present work have a value of $1.31 \cdot 10^{-10}$ cm/s, about 3-times greater. The difference in membrane composition may be the reason for this difference. A more recent paper of Brunner and co-workers [34] shows very close agreement with the present results. Those investigators reported the permeability of vesicular phosphatidylcholine bilayers to glucose at 25°C to be $(3.0 \pm 2) \cdot 10^{-11}$ cm/s and of planar membranes at 26 ± 2°C, about $(1.1 \pm 0.3) \cdot 10^{-10}$ cm/s both of which can be compared to the value of $4.2 \cdot 10^{-11}$ cm/s for DMPC/dicetylphosphate (95 : 5 mol%) at 26°C in the present work.

Only few investigators refer to a relation between permeability and membrane composition, especially in relation to saturated lipids. De Gier et al. [35] studied the permeability of liposomes of various composition to some polyhydroxy alcohols. The qualitative results revealed an increase

of permeability (osmotic swelling rate) on decreasing the saturated hydrocarbon chain length. Inoue [7] examined the permeability of DMPC and DPPC bilayers to glucose near the lipid phase transition temperature. Although no permeability constants were given, that paper described a drastic enhancement of glucose release at the lipid transition temperature of 24°C for DMPC and 38 to 40°C for DPPC, which is in line with the quantitative results reported here. An equimolar mixture of DMPC and DPPC with 10 mol% dicetylphosphate showed a permeability change at about 30°C which is close to 32°C, the temperature at which the break in the Arrhenius plot of DMPC/DPPC/dicetylphosphate (49 : 46 : 5 mol%) is displayed by these permeability experments. Unfortunately, Inoue confines himself to permeation measurements in the proximity of the phase transition temperature and no data on the activation energy of the process are given.

The sharpness of the drop in the Arrhenius plot for DPPC/dicetylphosphate (95 : 5 mol%) compared to the more extended discontinuity in the permeability data for DMPC/dicetylphosphate (95 : 5 mol%) may be explained by the exertion of a stronger hydrophobic interaction by the long C_{16} fatty acid chains than by the shorter C_{14} chains [36]. The curvature of the graphs for DMPC/dicetylphosphate (95 : 5 mol%) and DMPC/DPPC/dicetylphosphate (49 : 46 : 5 mol%) below the lipid phase transition (Fig. 5) may give erroneously high overall activation energies as has already been pointed out by De Gier et al. [37] and consequently no reliable values can be calculated in this temperature range. Although an early report of Sweet and Zull [38] suggests an activation energy for glucose permeability of about 46 kJ/mol, Papahadjopoulos et al. [2] estimated it to be about 82 to 94 kJ/mol. These latter values are comparable with the present calculated values of 103 to 109 kJ/mol.

Some authors propose a relationship between the activation energy for membrane permeation and the ability of the permeant to form a number of hydrogen bonds in water [39,40]. To penetrate a hydrophobic membrane, a polar molecule should be stripped off its water molecules. According to the criteria of Stein [39], ten hydrogen bonds should be broken simultaneously for complete dehydration of glucose. Using a contribution of 8.4 to 11.3 kJ/mol for each hydrogen bond to be broken [39], the calculated values of 84 to 113 kJ/mol are in close agreement with the present experimentally-determined activation energies. De Gier et al. [35] found an activation energy of 60, 87 and 76 kJ/mol for the permeation of glycol, glycerol and erythritol through liposomal bilayers, respectively. Considering the capability of these molecules to form 4, 6 and 8 hydrogen bonds respectively (some of which should be intra-molecular), an activation energy of about 105 kJ/mol for the penetration of glucose through such membranes is not unreasonable. In a very recent paper Brunner et al. [34] give an activation energy for the permeation of fructose of 90 kJ/mol.

The observation of a constant activation energy, irrespective of membrane composition or of physical state of the lipids, suggests a rate-limiting step in the process prior to penetrating the membrane. The need for a preliminary dehydration of the permeant may account for this. However, the fact that changing the membrane composition or the physical state of the bilayer is attended by significant change in the permeability indicates the importance of the hydrophobic core in the permeation process. This can be explained in terms of the well-known equation [41]

$$P = A\, e^{\Delta S^{\neq}/R} e^{-\Delta H^{\neq}/RT} \tag{5}$$

in which $\Delta S^{\neq}$ and $\Delta H^{\neq}$ are the molar entropy and molar enthalpy of activation, respectively, and A is a pre-exponential constant. For a process in a condensed phase, $\Delta H^{\neq}$ nearly equals E_a, which was here found to be independent of membrane composition. Consequently, the difference in permeation rate is largely determined by the magnitude of the entropy of activation, $\Delta S^{\neq}$. As a bilayer system resembles a more structured system than the surrounding water, the transfer of glucose into this hydrophobic barrier would be accompanied by a unfavourable entropy change. This decrease in entropy is expected to be smaller for the penetration into a liquid-like membrane than into one in the solid-like state. These differences would result in a permeability change without change in the activation energy as here reported for DPPC/dicetylphosphate (95 : 5 mol%). The same

reasoning would apply to the decreased permeation rate of glucose through a highly structured DPPC bilayer as compared to a more disordered DMPC membrane.

Although these effects may account for all the observed results, further experiments are performed to elucidate them.

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