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DIFFUSION PROCESSES IN LIPID–WATER LAMELLAR PHASES

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SUMMARY

The diffusion of ^3HHO in lamellar lecithin–water and lecithin–cholesterol–water phases was measured as a function of water content and temperature. The diffusion coefficient was found to increase rapidly with the water content above 32 % in lecithin–water systems and above 20 % in lecithin–cholesterol–water systems. Below these values the diffusion coefficient was found to be essentially independent of water content. The evolution of the diffusion rate with water content can be accounted for in terms of the known structural parameters as well as the state of water in both systems.

The diffusion of benzene in the lipid region of the phases was measured in both systems and found to increase with the water content. The evolution of the diffusion rate of benzene paralleled the variation in the fluidity of the hydrocarbon chains.

INTRODUCTION

Phospholipid–water systems have become the subject of growing interest over the past several years. They provide an attractive model for biological membranes with the advantage that their microscopic structure is known over a wide range of concentration and temperature. A study of the permeability properties of these systems will give information about the relationship between these properties and the known structure. Such an understanding in this relatively simple system will undoubtedly give insight into related processes in more complex biological systems.

The study of the permeability properties of phospholipid–water systems yields information somewhat different from that obtained with lipid bilayers. The system differs from bilayers in the important respect that an aqueous region is present. The lipid–water spacing is known from X-ray diffraction studies and may be varied by changing the amount of water present.

In this paper we present the results of studies on lecithin–water and lecithin–cholesterol–water phases. A preliminary report has already been published[1]. The diffusion of ^3HHO in the phases was measured as a function both of water content and of temperature. It is known that the state of the hydrocarbon chains in lipid–water phases depends on the amount of water present. For this reason the diffusion of a lipophilic substance, benzene, was also determined as a function of the water content of the phase.

MATERIALS AND METHODS

Lecithin was extracted from egg yolk according to the method of Singleton *et al.*[2] and checked for purity by thin-layer chromatography.

Preparation of phases

Two different methods were used, both giving homogeneous phases, free of air bubbles. The first method involved the manual mixing of lecithin and water and was used to prepare phases containing more than 25 % water. Lecithin was weighed in a syringe, the plunger inserted and the syringe attached to a three-way stopcock and evacuated. The appropriate quantity of water was introduced into a second syringe, using a λ-pipette. This syringe was also connected to the stopcock and evacuated. Finally, the stopcock was positioned so that the two syringes were in contact and the lecithin and water were mixed by pushing the plungers back and forth.

The viscosity of phases of low water content was so great as to make manual mixing difficult and therefore a second method was used in their preparation. A weighed amount of lecithin was dissolved either in chloroform or in ethanol and the required amount of water added. The solvent was then removed in a rotary evaporator and the phase recuperated and placed in a syringe. The syringe was evacuated so as to obtain a compact phase, free of air bubbles. This method was always used in the preparation of lecithin–cholesterol–water phases. Good mixing of the lecithin and cholesterol was ensured by their dissolution in solvent.

After preparation, the phases were always kept for at least 24 h before use. This time was required to ensure that the ordering of the phases was stable.

The water content of the phase was initially determined by drying to constant weight under vacuum at room temperature. This process was lengthy so in most cases the drying was accomplished in an oven at 90° for 15–20 min. Both methods gave the same result.

Measurement of diffusion coefficient

Diffusion coefficients of water were determined using the radioactive isotope, ^{3}HHO. Experimental boundary conditions were chosen to simplify the solution of the diffusion equation,

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} \tag{1}$$

where D is the diffusion coefficient and c the concentration of diffusing substance, ^{3}HHO. In the case of a semi-infinite cylinder with a plane source at $x = 0$, the solution[3] of Eqn. 1 is,

$$c(xt) = \frac{M}{(\pi Dt)^{1/2}} e^{-x^2/4Dt} \tag{2}$$

where M is the amount of diffusing substance deposited at time $t = 0$, in the plane $x = 0$.

The diffusion experiments were carried out in a capillary filled with the phase. A small amount of radioactive tracer was introduced at one end of the capillary and

allowed to diffuse down the column of phase. At a subsequent time, axial slices of the phase were made along the length of the column and analyzed for radioactivity. The diffusion coefficient was then calculated using Eqn. 2.

The diffusion chamber consisted of a hollow, cylindrical lucite sleeve, C (Fig. 1), in which a number of lucite discs with axial holes (diameter 1.2 mm) could be stacked. In general, several thick discs were used (B in Fig. 1), together with 10 thinner discs of identical thickness (approx. 1 mm). The central holes of the discs formed a capillary approx. 5 cm long. The base of the sleeve contained a threaded hole into which could be placed the threaded tip of a syringe needle for the introduction of the phases to be studied. Alternatively, a screw (V in Fig. 1) could be inserted to advance the discs in the sleeve. A circular lucite end piece could be attached with screws to the open end of the sleeve to close the system.

At the start of an experiment the phase was introduced into the capillary with a syringe. The needle of the syringe was chosen so that the cross-section of the emerging ribbon of phase was slightly larger than that of the capillary. In this way, the capillary was well filled and free of air bubbles. Sufficient phase was introduced to cause slight protrusion at the end of the capillary. The syringe was then removed and replaced by the screw, which was used to advance the stack of discs until the end surface was flush with the edge of the sleeve. The system was then closed with the lucite end piece and left at constant temperature for at least 6 h, the time necessary for equilibration of the phase to occur (see next section). The end piece was then

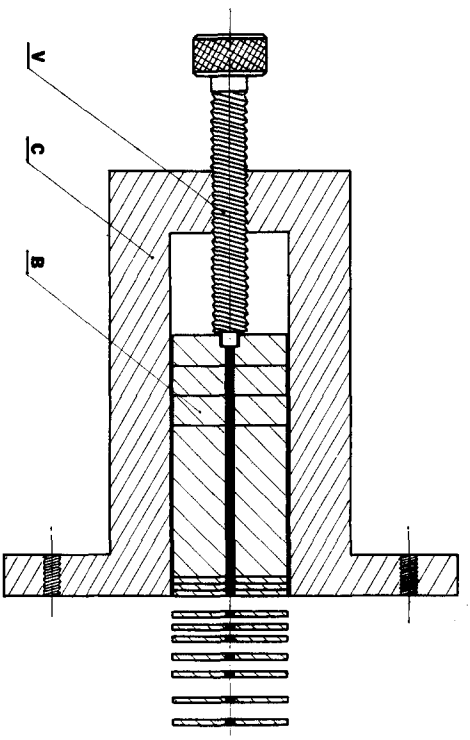

Fig. 1. Schematic of chamber in which diffusion measurements were made, see text.

removed and the surface of the phase cut flush with the surface of the first disc.

To introduce the radioactive tracer into the system, approx. 1 μl of a solution of ^{3}HHO (approx. 1 mC/ml) on a small piece of filter paper was placed on the first disc, in contact with the phase. In order for the solution (Eqn. 2) of the diffusion equation (Eqn. 1) to pertain, it was necessary that the radioactive source be localized and of infinitesimal thickness. The filter paper was therefore kept in contact with the phase for a length of time such that the radioactivity in the phase remained in the first disc and yet was sufficient in amount to give a measurable concentration profile at a subsequent time. The optimum time of contact was determined experimentally to be approx. 1 min. After removing the filter paper it was replaced by a small glass cover slip and the system was tightly closed with the lucite end piece. The glass cover slip was necessary to ensure that the system was air tight so that there was no loss of radioactivity by evaporation from the first disc. The chamber was kept at constant temperature and diffusion was allowed to occur for a time long enough so that there was a detectable amount of radioactivity in the first ten 1-mm discs but too short for the tracer to diffuse to the end of the capillary. In this way, the capillary was effectively semi-infinite, as required for the solution (Eqn. 2) to be valid. The time allowed for diffusion varied with the diffusion coefficient, namely with the composition of the phase, but was always of the order of hours.

After this period of time had elapsed, the 1-mm discs were removed and the amount of ^{3}HHO in the phase contained in each disc determined. The discs were removed one at a time by advancing the screw until a single disc protruded from the containing sleeve. The disc was removed by a lateral displacement so as to separate it cleanly from the remaining column. The disc was then placed on a scintillation vial. The phase was ejected from the disc into the vial by a small amount of water (approx. 0.4 ml) contained in a syringe fitted with a short piece of rubber tubing. 10 ml of Bray's scintillation fluid was then added to the vial for counting.

The diffusion coefficient was determined using Eqn. 2. As each disc was of finite thickness, it was necessary in principle to integrate Eqn. 2 to obtain the average concentration in each slice. However, a simple calculation showed that the error involved in taking the average value of x for each slice was very small compared to the overall error involved in the measurement. The main errors resulted from the uncertainty in the precise position of the point $x = 0$ due to the fact that the radioactive source was not perfectly localized and the sampling error. The latter was determined using a phase prepared with water containing ^{3}HHO. The phase was introduced into the diffusion chamber and sampled as usual. The variation observed in the radioactive content of each disc was due both to the inhomogeneity of the phase and to the sampling error. A standard deviation of 3 % was obtained.

Eqn. 2 can be written in the form:

$$\ln c(xt) = \ln M - \ln (\pi Dt)^{\frac{1}{2}} - \frac{x^2}{4Dt}$$

A plot of the radioactive counts in each slice against the square of the average value of x for that slice yields a straight line. D was determined from the slope of the least mean square fit and the time of diffusion.

The diffusion of benzene was measured by the same method as was used to

measure the diffusion of water. The lucite discs were replaced by stainless steel discs to avoid any loss of benzene from the system.

The diffusion experiments were carried out in a water bath; temperature was controlled to within 0.05°.

^{3}HHO and [^{14}C]benzene were obtained from C.E.A., France.

Counting was done in a liquid scintillation counter (Nuclear Chicago Corp.)

RESULTS

To check the validity of the method of measuring diffusion coefficients it was applied to the diffusion of ^{3}HHO in Agar gels. At 5 % (by weight) Agar, the diffusion coefficient was found to be $(1.91 \pm 0.03) \cdot 10^{-5}$ cm$^2 \cdot$sec^{-1} at 22°. This value is in good agreement with the value of $1.93 \cdot 10^{-5}$ cm$^2 \cdot$sec^{-1} obtained by extrapolation of the data of Nakayama and Jackson[4] whose measurements were made using a different method. With this assurance that the method yielded good results, it was applied to the measurement of diffusion coefficients of ^{3}HHO in lecithin–water and lecithin–cholesterol–water phases.

The applicability of Eqn. 2 was first checked in phases containing 25–30 % water. Table I gives the results of a series of measurements in which the time of diffusion was varied. From the reproducibility of the results and the values of χ^2, we conclude that the equation is valid, within the experimental error.

The possibility existed that some ordering of the phases was induced by filling the capillary, which could affect the diffusion coefficient. The diffusion coefficient was measured as a function of the time elapsed between introduction of the phase into the capillary and the addition of the isotope and found to be constant for times between 6 and 260 h. In all subsequent experiments, the phase was allowed to equilibrate in the capillary for at least 6 h before initiating the diffusion.

The solvent used in preparing the phases is probably not completely removed by evaporation, leaving small amounts in the hydrophobic region, in the case of chloroform and in the hydrophilic region, in the case of ethanol. The presence of small amounts of solvent could affect the diffusion coefficient measured. ^{3}HHO diffusion measurements were made using phases of varying water content prepared by manual mixing (in the absence of solvent) and by dissolution in ethanol or chloroform. The diffusion coefficient, D, is plotted as a function of, Φ_w, the water content in g/g phase in Fig. 2. It can be seen that the diffusion coefficients measured in phases

TABLE I

DIFFUSION COEFFICIENT IN LECITHIN–WATER PHASES

Verification of applicability of Eqn. 2 by Chi-Square Test (n = 10).

Water content (g/g phase)	*No. of experiments*	$D \times 10^6$ (cm$^2 \cdot$sec^{-1})	χ^2
0.30	17	0.87 ± 0.08	15–80
0.29	5	0.83 ± 0.03	22–50
0.26	5	0.79 ± 0.03	15–180
0.25	3	0.76 ± 0.03	10–50

prepared by the three different methods all fall on the same curve. It may be concluded that any small amount of solvent remaining in the phase has no effect on the diffusion of water to within the accuracy of our measurements.

Reiss-Husson[5] and Lecuyer and Dervichian[6] have carried out a structural analysis of lecithin–water and lecithin–cholesterol–water systems by X-ray diffraction. They found that both systems exhibit a lamellar structure over a wide range of water content, from about 10 to 45 % for lecithin–water systems and from 10 to 35 % in systems with a lipid component of equimolar quantities of lecithin and cholesterol. The thicknesses of the lipidic and aqueous layers may be computed from the X-ray diffraction data. The former decreases and the latter increases as the water content of the phase increases.

Water can diffuse both in the lipidic and in the aqueous layers of the phase. However, because of the very small solubility of water in lipid, it may be assumed that the measured diffusion coefficient for ^{3}HHO exclusively reflects diffusion in the aqueous layer in spite of the fact that the diffusion rate of water in lipid is of the same order of magnitude as that in water[7].

The diffusion coefficient of ^{3}HHO in the phase increases with the water content, Φ_w shown in Fig. 2. The increase is due in part to the fact that the area available

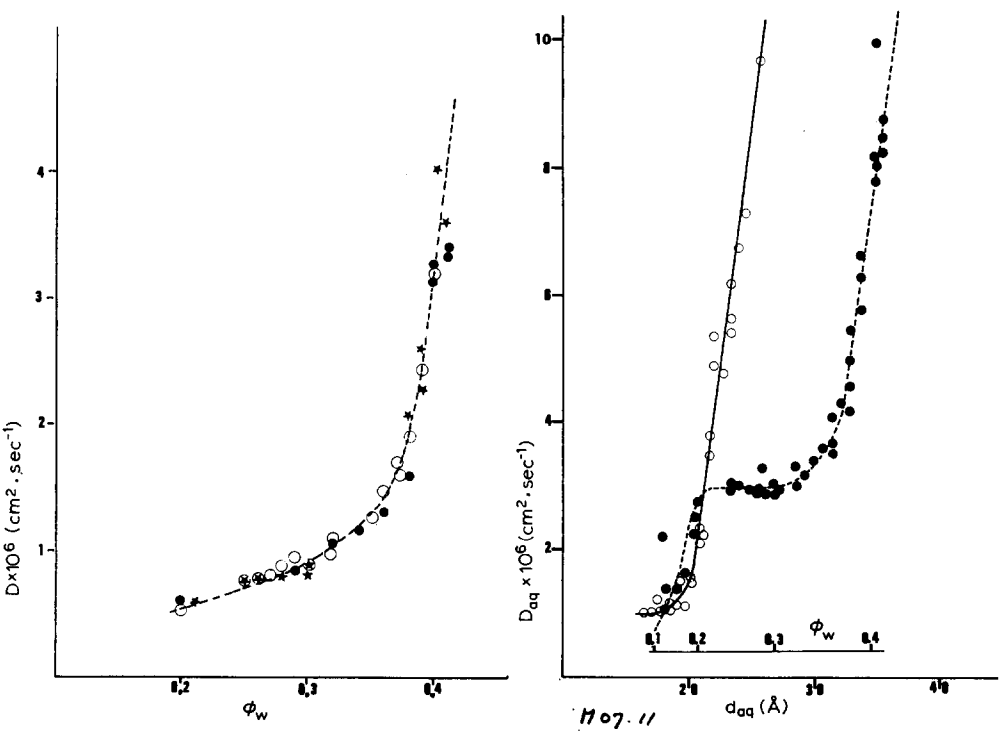

Fig. 2. Diffusion coefficient of ^{3}HHO at 22° in lecithin–water phases prepared by different methods as a function of phase water content. Phase prepared in: ethanol (✱), chloroform (○) and without any solvent (●).

Fig. 3. D_{aq} for ^{3}HHO in lecithin–water (●) and lecithin–cholesterol–water (○) phases as a function of d_{aq} and Φ_w at 22° (see text for definition of parameters).

for diffusion increases with Φ_w. The dependence on Φ_w of the density of the aqueous region of the phase is not known, but it may reasonably be assumed to vary in a relatively narrow range around 1. Assuming that the density is 1, the ratio $D/\Phi_w = D_{aq}$ can be taken as the diffusion coefficient of ^{3}HHO per constant area of the aqueous region of the phase.

In Fig. 3 the quantity D_{aq}, measured both in lecithin–water and in lecithin–cholesterol–water systems containing equimolar amounts of lecithin and cholesterol, is plotted as a function of d_{aq}, the thickness of the aqueous layer computed from the data of Lecuyer and Dervichian[6].

Three regions of the curve can be distinguished for the lecithin–water system (closed circles). For Φ_w between 0.1 and 0.2, which corresponds to d_{aq} between 17 and 21 Å, there is a rapid increase in D_{aq}. For Φ_w between 0.2 and 0.3, which corresponds to d_{aq} between 21 and 27 Å, D_{aq} remains essentially constant at $3 \cdot 10^{-6}$ cm$^2 \cdot$sec^{-1}. When Φ_w exceeds 0.33 there is another region where D_{aq} increases rapidly to a value of $8.20 \cdot 10^{-6}$ cm$^2 \cdot$sec^{-1} at $\Phi_w = 0.40$. According to the results of Lecuyer and Dervichian[6], the lamellar phase persists up to 45 % water. However, due to difficulties in obtaining homogenous phases of high water content, diffusion measurements in lecithin–water phases containing more than 40 % water are not reliable.

The domain of the lamellar phase may be extended by the addition of a charged group, such as phosphatidic acid, to the system. Lamellar phases containing 10 % phosphatidic acid were prepared with 50 %, 66 % and 72 % water. D_{aq} measured in these phases had values of 7.8, 9.0 and $9.4 \cdot 10^{-6}$ cm$^2 \cdot$sec^{-1}, respectively, indicating that a new plateau is reached when the water content of the phase exceeds 40 %. This value can be compared with the value of $2.3 \cdot 10^{-5}$ cm$^2 \cdot$sec^{-1} obtained by Wang[8] for the diffusion coefficient of ^{3}HHO in bulk water at 22°.

In the lecithin–cholesterol–water system (open circles in Fig. 3), D_{aq} remains constant at about $1.0 \cdot 10^{-6}$ cm$^2 \cdot$sec^{-1} in the region of low water content up to $\Phi_w = 0.18$ ($d_{aq} = 19.8$ Å) and then increases rapidly at a rate which roughly parallels the lecithin–water curve. Here again, the lamellar region does not persist above $\Phi_w = 0.35$, but it is reasonable to assume that a new plateau would be reached, the asymptotic value for $\Phi_w \rightarrow 1.0$ again being $2.3 \cdot 10^{-5}$ cm$^2 \cdot$sec^{-1}. As in the case of the lecithin–water systems no measurements were made for Φ_w below 0.1, due to the difficulty in obtaining homogeneous phases.

In order to interpret the evolution of D_{aq} with the water content of the phase, steric factors as well as the interactions of water molecules in the system have to be taken into account. The quantity D_{aq} measured in these structured systems cannot directly be compared with the diffusion coefficient of ^{3}HHO in bulk water due to several considerations. D_{aq} depends not only on water–water interactions, but also on lecithin–water and cholesterol–water interactions. On a more macroscopic scale, D_{aq} depends on the tortuosity of the aqueous pathway due to folding of the lamellae. The extent of this effect is difficult to assess, but its importance could be considerable, particularly if the tortuosity varied with the water content of the phase. If this were the case, the abrupt changes in D_{aq} could be simply a reflection of changes in the macroscopic structure of the phase. Such changes would be expected to affect the diffusion of all small hydrophilic molecules in a similar manner. The diffusion of several non-electrolytes in the phases was studied and it was found that in some cases the curves exhibited abrupt changes in slope, similar to those found for D_{aq}

but in other cases, for example, formamide (see Fig. 4), the diffusion coefficient varied monotonically with Φ_w. The importance of tortuosity should not be discounted but the evolution of D_{aq} with water content cannot be accounted for in these terms. It should be noted that at Φ_w about 0.4, D_{aq} for ^{3}HHO is only one third of the self-diffusion coefficient in pure water. If this difference could be ascribed entirely to the tortuosity of the system, this implies that at this water content, the real path length for diffusion is only three times greater than the geometric length used.

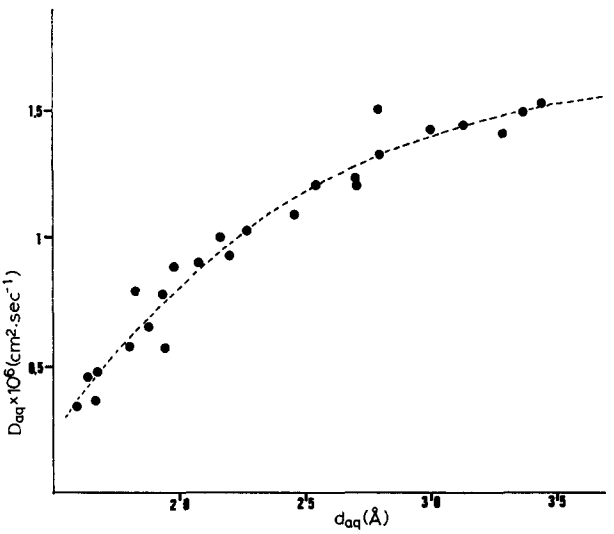

Fig. 4. D_{aq} for formamide in lecithin–water phases as a function of d_{aq} at 22° (see text for definition of parameters).

The behavior of D_{aq} can be related to interactions and steric hindrance at molecular level. Lecuyer and Dervichian[6] pointed out that in lecithin–water the lamellar phases the aqueous region is not pure water, but contains the choline phosphate group of the lecithin molecule. The length of the choline phosphate group is about 10 Å when fully extended. It is, therefore, reasonable to expect that as long as d_{aq}, the thickness of the aqueous layer, is less than 20 Å the water region is crowded by bulky polar groups which impede water diffusion. As soon as d_{aq} exceeds 20 Å, however, a pure water region can be formed and diffusion is accelerated.

Another factor to be considered is the state of the water in the hydrophilic region. Chapman *et al.*[9] have shown by differential scanning calorimetry that at least up to 20% of the water in a lecithin–water phase cannot freeze. In a study of the water sorption isotherms of egg yolk lecithin at 25°, Elworthy[10] determined that 1 g of lecithin binds up to 0.48 g water. In other words in a lecithin–water phase, all the water up to $\Phi_w = 0.32$ is hydration water. It is reasonable to expect that the mobility of this water is notably reduced.

For a system whose lipid portion is composed of equimolecular amounts of lecithin and cholesterol, the number of polar choline phosphate groups is halved. The steric hindrance in the aqueous region and the amount of hydration water are therefore reduced in such a system.

The dependence of D_{aq} on the phase water content in both the lecithin–water and the lecithin–cholesterol–water phases can be explained qualitatively in terms of these simplified considerations.

In the lecithin–water system of low water content ($\Phi_w < 0.20$), diffusion of ^3HHO occurs in a dense maze of choline phosphate groups. As the amount of water is increased, the thickness of the aqueous layer increases, resulting in a greater separation between the choline phosphate groups located on opposite sides of the aqueous layer and a rapid increase in the diffusion rate D_{aq}. When Φ_w reaches about 0.20, the thickness of the aqueous layers is such that a pure aqueous region, unencumbered by choline phosphate groups begin to appear. At the same time, the terminal $-N^+(CH_3)_3$ groups of the choline phosphates on opposite sides of the layer face each other, which is an unfavourable situation. It is probable that at this point the fully extended groups begin to bend over in order to juxtapose their terminal positively charged group with the nearest negatively charged group, that is the phosphate group of the adjacent lecithin molecule. Santis and Rojas[11] have described a similar process for lecithin monolayers. As a result of this phenomenon, the thickness of the pure water layer could be considerably greater than the thickness computed from the difference between d_{aq} and the length of the two fully extended choline phosphate groups. This effect could be expected to result in a rapid increase of diffusion, as the steric hindrance due to the choline phosphate groups would be considerably reduced. However, as can be seen from Fig. 3, D_{aq} remains essentially constant for Φ_w between 0.20 and 0.32. The occurrence of this plateau can be explained by the fact that up to 32 %, all the water in the phase is hydration water. Therefore, in spite of the increase in effective thickness of the water layer, diffusion is limited by the structure of the water. Above $\Phi_w = 0.32$ free water appears and D_{aq} increases rapidly.

The interpretation of the evolution of water diffusion coefficient with water content in the lecithin–water system in terms of steric effects and hydration water is confirmed by the results obtained in the lecithin–cholesterol–water system. The latter system contains half as many choline phosphate groups as the former. The steric hindrance effect is therefore smaller. Furthermore, the amount of hydration water is less and as soon as Φ_w exceeds 0.20 free water appears in the system. The rapid increase of D_{aq} for $\Phi_w > 0.20$ observed in this system is thus readily understood.

To test the importance of the hydration effect, D_{aq} was measured in lecithin–water systems at 10° and at 30° in addition to 22°. The data obtained at these different temperatures may be compared directly as the structural parameter, d_{aq}, does not change appreciably over the temperature range 5–35° (ref. 5). The results of these measurements are given in Fig. 5. D_{aq} increases with temperature, the general features of the three curves are similar. However, the range of water content over which D_{aq} remains constant decreases with increasing temperature. This is consistent with the hypothesis, as the hydration of lecithin decreases with increasing temperature.

The apparent activation energy for water diffusion in the system was estimated in the range Φ_w between 0.22 and 0.26. The mean values for D_{aq} in this region are $1.80 \cdot 10^{-6} \pm 0.1 \cdot 10^{-6}$, $3.0 \cdot 10^{-6} \pm 0.1 \cdot 10^{-6}$ and $4.3 \cdot 10^{-6} \pm 0.2 \cdot 10^{-6}$ at 10°, 22° and 30°, respectively. An Arrhenius plot of these values yielded a straight line from which an apparent activation energy of 8.0 ± 1.0 kcal/mole was obtained.

This value is significantly higher than the value of 4.8 kcal/mole obtained by Wang[8] for the activation energy of ^{3}HHO diffusion in bulk water. This difference can be interpreted as meaning that more numerous (or stronger) H bonds have to be broken by ^{3}HHO diffusing in the lipid–water phase and is therefore consonant with the supposed role of water organization in this phase.

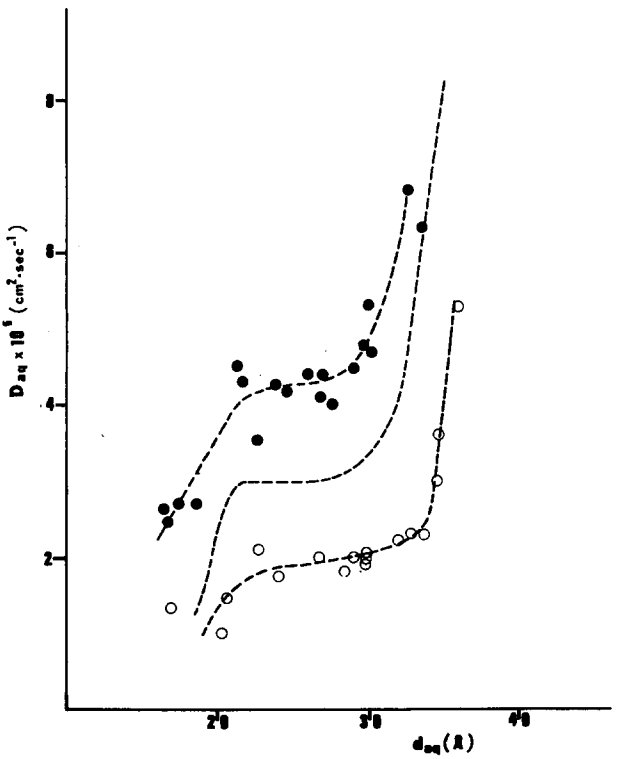

Fig. 5. D_{aq} for ^{3}HHO in lecithin–water phases as a function of d_{aq} at 10° (○) and 30° (●). The curve without experimental points are the data at 22° redrawn from Fig. 3. (See text for definition of parameters).

Diffusion of benzene

The diffusion of benzene was measured at 22° using the same method as was used to measure water diffusion. Benzene is able to diffuse in both lipidic and aqueous regions. However, the partition coefficient between these regions is largely favorable to the lipids (about 10^{-3} as estimated from the partition coefficient between oleic acid and water). It may be assumed, as it was done in the case of water for the aqueous region, that in first approximation, the diffusion measured reflects mainly the movement of benzene in the lipidic region of the phase.

Therefore, it is the ratio $D_{lipid} = D/\Phi_{lipid}$, where Φ_{lipid} is the amount of lipid in g/g of phase, which is of interest. The thickness of the lipid layer may be computed from X-ray diffraction measurements. However, the variation of this thickness with the water content, Φ_w, does not have a simple and direct significance. The thickness of the lipidic lamellae reflects changes in the state of the hydrocarbon chains which could have a more important effect on diffusion than merely changing the area avail-

able. Since the molecular organization of the lipid is diminished by the presence of water in the phase, D_{lipid} *versus* Φ_w in both lecithin–water (closed circles) and lecithin–cholesterol–water (open circles) systems is given in Fig. 6.

In lecithin–water lamellar phases, increasing water content leads to greater fluidity of the hydrocarbon chains. It is known from X-ray diffraction studies that as the amount of water in the phase is increased, the mean area per lipid molecule increases and the thickness of the lipid leaflet decreases. The hydrocarbon chains have greater freedom of movement and can coil, bend or develop "kinks". Levine and Wilkins[12] have shown from electron density measurements that increasing hydration leads to desorientation of the chains and delocalization of their ends.

Electron spin resonance studies[13,14] confirm that the mobility of the chains increases with increasing hydration. Hubbell and McConnell[15] have shown from electron spin resonance measurements that the hydrophobic region of the lipid bilayer becomes increasingly fluid in the direction of the terminal methyl group, the region near the interface remaining rigid.

As could be expected from these observations, the diffusion coefficient of benzene was found to increase with Φ_w. Two regions of sudden change may be distinguished in the curve. The first one occurs at Φ_w approx. 0.20, it is rather small and possibly artefact. The second one, at Φ_w approx. 0.30 seems more significant and could indicate the onset of a new level of fluidity. The addition of an equimolar amount of cholesterol to the lamellar phase is known to decrease the disordering effect of water. Cholesterol prevents the decrease in thickness of the lipid layer with increasing water content observed in lipid–water system up to 20–22 % water. Accordingly, it prevents the delocalization of the methyl end group of the chain. However, in phases containing more than 20–22 % water the condensing effect of cholesterol disappears and the lipid layer thickness decreases with increasing water content even more

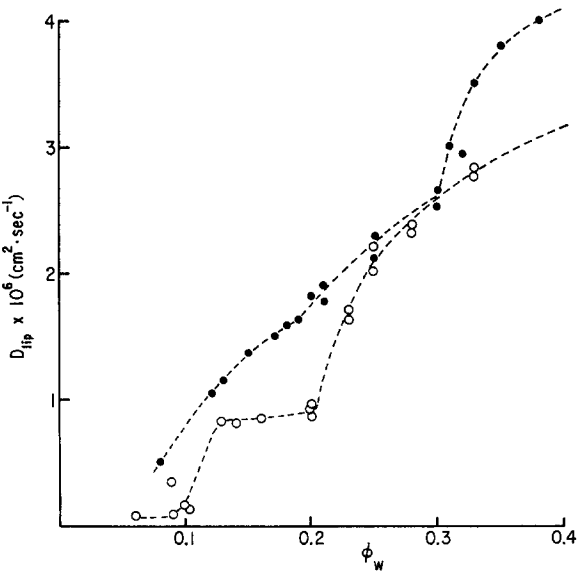

Fig. 6. D_{lip} for benzene in lecithin–water (●) and lecithin–cholesterol–water (○) phases as a function of Φ_w at 22° (see text for definition of parameters).

rapidly than is the case in pure lecithin systems[6]. However, even in the presence of a large excess of water, namely in lecithin–cholesterol vesicles dispersed in water, Hubbell and McConnell[15] showed that the hydrocarbon chains in the region extending about 8 carbon atoms from the polar head may be considered as relatively rigid rods.

It was found (see open circles in Fig. 6) that the diffusion coefficient of benzene in the lecithin–cholesterol system was always smaller than in the pure lecithin system. This is a reflection of the condensing effect of cholesterol. The curve exhibits a plateau in the range Φ_w between 0.1 and 0.2 where the cholesterol is able to maintain a condensed state in spite of the increasing water content.

It may be concluded that the results of the present diffusion measurements carried out in both systems are consistent with the structural data obtained by other authors using spectroscopic techniques.

CONCLUSION

The diffusion measurements in lipid–water systems described here yield information which is very different from that obtained from similar studies on bilayers isolated between two water phases. For example, the diffusion of benzene could be determined by different factors in the two cases. In lipid–water lamellar systems benzene diffuses transversely along a lipidic leaflet whereas in bilayers it diffuses perpendicularly to the plane of the bilayer, along the axis of the hydrocarbon chains. In view of this difference, a theory such as Träuble's[16] theory for diffusion through bilayer hydrocarbons by "kink" formation could require modification to be applicable. Moreover, since the state of hydrocarbon chains in lipid-water systems varies with the water content, the bilayer isolated in bulk water can be considered only as a limiting case. The study of diffusion processes in the lipid region of lipid–water systems could yield additional information about the state of the lipids, complementary to that obtained by spectroscopic methods.

One of the most striking features of water diffusion in lamellar phases is the dramatic increase of the diffusion coefficient above a certain water content. As can be seen from Fig. 3, an increase in water content of only several percent results in an increase in diffusion coefficient of 300 or 400 %. This phenomenon could provide a means for very sensitive regulation of the permeability of natural membranes by control of their water content.

More generally lipid–water lamellar phases offer the possibility of studying diffusion processes of solutes in water organized near a polar surface in a restricted region. Information about such processes is of primary importance from a biological viewpoint. There is considerable evidence that water and small hydrophilic solutes cross biological membranes through hydrophilic pathways which are sensitive to molecuar size and polarity[17]. The lipid–water phases discussed here provide a valuable system of known structure which will permit an assessment of the factors governing transfer of various solutes in hydrophilic channels a few angstroms wide.

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