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KINETICS OF WATER DIFFUSION ACROSS PHOSPHOLIPID MEMBRANES

^{1}H- AND ^{17}O-NMR RELAXATION STUDIES

C. LIPSCHITZ-FARBER and H. DEGANI

Isotope Department, Weizmann Institute of Science, Rehovot (Israel)

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Summary

Nuclear relaxation measurements of ^{1}H and ^{17}O of water have been applied to study the kinetics of water diffusion across vesicular lipid membranes. Differentiation between the intra- and extravesicular media was achieved by entrapping Mn^{2+} inside the vesicles.

The water permeability of egg phosphatidylcholine vesicles was found to be $2.9 \cdot 10^{-3}$ cm/s at 25°C, with an activation energy of 10.5 kcal/mol which remains constant through the temperature range 0—65°C.

The water permeability across vesicular bilayers of L-α-dipalmitoyl phosphatidylcholine exhibited a sharp change through the lipid phase transition. The permeability in the lipid crystalline phase (45°C) was found to be $7.2 \cdot 10^{-3}$ cm/s with an activation energy of 7.2 kcal/mol. Below the transition at the gel phase (35°C) a permeability of $1.0 \cdot 10^{-3}$ cm/s was determined.

The results indicate that water diffuses through lipid membranes in the liquid crystalline phase in a similar fashion to its diffusion in hydrocarbon liquids. However, when the lipids undergo a phase transition to the gel state, this similarity does not hold any more and water diffusion becomes much more restricted than in hydrocarbon liquids. The change in water permeability through the phase transition was correlated with the changes observed in the lipid segmental motion determined from ^{13}C T_1 measurements.

Introduction

Kinetic studies of water diffusion rates across model membranes are useful in understanding the mechanism of water transport. In particular, it enables the

correlation of water permeation with the dynamic properties of membrane constituents. NMR spectroscopy has been shown to be a successful method for determining fast water permeation processes. ^{1}H and ^{17}O relaxation studies have been employed to measure water permeability of red blood cells [1—4] as well as that of lipid vesicles [5,6]. A necessary condition for the NMR experiments is a difference in some NMR parameter (relaxation or shift) of the water nuclei at the compartments on both sides of the membrane barrier. Such a difference may arise in vivo due to a difference in chemical composition of the external and internal cell media but can also be achieved artificially by adding small amounts of non-penetrating paramagnetic reagents which enhance the water relaxation or shift its signal.

This study presents the employment of both ^{1}H and ^{17}O relaxation measurements to follow the kinetics of water diffusion across egg phosphatidylcholine and L-α-dipalmitoyl phosphatidylcholine vesicles. Differentiation between the intra- and extravesicular media was achieved by loading the vesicles' interior with Mn^{2+}. A similar method, using ^{1}H relaxation in DPPC vesicles, has been previously employed by Andrasko and Forsen [5]. However, by the application of ^{17}O relaxation studies, it was possible to increase the kinetic range by an order of magnitude, thus enabling the extension of the measurements over a broad temperature range. In particular, it became feasible to measure water diffusion rates across dipalmitoyl phosphatidylcholine vesicles above the phase transition and through it.

Experimental Procedure

Vesicle preparation

Vesicles were prepared from pure egg phosphatidylcholine obtained from Makor and from L-α-dipalmitoyl phosphatidylcholine from Sigma. The dry lipids were dispersed in an aqueous solution of $MnCl_2$ (50 mg lipid/ml) and then sonicated under N_2 for 10—20 min with a Heat System W-375 sonifier (0.375 inch tip, power level 5, pulsed mode, 40% fractional power). Egg phosphatidylcholine dispersions were sonicated in an ice bath and centrifuged for 30 min at 4°C. Dipalmitoyl phosphatidylcholine dispersions were both sonicated and centrifuged at 45°C. After centrifugation the zone containing clear supernatant was removed for dialysis. 2 ml of vesicles were dialysed against a 1 l aqueous solution at pH 6 containing Na_4EDTA in an amount equivalent to that of $MnCl_2$. Dialysis of egg phosphatidylcholine vesicles was carried out overnight at 4°C, while dipalmitoyl phosphatidylcholine vesicles were dialysed at 45°C for about 2—3 h. An additional dialysis against 25 ml of $H_2{}^{17}O$ (3%) was carried out prior to the ^{17}O measurements.

In Fig. 1 we present an example of ^{1}H spectra of egg phosphatidylcholine vesicles before and after dialysis. The appearance of the signal due to the choline groups at the external surface of the vesicles (trace B) is an indication that most of the Mn^{2+} has been removed from the external medium.

A control solution of $MnCl_2$ of the same concentration as that used in the preparation of the vesicles was dialysed simultaneously with the vesicles. Measurements of the control solution provided information on the relaxation in the external vesicular medium without exchange.

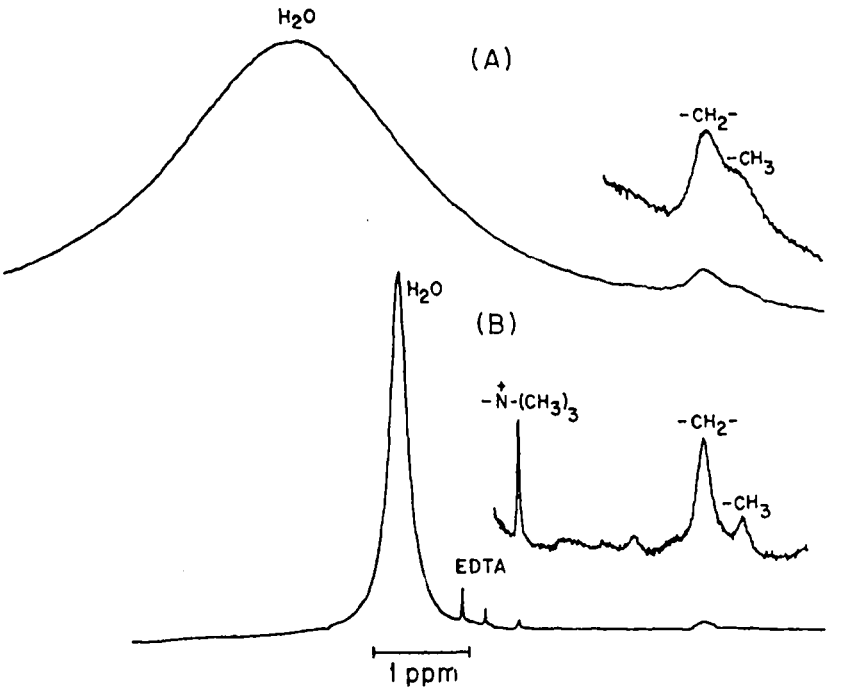

Fig. 1. 270 MHz ^1H spectra of egg phosphatidylcholine vesicles in a water solution containing 90 mM $MnCl_2$, at 28°C. B, same as A after dialysis of the vesicle suspension as explained in Experimental Procedure.

The phospholipid content of the vesicles was determined by ashing and analyzing for inorganic phosphate.

NMR measurements

Proton relaxation studies were carried out at 270 MHz on a Bruker WH-270 pulse-FT spectrometer equipped with variable temperature accessories which maintain the temperature to within ±1.0°C. The field was locked on an external ^2H_2O solution. Transverse relaxation rates were determined from the half-width at half-height, $\Delta\nu_{1/2}$, according to $1/T_2 = 2\pi\Delta\nu_{1/2}$. Longitudinal relaxation rates were determined by the inversion recovery method.

^{17}O studies were performed at 12.2 MHz on a Bruker WH-90 pulse-FT spectrometer with a multinuclear probe. The field was locked on an external ^2H_2O solution. A variable temperature unit maintained the temperature to within ±1.0°C. Transverse relaxation rates were determined from the line-width at half-height.

Analysis of Data

The spherical closed membranes of a vesicle suspension form a separation between internal water and external water environments. In general, ions diffuse very slowly across the vesicular walls. Therefore, we can differentiate between the inner and outer water media by preparing vesicles with an excess

of paramagnetic ions in the inner medium. Due to the presence of paramagnetic ions, such as Mn^{2+}, inside the vesicles, the NMR relaxation rates of either protons or ^{17}O of the inner water become much faster than the corresponding relaxation rates in the external medium. The process of water diffusing across the vesicular membrane induces an exchange of water between the two media which can be treated in an analogous way to a two-site chemical exchange process.

In our system, the fraction of the fast-relaxing inner water nuclei is small relative to the slow-relaxing external water nuclei (5%). Therefore, we can apply the Swift and Connick treatment for analyzing the T_2 relaxation results [7] and the Luz and Meiboom derivation for T_2 relaxation [8]. Since the shifts of the water nuclei in the presence of Mn^{2+} are small relative to the corresponding relaxation enhancements, we can neglect the shift contribution and obtain the following approximate equation:

$$1/T_j = 1/T_j^{\circ} + p_i/p_o[1/(T_j^{i\circ} + \tau^i)] \qquad j = 1,2 \tag{1}$$

where $1/T_j$ = relaxtion rate in external water medium under exchange, $1/T_j^{\circ}$ = relaxation rate in external water medium without exchange, $1/T_j^{i\circ}$ = relaxation rate in internal water medium without exchange, p_i = water fraction of internal medium, p_o = water fraction of external medium and τ^i = mean lifetime of water inside the vesicles. The exchange rate, $1/\tau^i$, can be accurately determined when $1/T_j^{i\circ} > 1/\tau^i$. In order to measure the fast kinetics of water diffusion across membranes, $1/T_j^{i\circ}$ should be increased as much as possible. $1/T_j^{i\circ}$ for water nuclei in the presence of Mn^{2+} is given by [7]:

$$1/T_j^{i\circ} = 1/T_j^{(\circ)} + p_m/(T_{jm} + \tau_m) \qquad j = 1,2 \tag{2}$$

where $1/T_j^{(\circ)}$ = relaxation rate in pure water, p_m = fraction of water coordinated to Mn^{2+}, τ_m = lifetime of a water molecule in the hydration sphere and $1/T_{jm}$ = relaxation rate of water nuclei in the hydration sphere. $1/T_j^{i\circ}$ can therefore be increased either by adding more Mn^{2+} into the internal medium and increasing p_m, or by increasing $1/T_{jm}$. $1/T_{jm}$, i.e., $1/T_{1m}$ and $1/T_{2m}$ are given by the Solomon-Bloembergen equations [9]:

$$1/T_{1m} = \tfrac{2}{15} S(S+1) A_a^2 \left(\frac{\tau_a}{1 + (\omega_I - \omega_S)^2 \tau_a^2} + \frac{3\tau_a}{1 + \omega_I^2 \tau_a^2} + \frac{6\tau_a}{1 + (\omega_I + \omega_S)^2 \tau_a^2} \right)$$
$$+ \tfrac{2}{3} S(S+1) A_I^2 \left(\frac{\tau_i}{1 + (\omega_I - \omega_S)^2 \tau_i^2} \right) \tag{3a}$$

$$1/T_{2m} = \tfrac{1}{15} S(S+1) A_a^2 \left(4\tau_a + \frac{3\tau_a}{1 + \tau_I^2 \tau_a^2} + \frac{\tau_a}{(\omega_i - \omega_S)^2 \tau_a^2} + \frac{6\tau_a}{1 + \omega_S^2 \tau_a^2} \right.$$
$$\left. + \frac{6\tau_a}{1 + (\omega_I + \omega_S)^2 \tau_a^2} \right) + \tfrac{1}{3} S(S+1) A_I^2 \left(\tau_i + \frac{\tau_i}{1 + \omega_S^2 \tau_i^2} \right) \tag{3b}$$

where S = the magnitude of the electron spin ($S = 5/2$), ω_I = precession frequency of the nuclei, ω_s = precession frequency of the electrons, A_i = the magnitude of the isotropic scalar part of the nucleus electron hyperfine interaction tensor, A_a = magnitude of the anisotropic dipolar part and τ_i and τ_a = correla-

tion times given by:

$$\tau_i^{-1} = \tau_m^{-1} + T_{1e}^{-1}$$

$$\tau_a = \tau_i^{-1} + \tau_r^{-1}$$

where τ_r is the correlation time for the rotation of the hydrated complex, T_{1e} is the longitudinal electronic relaxation time and τ_m was previously defined.

For $Mn^{2+} \cdot 6\,H_2O$, τ_r is equal to $3 \cdot 10^{-11}$ s [10]. τ_m was determined to be approx. $3 \cdot 10^{-8}$ s [7,10,11]. T_{1e} ranges from $1 \cdot 10^{-7}$ to $1 \cdot 10^{-8}$ s depending on the field [12]. The dipolar coefficient, $A_a = g\beta\gamma_I/r^3$, is in the range of $2 \cdot 10^7$ rad/s ($r = 2.8$ Å) for ^1H and $8 \cdot 10^6$ rad/s ($r = 2.2$ Å) for ^{17}O. The scalar interaction coefficient, A_i, is $3.8 \cdot 10^6$ rad/s for ^1H [10] and $2.8 \cdot 10^8$ rad/s for ^{17}O [11]. Thus, at both fields used in our measurements (65 kG for ^1H and 21 kG for ^{17}O) we have: $\omega_S^2\tau_i^2 >> \omega_S^2\tau_a^2 >> 1$ and $\omega_I^2\tau_a^2 << 1$, so that Eqns. 3a and 3b can be simplified to the following approximate equations:

$$1/T_{1m} = \tfrac{2}{5}\,S(S+1)\,A_a^2\tau_a \tag{4a}$$

$$1/T_{2m} = \tfrac{7}{15}\,S(S+1)\,A_a^2\tau_a + \tfrac{1}{3}\,S(S+1)\,A_i^2\tau_i \tag{4b}$$

The scalar term is larger than the dipolar one for both ^1H and ^{17}O, therefore, $1/T_{2m} > 1/T_{1m}$, and T_2 studies provide data for a larger kinetic range than T_1 measurements. Since the scalar term of ^{17}O is larger by about two orders of magnitude than that of ^1H, the advantage in following the T_2 relaxation of ^{17}O is obvious. Thus, it is possible to extend the kinetic range using moderate Mn^{2+} concentrations by measuring T_2 relaxation of ^{17}O.

The diffusion rate of the water is related to the permeability, P, by:

$$P = V/A\ (1/\tau^i) \tag{5}$$

where V = inner volume of the vesicles and A = surface area of the vesicles. For spherical vesicles with radius r, Eqn. 5 reduces to:

$$P = \tfrac{1}{3}r(1/\tau^i) \tag{6}$$

Results and Interpretation

The variation with temperature of the ^1H and ^{17}O relaxation rates of water nuclei in a suspension of egg phosphatidylcholine vesicles, containing excess intervesicular Mn^{2+}, is shown in Fig. 1.

As previously discussed, the ^{17}O relaxation rate ($1/T_2$) is much faster than that of ^1H in the presence of the same Mn^{2+} concentration. In our system, the diffusion exchange rate across the membrane ($1/\tau^i$) was slower than $1/T_2^{i\circ}(^{17}O)$, but was of the same order of magnitude as $1/T_2^{i\circ}(^1H)$. Thus, the ^1H data were analyzed according to Eqn. 1 while the ^{17}O results were analyzed using the following approximation: $1/\tau^i << 1/T_2^{i\circ}$, which led to the simplified equation:

$$1/T_2 = 1/T_2^\circ + p_i/p_o(1/\tau^i) \tag{7}$$

$1/T_2^\circ$ was determined by measuring the relaxation in a control solution identical in composition to the external medium of the vesicles (see Experimental procedure). p_i and p_o were calculated from the lipid concentration and the partial

specific volume of the vesicles [13]. $1/T_2^o(^1H)$ was found to be very small relative to the measured $1/T_2$ in the vesicles (less than 4%) and could therefore be neglected. $1/T_1^o(^1H)$ was found to be approx. 30% of the measured longitudinal rate in the vesicles and was therefore taken into account in the calculations. In order to analyze the ^1H data, the value of the relaxation rate ($1/T_j^{i\circ}$) had to be determined. This could be calculated from the data obtained in the fast-exchange region, where $1/T_j^{i\circ} << 1/\tau^i$. In this region, Eqn. 1 can be approximated according to:

$$1/T_j = 1/T_j^o + p_i/p_o(1/T_j^{i\circ}) \qquad j = 1,2 \tag{8}$$

Based on previous measurements of the behaviour with temperature of the ^1H relaxation of water in Mn^{2+} solutions [14], it was possible to assume a linear Arrhenius behaviour for the ^1H relaxation of the inner water nuclei in our system. Thus, by extrapolation from the fast-exchange region (broken lines in Fig. 1) we could determine $1/T_j^{i\circ}$ over the entire temperature range studied. The temperature-dependence of the calculated exchange rates is plotted in Fig. 2. Rates between 40° and 65°C were calculated from ^{17}O data, while rates between 0° and 20°C were determined from ^1H data. The activation energy of 10.5 kcal/mol remained constant throughout the entire temperature range studied (Fig. 3).

The temperature-dependence of ^{17}O and ^1H transverse relaxation rates of the water nuclei in a suspension of dipalmitoyl phosphatidylcholine vesicles containing excess intervesicular Mn^{2+} is presented in Fig. 4. $1/T_2^o$ for both ^{17}O and ^1H was determined as for the egg phosphatidylcholine suspensions. At about 40°C there is a conspicious change in the shape of the curve, which does not

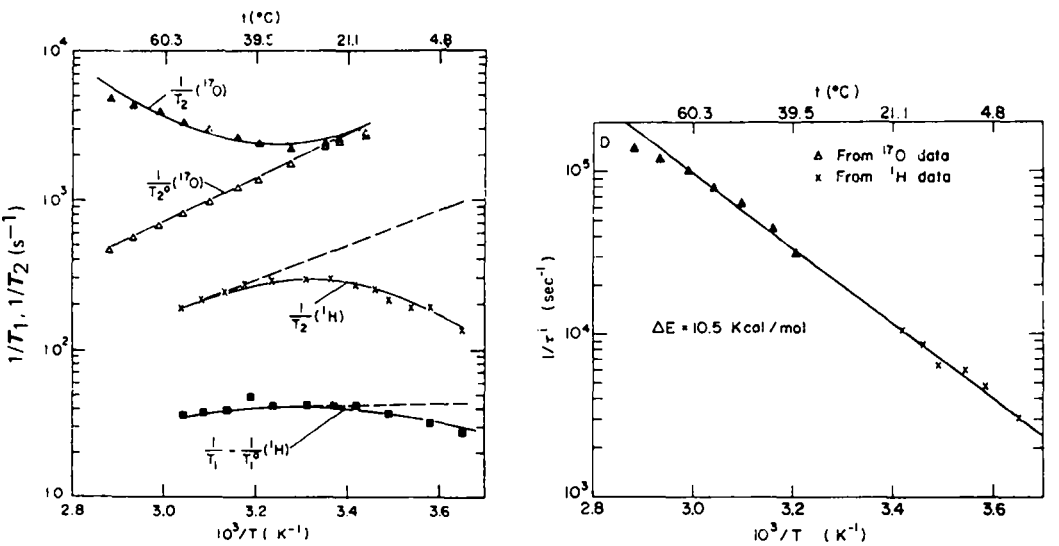

Fig. 2. Temperature-dependence of ^1H and ^{17}O longitudinal ($1/T_1$) and transverse ($1/T_2$) relaxation rates in a vesicular suspension of egg phosphatidylcholine. Vesicles prepared with 50 mM $MnCl_2$ after dialysis (▲); 50 mM $MnCl_2$ solution after dialysis (△); vesicles prepared with 90 mM $MnCl_2$ after dialysis (X, ■).

Fig. 3. Temperature-dependence of diffusion exchange rates across egg phosphatidylcholine vesicles.

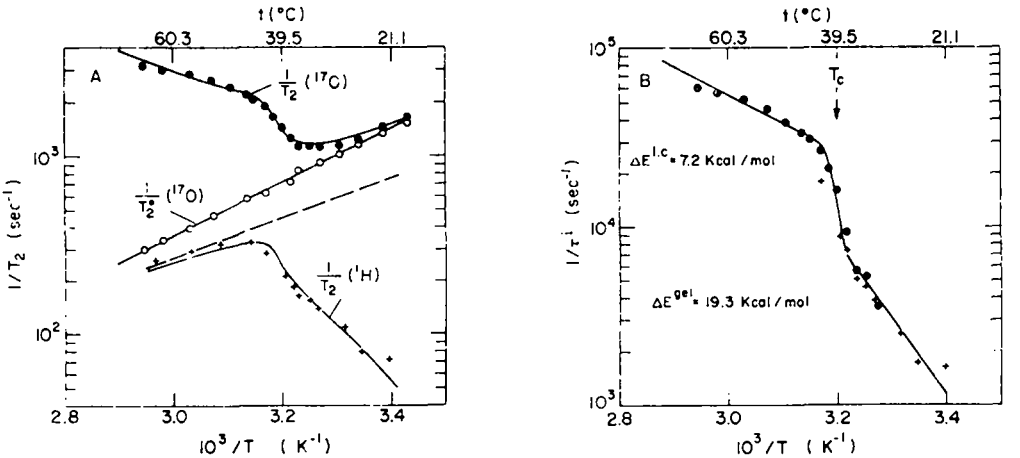

Fig. 4. Temperature-dependence of ^{1}H and ^{17}O transverse relaxation rate ($1/T_2$) in a vesicular suspension of dipalmitoyl phosphatidylcholine. Vesicles prepared with 50 mM $MnCl_2$ after dialysis (•); 50 mM $MnCl_2$ solution after dialysis (○); vesicles prepared with 90 mM $MnCl_2$ after dialysis (+).

Fig. 5. Temperature-dependence of diffusion exchange rates across dipalmitoyl phosphatidylcholine vesicles. ^{17}O data (•); ^{1}H data (+).

occur for egg phosphatidylcholine vesicles (Fig. 1). This change is more pronounced in the curve representing the temperature-dependence of the diffusion exchange rate ($1/\tau^i$) in Fig. 5. A sharp reduction in the rate (by about a factor of 3) occurs from 40° to 37°C with a concomitant large increase in the activation energy. It is known that at this temperature range dipalmitoyl phosphatidylcholine membranes undergo a liquid crystalline-gel phase transition [15].

Discussion

Previous measurements of water permeability coefficients across lipid model bilayers as well as our results are summarized in Table I. There is a good agree-

TABLE I

WATER PERMEABILITY AND ACTIVATION ENERGY FOR DIFFUSION ACROSS LIPID MEMBRANES

Lipid	Temperature (°C)	$10^3 \times P$ (cm/s)	E_a (kcal/mol)	Method
Egg phosphatidylcholine	36	4.2		tritiated water across lipid films [17]
	25	2.4		
	25	2.9	10.5	NMR (^{17}O, ^{1}H), vesicles, this work
Dipalmitoyl phosphatidylcholine	25	0.30	15	NMR (^{1}H), vesicles [5]
	44	1.6		
	25	0.34	19.3	NMR (^{17}O, ^{1}H), vesicles, this work
	45	7.2	7.2	
Gel phase			24.9	osmotic shrinkage of multilayered liposomes [1]
Liquid crystalline phase			8.6	

ment between the permeabilities obtained by the NMR and tritiated water exchange methods as well as between the two NMR results for dipalmitoyl phosphatidylcholine in the gel state (25°C). However, for dipalmitoyl phosphatidylcholine in the liquid crystalline phase (45°C), the permeability determined by us, using ^{17}O relaxation measurements, is larger by a factor of approx. 4 than that determined by Andrasko and Forsen [5] using ^{1}H relaxation data. It should be noted that in the liquid crystalline phase, the ^{1}H relaxation is confined to the so-called 'fast-exchange' limit in which the contribution of the exchange term to the relaxation is rather negligible. However, the ^{17}O relaxation in this phase is in the 'slow exchange' limit and is dominated by the water exchange rate. It is therefore suggested that for the liquid crystalline phase, the permeabilities determined in our work from ^{17}O measurements are more accurate.

The NMR data for the activation energies for water diffusion above and below the phase transition of dipalmitoyl phosphatidylcholine membranes are in close agreement with those obtained by the osmotic shrinkage method [16]. This is to be expected in view of the findings of Finkelstein and Cass [17] that the water osmotic permeability and the tagged water permeability are the same.

A general mechanism for the permeation of water and small non-electrolytes across lipid membranes is provided by the solubility diffusion model of Diamond et al. [18]. These authors derived an expression for the permeability coefficient, P_j, of a permeating species, j, in terms of the interfacial resistances, r_j' and r_j'', the partition coefficient, $K_j(x)$, the diffusion coefficient, $D_j(x)$, and the membrane thickness, x_o, taking the plane of the membrane perpendicular to the x-axis:

$$P_j = r_j' + \int_0^{x_0} dx/K_j(x)\, D_j(x) + r_j'' \tag{9}$$

both K_j and D_j are likely to be a function of position. Assuming that the interfacial resistances are small and that $K_j(x)$ and $D_j(x)$ can be approximated by average values K and D, Eqn. 4 can be simplified to:

$$P_j = KD/x_o \tag{10}$$

It is possible to check whether Eqn. 5 provides a good estimate for water permeability coefficients, as previously suggested by Finkelstein and Cass [17]. We assume that the rate-limiting barrier for water permeation is provided by the diffusion across the bilayer and that solubility and diffusion of water in the bilayer interior and in a bulk hydrocarbon are similar. In Table II the measured P values are compared with values for KD/x_o in hexadecane using Schatzberg's data for K and D [19] and x_o is 40 Å [20]. It is clear that for the egg phosphatidylcholine membranes the ratio between the measured and the calculated permeabilities is rather close to 1 and is constant over the temperature range studied. This suggests that our previous assumptions were valid and that water diffuses across lipid membranes in the liquid crystalline phase according to a mechanism similar to the diffusion in fluid hydrocarbons.

For the dipalmitoyl phosphatidylcholine lipid bilayers in the liquid crystalline phase the ratio between the measured and the calculated permeability is

TABLE II

MEASURED AND CALCULATED WATER PERMEABILITIES

$P_{calc.} = KD/x_0$ in Hexadecane. K, D [19] $x_0 = 40$ Å [20].

	Temperature (°C)				
	25	30	35	40	42
$P_{calc.} \times 10^3$ (cm/s)	4.3	6.0	7.9	10.7	14.2
$P/P_{calc.}$, egg phosphatidylcholine	0.67	0.67	0.66	0.63	0.62
$P/P_{calc.}$, dipalmitoyl phosphatidylcholine	0.08	0.10	0.13	0.24	0.51

still close to 1, although somewhat smaller than that obtained for egg phosphatidylcholine at the same temperature. This difference may be due to the presence of unsaturated chains in the latter lipid. However, a marked decrease in the above ratio is observed through the lipid phase transition and in the gel phase. This behaviour suggests that the similarity to the diffusion in hydrocarbon fluids does not hold as the lipids undergo a phase transition to the gel phase and that lipid order and motional freedom determine the kinetics of water diffusion across lipid membranes. Similar conclusions are derived from the difference in the activation energy in both phases.

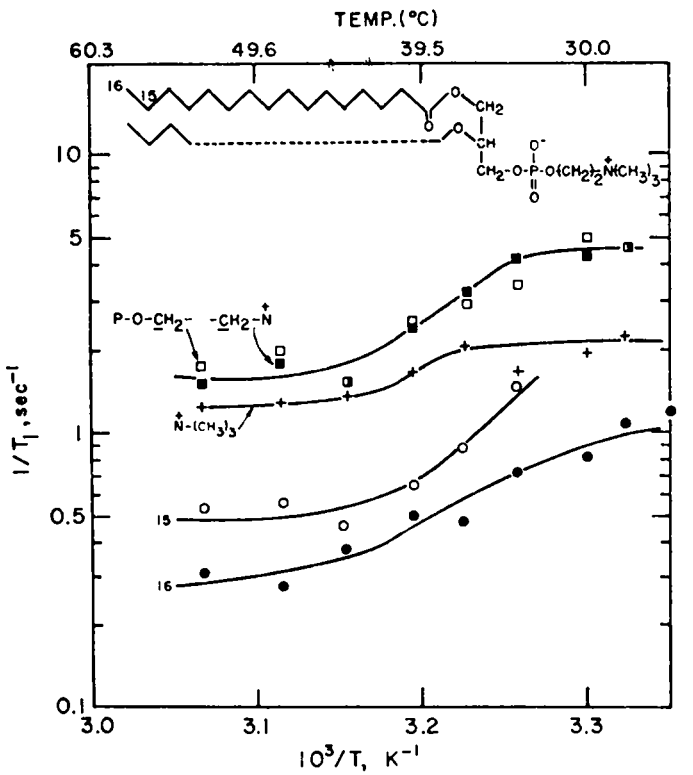

Fig. 6. Temperature-dependence of the ^{13}C spin lattice relaxation rates ($1/T_1$) of dipalmitoyl phosphatidylcholine vesicles. Measurements were performed at 67.89 MHz using the inversion recovery method.

In order to correlate the changes in permeability with changes in the lipid dynamics at the phase transition, we have measured the temperature dependence of the ^{13}C T_1 relaxation rates through the phase transition. It was previously shown that ^{13}C T_1 relaxation of lipid membranes is determined by the segmental reorientation of the CH_2 fragments of the lipid molecules, for example by β-coupled *gauche* isomerization ('kink' formation) [21]. The results in Fig. 6 for part of the carbons clearly indicate that changes in T_1 through the phase transition are about the same order of magnitude as the change in water permeability. This comparison lends support to the preposition of Traüble [22] that water diffusion across membranes is associated with the fast segmental motion of the lipids and the formation of mobile kinks.

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References

1 Conlon, T. and Outhred, R. (1972) Biochim. Biophys. Acta 288, 354—361
2 Conlon, T. and Outhred, R. (1978) Biochim. Biophys. Acta 511, 408—418
3 Morariu, V.V. and Benga, G. (1977) Biochim. Biophys. Acta 469, 301—310
4 Shporer, M. and Civan, M.M. (1975) Biochim. Biophys. Acta 385, 81—87
5 Andrasko, J. and Forsen, S. (1974) Biochem. Biophys. Res. Commun. 60, 813—819
6 Haran, N. and Shporer, M. (1976) Biochim. Biophys. Acta 426, 638—646
7 Swift, T.J. and Connick, R.E. (1962) J. Chem. Phys. 37, 307—313.
8 Luz, Z. and Meiboom, S. (1964) J. Chem. Phys. 40, 2686—2692
9 Solomon, I. and Bloembergen, N. (1956) J. Chem. Phys. 25, 261—266
10 Luz, Z. and Shulman, R.G. (1965) J. Chem. Phys. 43, 3750—3756
11 Grant, M., Dodgen, H.W. and Hunt, J.P. (1971) Inorg. Chem. 10, 71—73
12 Rubinstein, M., Baram, A. and Luz, Z. (1971) Mol. Phys. 20, 67—80
13 Huang, C. (1969) Biochemistry 8, 344—351
14 Bloembergen, N. and Morgan, L.O. (1961) J. Chem. Phys. 34, 842—849
15 Chapman, D., Williams, R.M. and Ladbrooke, B.D. (1967) Chem. Phys. Lipids 1, 445—475
16 Blok, M.C., van Deenen, L.L.M. and de Gier, J. (1976) Biochim. Biophys. Acta 433, 1—12
17 Finkelstein, A. and Cass, A. (1968) J. Gen. Physiol. 52, 145S—172S
18 Diamond, J.M., Szabo, G. and Katz, Y. (1974) J. Membrane Biol. 17, 148—152
19 Schatzberg, P.J. (1965) Polymer Sci. Part C 10, 87—92
20 Small, D.M. (1967) J. Lipid Res. 8, 551—557
21 Gent, M.P.N. and Prestegard, J.H. (1977) J. Magn. Resonance 25, 243—262
22 Traüble, H. (1971) J. Membrane Biol. 4, 193—208