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Ion and Sugar Permeabilities of Lecithin Bilayers: Comparison of Curved and Planar Bilayers*

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Summary. Na^+ and sugar permeabilities of egg lecithin bilayers were measured using curved bilayers and planar bilayers as represented by single-bilayer vesicles and black lipid films, respectively. The Na^+ permeability coefficient measured with single-bilayer vesicles at 25 °C is $(2.1 \pm 0.6) \times 10^{-13}$ cm sec^{-1}. Because of technical difficulties it has been impossible to measure ionic permeabilities of values lower than about 10^{-10} cm sec^{-1} in planar (black) lipid bilayers using tracer methods. The D-glucose and D-fructose permeabilities were measured with both curved and planar bilayers. The permeability coefficients measured with vesicles at 25 °C are $(0.3 \pm 0.2) \times 10^{-10}$ cm sec^{-1} for glucose and $(4 \pm 1) \times 10^{-10}$ cm sec^{-1} for D-fructose; these are in reasonable agreement with the corresponding values obtained for planar (black) lipid bilayers which are $(1.1 \pm 0.3) \times 10^{-10}$ cm sec^{-1} for D-glucose and $(9.3 \pm 0.3) \times 10^{-10}$ cm sec^{-1} for D-fructose, respectively.

Knowledge of the permeability properties of phospholipid bilayers is a prerequisite for the study of permeability changes induced by incorporating into bilayers integral membrane proteins, hydrophobic peptides, or active transport proteins giving rise to "carrier-mediated" diffusion of permeants.

Here we primarily study ion and sugar permeabilities of highly curved lecithin bilayers such as those in single-bilayer vesicles. The permeability of these bilayers will be compared with the permeability of planar lecithin bilayers (black lipid films). Such a comparison should shed light on the question of whether or not the molecular packing and motion in the two types of bilayers are significantly different. Differences in the packing and thus the fluidity of the lipid bilayer can be expected to be reflected in different permeability properties. This question is important because of the extensive use of single-bilayer vesicles in the study of bilayer properties and also as a model for biological membranes. This use of single-bilayer vesicles has been criticized on the basis that highly curved bilayers are atypical of plasma membranes and have properties different from planar bilayers.

Materials and Methods

Hen egg lecithin was purchased from Lipid Products, South Nutfield, UK and used without further purification. The purity of the phospholipid was monitored by thin-layer chromatography (TLC) and by determining the oxidative index before and at the end of the permeability measurement. For the TLC analysis 0.3–1 mg lipid were applied to silicagel H plates [10]. ^{22}NaCl, D-(1-^{3}H) glucose and D-(U-^{14}C) fructose (specific activities 4 μCi mg^{-1}, 22 mCi mg^{-1} and 495 μCi mg^{-1}, respectively) were obtained from Radiochemical Centre, Amersham, UK. The nominal purity of radiolabeled D-glucose and D-fructose was better than 98 and 99%, respectively. Since permeability studies gave consistently larger values for D-fructose than for D-glucose, the purity of D-fructose was checked before the permeability measurement using silicagel 60 plates from Merck (Darmstadt, G.F.R.) and isopropanol/ethyl acetate/water = 7:1:2 (by vol). Thin layer chromatography gave a single radioactive spot with the same R_F as that of cold D-fructose.

n-Decane was purissimum grade from Koch Light, UK, and all other chemicals were analytical grade. The water used for the planar lipid bilayer work was deionized, distilled from $KMnO_4$ under N_2 and redistilled in an all-glass apparatus. For other work deionized water twice-distilled in an all-quartz apparatus was used.

Permeability Measurements using Single-Bilayer Vesicles

Bilayer permeabilities were determined by both efflux and influx measurements using single-bilayer vesicles. The vesicles were either

* This paper is dedicated to the memory of Walther Wilbrandt, *cuius nomini nullum par elogium.*

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prepared by sonication [12] (method I) or without sonication using a detergent method described before [2–4] (method II).

To monitor the movement of radioactively labeled compounds across the membrane of vesicles a microgel filtration technique was developed which allows the rapid and quantitative separation of vesicles and hence of the radioactivity entrapped from the radioactivity in the bulk phase. Two-milliliter graduated pipettes were treated with 1–2% solutions of dimethyl-dichlorosilane in benzene at 60 °C for 10 min prior to being filled with Sephadex G-50 (medium). The pipettes were then packed with the gel, the dimensions of the column (145 × 3.6 mm) being chosen so that the separation of the vesicles from the external medium was completed within 4 min. An aliquot of the incubation medium (10 µl) was injected with a Hamilton syringe into the column matrix (approximately 1.4 cm from the top of the gel bed) without interrupting the flow of the column, and the vesicles were eluted with the same buffer as that used to equilibrate the column (0.1 M NaCl, 0.01 M Tris HCl, pH 7.5). The flow rate of the column was 0.12 ml min^{-1}. Before applying the sample, 1 ml of a 1% sonicated egg lecithin dispersion was passed through the column; it could be shown that after this initial "saturation" of the gel matrix with lecithin, subsequent lipid samples applied to the column were eluted quantitatively.

Efflux Measurements

In the case of efflux measurements, the lecithin vesicles were prepared in the presence of the radioactively labeled Na^+ or sugar to be monitored. Radioactivity present in the bulk phase was then removed by gel filtration on Sephadex G 50 (1.5 × 20 cm; 0.1 M NaCl, 0.01 M Tris HCl, pH 7.5). The peak fractions of vesicles were pooled and incubated at a given temperature. After certain periods of incubation 10.0 µl samples were withdrawn and gel filtrated using the microcolumn. Radioactivity trapped in the internal volume of the vesicle appeared in the void volume of the column and was well separated from the fraction of radioactivity released from the liposomes during the incubation time.

Influx Measurements

Vesicles were mixed with the radioactively labeled compound at a given temperature. After incubation for various periods of time, aliquots of 10.0 µl were subjected to microgel filtration and the fraction of radioactivity eluted with the vesicles was determined. Although operationally similar, there is a fundamental difference between efflux and influx measurements. For efflux measurements relatively little radioactivity is applied to the columns and changes in the ratio of radioactivity eluted with the lipid and in the total volume are measured. In contrast, for influx measurements 10^2–10^3 times more radioactivity has to be applied and the fraction of the radioactivity eluted with the vesicles is usually very small ($\lesssim 1\%$). In all experiments the amounts of radioactivity and the concentration of lipid in the incubation media were chosen so that at the beginning of efflux measurements or after reaching equilibrium during influx measurements 10^3 to 5×10^3 cpm were eluted with the vesicles originally contained in 10.0 µl dispersion.

The Na^+ permeability was measured under conditions of equal ionic composition on either side of the bilayer membrane, hence tracer diffusion rather than net fluxes were measured. In contrast, sugar permeabilities were measured under gradient conditions whereby the concentration difference across the bilayer was 10 mM at time zero.

Analysis of the Permeability Measurements using Single-Bilayer Vesicles

Efflux and influx measurements were analyzed as follows. Assuming that the permeation of Na^+ and sugars follows first-order kinetics, the following equations can be written for the amount of permeant crossing the vesicle membrane:

$$\textit{For efflux:}\quad -dx/dt = k\,(X - X_\infty) \tag{1}$$

where X and X_∞ are the concentrations of the radiolabeled compound in the internal vesicle cavity at time t and after reaching equilibrium, respectively, and k is the first-order rate constant. Integration gives

$$\ln \frac{X_0 - X_\infty}{X - X_\infty} = kt \tag{2a}$$

where X_0 is the concentration of radioisotope in the internal vesicle cavity at $t=0$. Since the experimental conditions of our efflux measurements are such that $X_\infty \to 0$, to a first approximation the above equation can be written as

$$\ln \frac{X_0}{X} = kt. \tag{2b}$$

$$\textit{For influx:}\quad dx/dt = k\,(X_0 - X) \tag{3}$$

which after integration gives

$$\ln \frac{X_0}{X_0 - X} = k_1 t \tag{4}$$

where X_0 is the concentration of radioactively labeled compound in the external medium, which to a good approximation remains constant during the experiment, X is the concentration in the intravesicular space at time t and k_1 is the first-order rate constant for influx. From the first-order rate constant k, the flux M and the permeability coefficient P can be calculated if the volume of the internal vesicle cavity v_i and the bilayer area A of the vesicle are known:

$$k = \frac{MA}{v_i X_0} = \frac{PA}{v_i}. \tag{5}$$

The internal volume v_i of the vesicle was determined from the fraction of radioactivity trapped at $t=0$ for efflux experiments and after reaching equilibrium in case of influx experiments. The average value v_i for sonicated vesicles is 3.1×10^{-4} cm^3 µmole^{-1} lipid which is in good agreement with data reported previously [12]. For vesicles prepared by method II, v_i varied within the range 3.0 to 4.4×10^{-4} cm^3 µmole^{-1} consistent with previous measurements [2]. For both types of single-bilayer vesicles an average value for the bilayer area $A = 2 \times 10^3$ cm^2 µmole^{-1} lipid was used as a first approximation [20, 25]. This value corresponds to the surface area of a sphere with radius $\frac{R_0 + R_i}{2}$ where R_0 and R_i are the outer and inner radius of the bilayer, respectively. It is equivalent to the interfacial area between the outer and inner monolayer of the bilayer.

Permeability Measurements using Planar Lipid Bilayers

A quartz cuvette consisting of two compartments separated by a Teflon partition wall with a hole of 2.4 mm radius (area = 0.18 cm^2) (Fig. 1) was used to measure permeabilities of planar phospholipid bilayers. At positions labeled 1–4 the tips of four gas tight Agla syringes were inserted; syringe 1 was used to inject 0.1 ml of solutions of the compound to be monitored into the

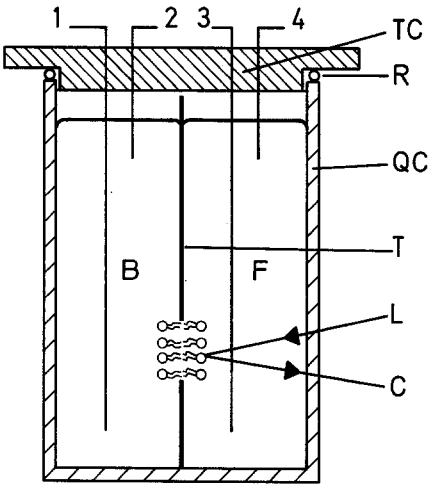

Fig. 1. Schematic diagram of apparatus used to measure permeabilities across planar(black)lipid bilayers. The quartz cuvette (*QC*) has a square base of dimensions ($1.5 \times 1.5 \times 2$ cm) and is separated into equal back (*B*) and front (*F*) compartments by a Teflon spacer (*T*) which has a 4.8 mm diameter hole, across which the planar bilayers are formed. 1–4 denote gas tight glass Agla syringe tips; *TC*, *R*, *L* and *C* denote Teflon cap, rubber seal, light source and eye position, respectively. Each compartment contained 1.50 ml of aqueous bathing solution (0.1 M NaCl, 0.01 M Tris HCl, pH 7.0, 0.02% NaN_3), which was 1.3 cm high

rear compartment while syringe 2 was used simultaneously to balance any curvature of the lipid bilayer. In order to measure the amount of radioactively labeled compound which had diffused across the lipid bilayer, 0.1 ml samples were withdrawn from the front compartment using syringe 3. Syringe 4 was manipulated simultaneously to maintain the planarity of the lipid bilayer. The syringes were connected to micrometer micromanipulators and by evenly and simultaneously manipulating syringes 1 and 2 during injection and 3 and 4 during sampling, the planarity of the bilayer was maintained and the vibration of the film was kept minimal. The bilayer was observed with a light microscope as shown schematically in Fig. 1. The addition and removal of 0.1 ml samples as described above did not give rise to any additional flux across the bilayer. Effective mixing of the sample in the rear compartment was achieved by a magnetic stirrer using a glass-sheathed rod of final nominal dimensions 1.2 mm diameter and 6 mm length. The 0.1 ml samples withdrawn from the front compartment were mixed with 20 ml of Bruno-Christian scintillator and counted in a 4,000 series Packard Tri-Carb Scintillation spectrometer. Twenty to 100 μCi of radiolabeled sugar were added to the rear compartment and the permeation of sugar was measured over a period of time sufficient to give final counting rates in the recipient (front) compartment of ~100 cpm. In the case of D-(U-^{14}C) fructose the counting efficiency was 94.0% and each sample was counted for 2.4×10^4 sec, whereas for D-(1-^{3}H) glucose the counting efficiency was 39.0%. Samples for flux measurements were taken immediately after stirring the injected radioactively labeled solution in the back compartment (15 sec) and then after prolonged periods of flux times (*cf.* Table 3).

The bilayer-forming solution was 1% (wt/vol) lecithin in *n*-decane and was prepared daily and stored under N_2 at 5 °C when not being used. Lipid bilayers were formed by brushing the lecithin solution across the Teflon hole as described before [19]. The electrical resistance of the lecithin bilayers was within the range of 5×10^7 to 1.1×10^8 Ωcm^2; bilayers with resistance values below this range were discarded. The bathing solution was 0.1 M NaCl, 0.01 Tris HCl, pH 7.0, 0.02% sodium azide. Sugar permeabilities were measured under conditions similar to those used with sonicated egg lecithin vesicles. The sugar concentrations in the rear and front compartment at zero time were 12.8 mM and zero, respectively; fluxes were thus measured under gradient conditions. The apparatus was not thermostated; all measurements were carried out at 26±2 °C.

Analysis of the Permeability Measurements using Planar Lipid Bilayers

The experimental conditions employed as described above are such that the analysis of the data according to the unidirectional flux equation appears to be justified:

$$\frac{dx}{dt} = -kX \tag{6}$$

where X is the concentration of radiolabeled compound remaining in the rear compartment at time t. Substituting for k the expression given in Eq. (5) and integrating gives

$$\ln X/X_0 = -P\frac{A}{v_i}t. \tag{7}$$

The mole fraction of the compound which has diffused across the bilayer into the front compartment during the time t is $X_D/X_0 = 1 - X/X_0$ (X_D=concentration in the front compartment at time t). Substituting for X/X_0 in Eq. (7) and expanding $\ln(1 - X_D/X_0)$ as a Maclaurin series with $X_D/X_0 \ll 1$ gives

$$X_D = \text{const. } t \tag{8}$$

where $\text{const.} = \frac{PA}{v_i}X_0$.

Results

Single-Bilayer Vesicles

The efflux and influx of Na^+ were measured with both types of vesicles at different temperatures and the data were analyzed according to Eqs. (1–4) (*see* Materials and Methods). In Fig. 2*a* the Na^+ influx measurements at three different temperatures are plotted according to Eq. (4). From the slope of the straight lines k is obtained which is related to the flux M and the permeability coefficient P by Eq. (5) The permeability coefficients thus derived are summarized in Table 1. Within the error of the measurement the Na^+ permeabilities determined with the two different types of vesicle preparations (methods I and II) are in good agreement. Furthermore the permeability coefficient of Na^+ derived from efflux measurements agrees within the experimental error with that derived from influx measurements (Table 1). The values for the Na^+ permeability coefficient are consistent with values published before [10, 11] and in good agreement with values of Papahadjopoulos et al. [20] who used mixtures of phosphatidylcholine containing small amounts of phosphatidic acid (4%) or

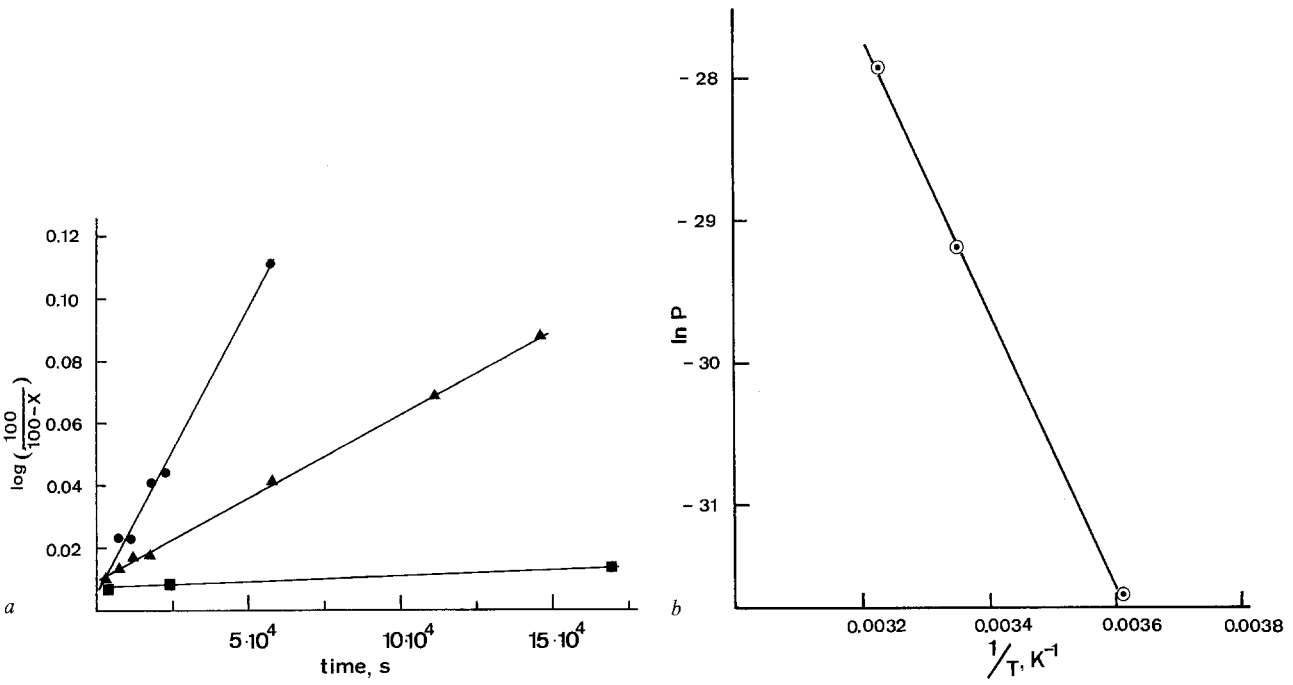

Fig. 2. (*a*): Influx measurements of Na^+; the influx of Na^+ was measured across bilayers of sonicated egg lecithin vesicles at three different temperatures. Data are plotted according to Eq. (4) assuming that the influx follows first-order kinetics. X=concentration in the vesicle cavity in per cent of the equilibrium concentration which in the case of influx measurements is identical to the initial (starting) concentration of Na^+ in the external medium. From the slope of the curve the first-order rate constant k is obtained (Eq. (5)) which is related to the permeability coefficient P, $k=PA/v_i$ where A is the mean vesicle surface area, and v_i is the internal vesicle volume. The values for A and v_i used to calculate P from k were 2.0×10^3 cm^2 μmole^{-1} and 3.1×10^{-4} cm^3 μmole^{-1}, respectively; (-■-) 4 °C, (-▲-) 25 °C, (-●-) 37 °C. The solid lines represent least-squares fit to the experimental data. (*b*): Arrhenius plot for the Na^+ permeability; ln P is plotted as a function $1/T$ according to Eq. (9). Data derived from Fig. 2*a* are used. The slope of the straight line gives an activation energy of 19.2 kcal

Table 1. Na^+ permeability of vesicular lecithin bilayers

Vesicle preparation	Permeability coefficient P (cm sec^{-1})	Temperature (°C)	Measurement
Sonicated (method I)	$1.8\pm0.5\times10^{-14}$	4	influx
Sonicated (method I)	$2.1\pm0.6\times10^{-13}$	25	influx
Sonicated (method I)	$7.4\pm2\times10^{-13}$	37	influx
Sonicated (method I)	$1.5\pm0.5\times10^{-14}$	4	efflux
Without sonication (method II)	$2.0\pm0.6\times10^{-14}$	4	efflux
Without sonication (method II)	$2.0\pm0.8\times10^{-13}$	25	influx

Results are presented as the mean ± SD. The internal vesicle volume and the bilayer area used in the calculation of fluxes and permeability coefficients were $v_i=3.1\times10^{-4}$ cm^3 μmole^{-1} and $A=2.0\times10^3$ cm^2 μmole^{-1}.

Table 2. Sugar permeabilities of vesicular lecithin bilayers

Sugar	Permeability coefficient P (cm sec^{-1})	Temperature (° C)
D-glucose	$0.30\pm0.2\times10^{-10}$	25
D-fructose	$0.25\pm0.1\times10^{-10}$	4
D-fructose	$0.7\pm0.3\times10^{-10}$	10
D-fructose	$1.3\pm0.5\times10^{-10}$	15
D-fructose	$2.5\pm1\times10^{-10}$	20
D-fructose	$4.0\pm1\times10^{-10}$	25
Sucrose	$8.0\pm3\times10^{-14}$	25

stearylamine (2%). The Arrhenius plot for the Na^+ permeability P according to Eq. (9)

$$P=A\exp(-E/RT) \tag{9}$$

(where E=activation energy, A=constant and R and T have their usual meaning) is shown in Fig. 2*b*. The value for E derived from this Figure is 19.2 kcal (correlation coefficient $r=1.00$).

Table 2 summarizes the permeability measurements of D-glucose, D-fructose and sucrose. The permeability coefficients were all obtained from influx measurements carried out with sonicated vesicles (method I). The influx measurements of all three sugars followed first-order kinetics. The plots for D-glucose and D-fructose are shown in Figs. 3*a* and *b*, respectively. The permeability coefficient for sucrose is of the same order as that of Na^+ while the permeabilities of the monosaccharides are at least three orders of magnitude larger. The permeability of D-

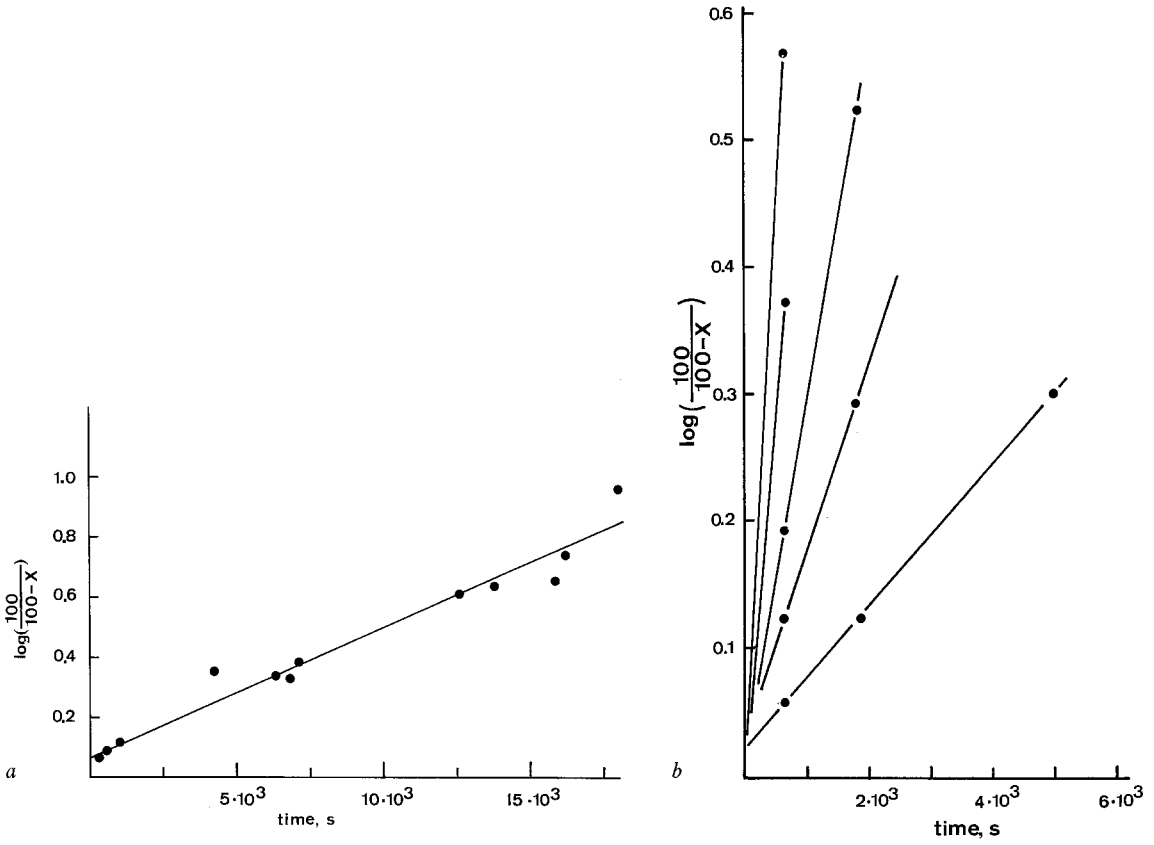

Fig. 3. (*a*): Influx measurements of glucose; the influx was measured across bilayers of sonicated egg lecithin vesicles at 25 °C. Data are plotted according to Eq. (4) assuming that the influx follows first-order kinetics; X=concentration in the vesicle cavity in per cent of the equilibrium concentration which, in this case, is identical to the glucose concentration in the external medium. The permeability P is derived from the slope of the straight-line relationship as described in the text. The solid line represents the least-squares fit to the experimental data. (*b*): Fructose influx measurements at different temperatures; the amount of fructose which diffuses across the bilayer of sonicated egg lecithin vesicles in t sec is plotted as per cent of the equilibrium concentration (in the case of influx measurements this concentration is identical to the external fructose concentration)

fructose (at 25 °C) exceeds that of D-glucose by a factor of about 10–15. The fructose permeability coefficient was determined as a function of temperature (Table 2) and from the Arrhenius plot (Fig. 4), the activation energy for the permeation of D-fructose is derived as $E=21.6$ kcal (correlation coefficient $r=0.99$).

Planar Lipid Bilayers

For comparison, the permeation of ions and monosaccharides through planar lipid bilayers of egg lecithin was measured. The time course of the diffusion of D-fructose is shown in Fig. 5*a*. The flux across the planar bilayer is adequately described by Eq. (8); the concentration in the front compartment increases linearly with time, and the permeability coefficient P is obtained from the slope of the straight-line relationship.

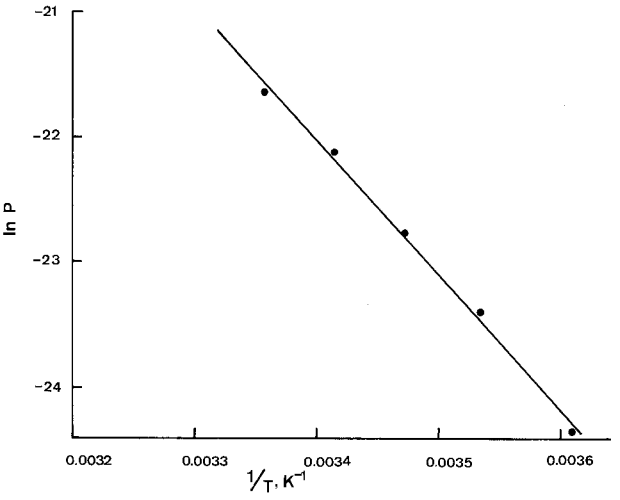

Fig. 4. Arrhenius plot for the fructose permeability. The permeability coefficients derived from the straight-line relationships presented in Fig. 3*b* are plotted according to Eq. (9). The slope of the least-squares fit gives an activation energy of 21.6 kcal

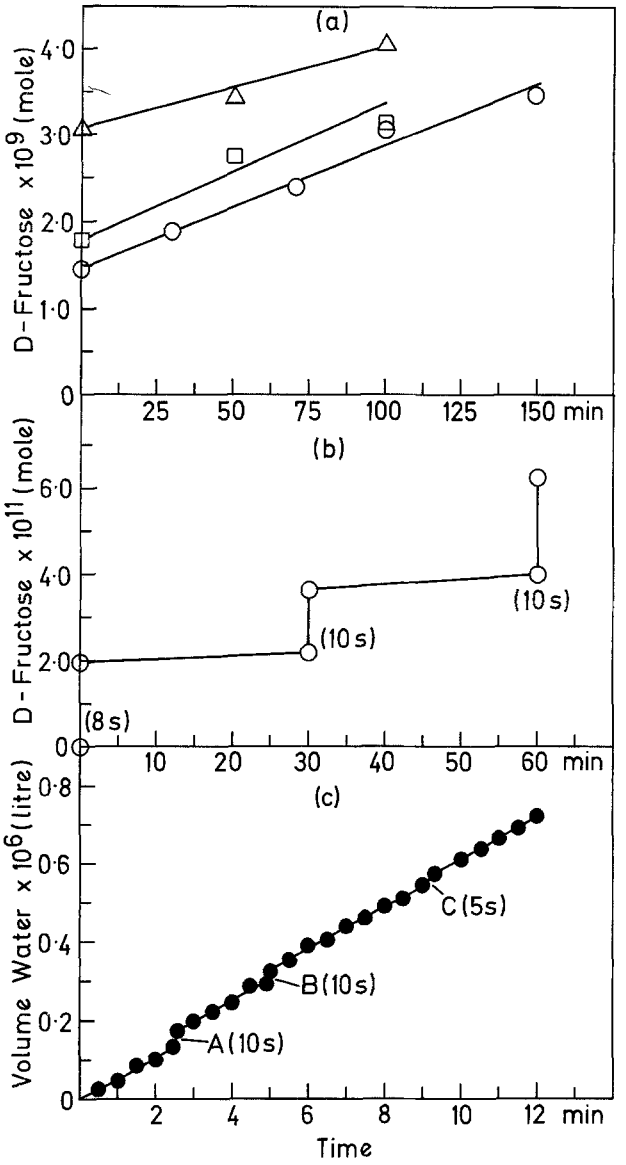

Fig. 5. Flux measurement of D-fructose and H_2O across planar lipid bilayers of egg lecithin. Measurements were carried out at 26 ± 2 °C, bilayer area = 0.18 cm^2. (*a*) The amount of radioactively labeled D-fructose which diffuses across the planar lecithin bilayer is plotted as a function of time. (*b*) The effect of stirring (8–10 sec) of the back compartment on the diffusion of D-fructose across planar lecithin bilayers. Stirring at 0, 30 and 60 min induces a leakage of D-fructose. (*c*) The volume of H_2O diffusing across planar egg lecithin bilayers in the presence of a sucrose gradient (0.65 OSM) is plotted as a function of time. The leakage of H_2O induced by mechanical stirring for 5 to 10 sec at points *A*, *B*, *C* is represented by the steps in the straight line (for experimental details *see* ref. [9, 13])

It is clear from Fig. 5*a* that during the initial 15-sec period of stirring, which is required to uniformly distribute D-fructose throughout the back compartment, the planar bilayer was highly permeable to fructose. That this initial flux is not related to the diffusion of fructose across the bilayer is further demonstrated in Fig. 5*b* where the leakage due to mechanical stirring and the diffusion of fructose are clearly differentiated. Such leakage due to mechanical stirring has also been observed during water permeability measurements (Fig. 5*c*) using an apparatus described elsewhere [9]. In this case, however, the water leakage induced by mechanical stirring is less disturbing because of the relatively large permeability of egg lecithin bilayers to water. The permeability coefficients for D-fructose (derived from the data shown in Fig. 5*a*) and for D-glucose (not shown) corrected for the initial leakage flux are summarized in Table 3. The average values for the permeability coefficients of D-glucose and D-fructose are $P = (1.1 \pm 0.3) \times 10^{-10}$ and $P = (9.3 \pm 0.3) \times 10^{-10}$ cm sec^{-1}, respectively. These values are 2 to 4 times greater than those measured with single-bilayer vesicles. As with single-bilayer vesicles, the planar bilayer was more permeable to D-fructose; the ratio of the permeability coefficients P(D-fructose)/P(D-glucose) is about 9 and is similar to that measured with sonicated vesicles (*cf.* Tables 2 and 3).

Table 3. Permeability coefficients of monosaccharides[a] measured with planar lecithin bilayers at 26 ± 2 °C

Sugar	Duration of flux measurement (min)	Permeability coefficient (cm sec^{-1})	Mean value ± SD
D-fructose[b]	100	0.66×10^{-9}	$9.3 \pm 0.3 \times 10^{-10}$ cm sec^{-1}
D-fructose	100	1.09×10^{-9}	
D-fructose	145	1.03×10^{-9}	
D-glucose[c]	180	1.49×10^{-10}	$1.1 \pm 0.3 \times 10^{-10}$ cm sec^{-1}
D-glucose	240	1.04×10^{-10}	
D-glucose	240	0.77×10^{-10}	

[a] The sugar concentrations in the rear compartment (Fig. 1) at time $t = 0$ were 12.8 mM. Since the sugar concentration in the front compartment was zero at time $t = 0$, net fluxes were measured under gradient conditions.
[b] Calculated from flux curves given in Fig. 5.
[c] Calculated from flux measurements in duplicate.

Discussion

Comparison of Permeabilities Measured in Curved and Planar Bilayers

Our value for the D-glucose permeability measured with sonicated lecithin bilayers is in good agreement with measurements carried out under comparable conditions [25] and also with the value obtained for sonicated phosphatidylserine single-bilayer vesicles [20] when corrections are made for differences in the temperature of the measurements. That the D-glucose

permeabilities of lecithin and phosphatidylserine bilayers are similar is not surprising because the ion permeabilities of the two bilayers were also found to be very similar [12]. Furthermore, our value for the D-glucose permeation through planar lecithin bilayers is in good agreement with similar measurements [27] and also compares well with flux measurements carried out with a single spherical lecithin bilayer of large surface area [15].

Using single-bilayer vesicles prepared by two different techniques, (I) by sonication and (II) by detergent removal, the following conclusions can be drawn. The observation that both types of vesicles give similar values for the Na^+ permeability indicates that these vesicles are similar in terms of structural arrangement (packing) [2]. Furthermore, within the error of our measurements the residual detergent retained in the lipid bilayer [2] has no effect on the Na^+ permeability.

The sugar permeabilities measured with the planar bilayer are 2 to 4 times larger than the equivalent values obtained with curved bilayers of vesicles. It is likely that the larger values are due to leakage through the torus region of the planar bilayer probably induced by vibrations and/or leakage induced by the presence of hydrocarbons in the lecithin bilayer. In spite of this error the agreement between the sugar permeabilities determined with curved (vesicular) bilayers and planar bilayers is good. In both curved and planar bilayers the D-fructose permeability exceeds that of D-glucose by a factor of about 10 to 15. The purity of the radioactively labeled sugars tested as described in Materials and Methods and the fact that different batches of radioisotopes gave consistent results in both curved and planar bilayers suggest that the difference in permeability between D-glucose and D-fructose is not an artifact due to radioisotopic impurities (*cf.* refs. [15, 27]). Stein [17, 23] has advanced an empirical theory which predicts that the rate of diffusion of a neutral molecule through a lipid bilayer is a function of the molecular volume, the number of CH_2-residues and the number of hydrogen-bond forming groups in the molecule. According to this theory the diffusion rates for the two monosaccharides are expected to be very similar and the difference in the permeability coefficients cannot be accounted for. However, the permeation process through a lipid bilayer is a composite of two independent events:

$$P=\frac{KD}{l} \tag{10}$$

where K is the partition coefficient, D is the diffusion coefficient for diffusion within the bilayer and l is the thickness of the bilayer. According to Eq. (10) these two events are (1) partitioning of the permeant between the external medium and the membrane and (2) diffusion across the membrane. The difference between D-glucose is then likely to be due to a difference in partition coefficient K. In support of this it is worth mentioning that the two sugars differ in their solubility properties and that on paper chromatography the two sugars are characterized by different R_F values in most solvent systems [18], fructose migrating farther.

Our values of the activation energies for the permeation of Na^+ and fructose compare well with data in the literature [20]. The magnitude of the activation energy is consistent with the three-step permeation mechanism discussed above [21, 28]. Since the details of this mechanism are still unknown, we have used, in both efflux and influx measurements, the interfacial area between outer and inner monolayer as effective bilayer area for the calculation of permeabilities.

As discussed previously [12], in principle there are two independent mechanisms which might contribute to the movement of ions across lipid bilayers: (1) diffusion across the bilayer and (2) collision-induced rupture and/or coalescence of vesicles leading to the exchange of their content with the external medium. The good agreement between the values obtained with sonicated lecithin vesicles and planar lecithin bilayers indicates that the contribution from the collision-induced diffusion mechanism is negligible. We can conclude that the measured permeabilities represent intrinsic bilayer permeabilities.

Limitations of the Use of Planar Lipid Bilayers for Permeability Measurements

It is clear that the use of planar bilayers for permeability studies is limited by technical difficulties. The major shortcomings are (I) the small surface area which, in many cases, does not allow passage of sufficient quantities of permeant to be accurately determined in the recipient compartment; and (II) the instability of the bilayer leading either to leakage fluxes or breakage of the bilayer itself so that permeability measurements are usually restricted to relatively short periods of time (1–2 hours). Fig. 5 shows that stirring gives rise to a leakage flux probably through the torus of the bilayer. Clearly, any perturbation of the planar lipid bilayer produced by vibration and/or manipulations can lead to such leakage fluxes. The contribution of this flux to the diffusional flux is not a serious source of error if, as in the case of H_2O, the permeability coefficient is of the order 10^{-4} to 10^{-3} cm sec^{-1}. With monosaccharides (permeability coefficient

10^{-11} cm sec^{-1}) the leakage induced by stirring becomes significant relative to the diffusion and corrections have to be made.

Attempts to measure Ca^{2+} and Cl^- permeabilities using radiolabeled ions and the equipment depicted in Fig. 1 were unsuccessful because of the small film area (~ 0.2 cm^2). The amounts of ions diffusing across the membrane into the recipient compartment were insufficient to be detected. Jung [15], using tracer techniques, estimated that spherical single bilayers with an area about 30 times as large as that of the planar bilayer are adequate for the precise measurment of permeabilities of the order of 10^{-7} to 10^{-11} cm sec^{-1}. In an attempt to increase the membrane area and so to determine Ca^{2+} and Cl^- permeabilities measurements were made in an apparatus described in ref. [13]. The main feature of this set-up is that the planar lipid bilayer is in a horizontal position with the bottom compartment sealed which gives the bilayer greater stability and thus allows the use of larger film areas. With a total lecithin bilayer area of 1.33 cm^2 (about 7 times as large as that of the planar bilayer in Fig. 1) the average values for the Ca^{2+} and Cl^- permeability coefficients measured were $P\,(Ca^{2+})=(1.22\pm 1)\times 10^{-7}$ cm sec^{-1} (duration of flux measurement 5 to 45 min) and $P\,(Cl^-)=(1.8\pm 0.9)\times 10^{-7}$ cm sec^{-1} (duration of flux measurement 1 to 180 min). Both measurements were characterized by relatively large standard deviations and the average permeability coefficients are several orders of magnitude larger than those measured with single-bilayer lecithin vesicles (for Cl^-, *cf.* refs. [11, 12, 25]; for Ca^{2+}, *cf.* ref. [26]).

The higher ion-permeabilities could be due to the presence of hydrocarbon (*n*-decane) in the lecithin bilayer and/or due to mechanical perturbations of the film which, because of the larger surface area of the film, are more critical than in the apparatus shown in Fig. 1. The former explanation seems, however, unlikely in the light of the sugar permeability measurements carried out with planar bilayers (*cf.* Table 3). These bilayers very likely contain residual hydrocarbons, yet the sugar permeabilities measured are several orders of magnitude lower than the above values for Ca^{2+} and Cl^-. From our permeability studies it may therefore be concluded that the equipment described in this work (Fig. 1) allows the measurement of permeability coefficients of the order of 10^{-10} cm sec^{-1} with reasonable accuracy. This value may be regarded as a threshold value below which permeability measurements become meaningless. The equipment described in Fig. 1 is unsuitable for the measurement of ionic permeabilities which are one to several orders of magnitude lower than the permeabilities of monosaccharides.

Molecular Packing in Curved and Planar Bilayers

The use of these vesicles as model membranes has been criticized on the basis that the radius of curvature corresponds at best to that of subcellular particles and that it is quite atypical of plasma membranes. Two schools of thought have developed in the past. Based upon the study of the geometry of the single-bilayer vesicle and NMR studies it was concluded that the molecular packing and motion in these bilayers is similar to those in lamellar multilayers and hence sonicated dispersions are a good system for studying bilayer and membrane properties [7, 8]. In essence this is in agreement with other ^{1}H-NMR studies [1] and with ^{2}H NMR quadropole splitting and relaxation time measurements [24]. The above conclusions are contrasted by those of Chan and his collaborators [6, 16, 22] and Horwitz et al. [14] who argued that because of the high curvature of the vesicle bilayer the regular packing of the hydrocarbon chain is disrupted and the degree of orientational order is significantly decreased. However, a large disorder in the hydrocarbon chain packing should produce a marked increase in the bilayer permeability. De Gier et al. [5] showed that introduction of a single double bond per hydrocarbon chain caused a significant increase in nonelectrolyte permeability by much more than a factor of 10. The permeability of glycerol increased in the order distearoyl lecithin < 1-stearoyl-2-oleoyl-lecithin < dioleoyl lecithin < dilineoyl lecithin. From these results it is clear that a large disorder in the hydrocarbon chain packing should manifest itself in a permeability increase significantly greater than one order of magnitude. This is not borne out by our experiments. The good agreement, within experimental error, between the permeability of curved and planar bilayers supports the notion that sonicated single-bilayer vesicles are a reasonable model system.

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