Lateral Diffusion in Spin-Labeled Phosphatidylcholine Multilayers[1a]

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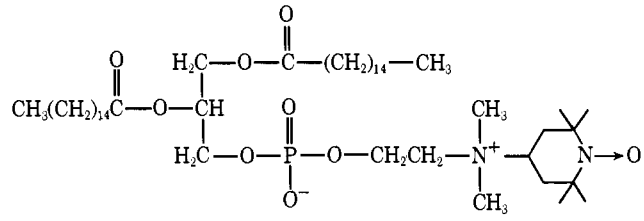
Abstract: The paramagnetic resonance spectrum of a highly concentrated region of spin-labeled phosphatidylcholine included in oriented bilayers of phosphatidylcholine (PC) changes dramatically in time. This time dependence of the spectra is due to the lateral diffusion of the oriented labeled molecules spreading in the planes of the corresponding monolayers. The resonance spectra can be analyzed in terms of a time-dependent superposition of spectra corresponding to the different concentrations of spin label. It is possible to estimate the diffusion constant: $D \simeq 1.8 \pm 0.6 \times 10^{-8}$ cm²/sec at room temperature (25°). If lateral diffusion is assumed to be due to pairwise exchange of neighboring molecules, then this diffusion coefficient corresponds to an exchange frequency which is of the order of 10^7 sec^{-1}. This rate is high enough to suggest that the lateral translation of molecules bound to membranes may sometimes have biological significance.

The membrane of a biological cell is involved in many essential processes that require molecular *motion*. For example, the active transport of ions and molecules through membranes doubtless requires the molecular motion of membrane components. There is much current interest in the question of the rate of *lateral diffusion* of molecules in membranes. This question arises in connection with membrane biosynthesis and, as discussed later, the possible lateral diffusion of "messenger" molecules in membranes. Since virtually all biological membranes contain lipids and many biological membranes are thought to contain lipid bilayers, we have carried out a study of the rate of lateral diffusion of a spin-labeled lipid in a phospholipid bilayer system.

It is very difficult to make an *a priori* estimate of the rate of lateral diffusion of lipids in phospholipid bilayers. Much evidence indicates that in, for example, egg lecithin bilayers the hydrocarbon chains are in a highly "fluid" state. That is, chain isomerizations take place rapidly ($\gtrsim 10^7$ sec^{-1}) and with relatively high probability.[2] However, this high degree of molecular motion is most pronounced toward the terminal methyl groups of the fatty acid chains,[3,4] whereas near the polar head groups the hydrocarbon chains appear to be relatively more rigid and tightly packed. The rate of inside–outside transitions of spin-labeled phospholipids through bilayers is remarkably slow,[5] of the order of $\sim$(24 hr)$^{-1}$. Thus, the bilayer appears to have the combined properties of a fluid and a rigid structure. This makes it difficult to give any plausible estimate of how rapid lateral diffusion might be. The first attempt to measure the rate of lateral diffusion in phospholipid bilayers was made by Kornberg and McConnell.[6] These investigators studied the *N*-methyl proton line broadening in phosphatidylcholine vesicles brought about by low concentrations of the spin label PC I.



PC I

It was concluded that the rate of the elementary step for diffusion is greater than 3×10^3 sec^{-1}.

In the present work a rather direct approach has been used to estimate the rate of lateral diffusion of phospholipids. It is known that, when certain phospholipids are hydrated and then squeezed between two flat surfaces, a highly ordered arrangement of parallel planar phospholipid bilayers is obtained.[2-4] These are briefly referred to as "multilayers." A large number of studies have been carried out on the resonance spectra of various spin labels included in low concentration in such multilayers.[2-4] When the concentration of spin label PC I in multilayers of lecithin is increased above a mole fraction of $\sim$0.05 it is found that the paramagnetic resonance line shapes are markedly changed, due to spin–spin interactions between unpaired electrons on different labels. One can then establish an *empirical* relationship between line shape and label concentration. In the present method, a multilayer sample is prepared so as to contain a small region of concentrated PC I. These labeled molecules then diffuse laterally, giving rise to time-dependent paramagnetic resonance spectra which can be analyzed to yield the diffusion constant *D*. By this method we have found that *D* is $\sim 1.8 \pm 0.6 \times 10^{-8}$ cm²/sec. The experiment is illustrated schematically in Figure 1.

Some of the experiments described in the present work were carried out using egg lecithin as the host lipid, but most experiments were carried out using didihydrosterculoyl phosphatidylcholine (DPC) as the

(1) (a) Supported by the National Science Foundation under Grant No. GP 26456. (b) NATO Postdoctoral Fellow, on leave from Laboratoire de Physique des Solides, Faculté des Science de Paris. This work has benefited from facilities made available by the Advanced Research Projects Agency through the Center for Materials Research at Stanford University.

(2) For a discussion of this subject and references to earlier work, see W. L. Hubbell and H. M. McConnell, *J. Amer. Chem. Soc.*, **93**, 314 (1971).

(3) B. G. McFarland and H. M. McConnell, *Proc. Nat. Acad. Sci. U. S.*, **68**, 1274 (1971).

(4) P. Jost, L. J. Libertini, V. G. Hebert, and O. H. Griffith, *J. Mol. Biol.*, **59**, 77 (1971). See also J. Seelig, *J. Amer. Chem. Soc.*, **92**, 3881 (1970). This increase in molecular motion toward the terminal methyl groups applies also to intact biological membranes. See W. L. Hubbell and H. M. McConnell, *Proc. Nat. Acad. Sci. U. S.*, **64**, 20 (1969).

(5) R. D. Kornberg and H. M. McConnell, *Biochemistry*, **10**, 1111 (1971).

(6) R. D. Kornberg and H. M. McConnell, *Proc. Nat. Acad. Sci. U. S.*, **68**, 2564 (1971).

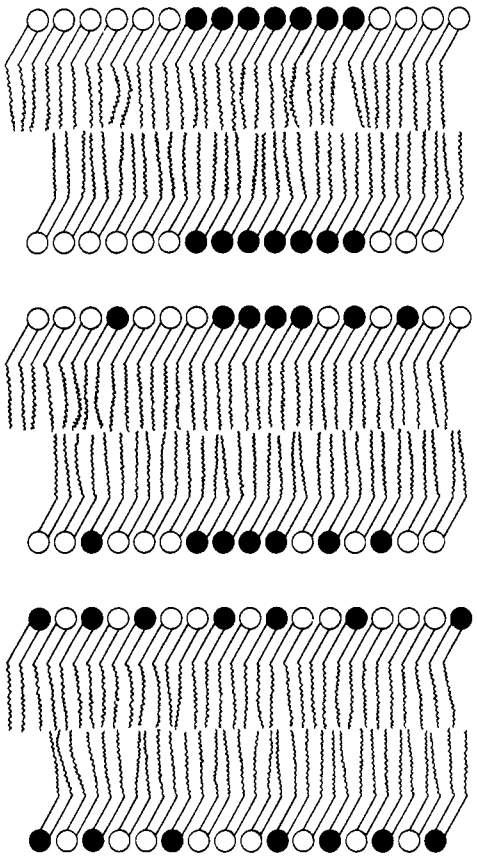


Figure 1. Schematic model of spin-labeled lipids (black polar head groups) diffusing in bilayer planes of the lipid didihydrosterculoyl phosphatidylcholine (white polar head groups).

host lipid. The phospholipid DPC has physical properties very similar to egg lecithin and has the advantage of not undergoing (free radical) oxidation as does egg lecithin.[6] This oxidation gradually changes the physical properties of the lecithin and also reduces the spin label concentration due to free-radical reactions. It was important to avoid these reactions in the present work since resonance line shapes depend on concentration in the concentration range of interest.

Not all pure phospholipids form well-ordered planar multilayers. (An example is phosphatidylserine.) Thus, an important requirement for PC I in the present work is that pure hydrated PC I form well-ordered multilayers. The visual appearance as well as the anisotropy of the resonance spectra of pure hydrated PC I between quartz plates provides evidence that PC I does form well-ordered multilayers.

Sackmann and Traüble have recently estimated the lateral diffusion coefficient of a spin-label derivative of androstane in small particles containing monolayers of dipalmitoylphosphatidylcholine.[7] Their method involves a theoretical analysis of resonance line shapes in terms of electron spin–spin interaction and the rate of lateral diffusion. Their line-shape analysis *is fundamentally different from that used in the present work.* For example, their spectra are time independent. They deduce a lateral diffusion coefficient D for the steroid label equal to $\sim 10^{-8}$ cm^2/sec, which is the same order of magnitude of the lateral diffusion coefficient for PC I determined in the present work.

(7) H. Traüble and E. Sackmann, *J. Amer. Chem. Soc.*, in press.

Experimental Section

Egg lecithin was isolated and purified from egg yolk by the method of Singleton, *et al.*[8]

Didihydrosterculoyl PC and PC I were kindly supplied by R. D. Kornberg; their preparation is described elsewhere.[5,6]

Hydration of egg lecithin, DPC, and PC I was carried out by leaving the dry samples overnight in an atmosphere of water-saturated argon. In some experiments a calibrated amount of water was introduced corresponding to 30% by weight of water. There were no apparent differences between the two methods of hydrating the films. This control could not be made with the pure spin-labeled PC I because of the small amount of material used. The hydration of PC I could be checked by the anisotropy of the resonance spectra of samples pressed between two plates.

Preparation of the Samples. Procedure 1. An 11 mM solution (1 or 2) μl of spin label in ethanol($\approx 10^{-2}$ mg) was deposited on an optically flat quartz plate previously cooled to approximately 0°. The ethanol was allowed to evaporate for a few minutes, leaving a *uniformly* thin spot of spin label of 1 or 2 mm in diameter. Residual ethanol was removed by placing the sample under vacuum for 1.5 hr. The initial spot was generally scratched with a razor blade in order to make an array of much smaller spots, the average size of which was checked with a microscope. The smallest diameter of spots obtained in regular arrays was ≈ 0.2 mm. The plate was then left overnight in an atmosphere of water-saturated argon. Just before the beginning of a measurement, a second quartz plate was covered with a thin layer of the previously prepared hydrated egg lecithin (or DPC) and the two plates were gently pressed together. The general orientation of the multilayers could be checked with a polarizing microscope, which clearly showed the main defects. In this procedure the actual size of the spots, once covered with lecithin, could only be estimated from their initial diameter. The shape of the first resonance spectrum indicated if the lecithin and spin-labeled lecithin were inadvertently mixed too much.

Procedure 2. About 2 μl of a more concentrated solution of spin label ($\sim$66 mM) was deposited on a plate and the ethanol removed under vacuum. Approximately 2 μl of distilled water was added with a micropipet and the suspension of spin-labeled lecithin in an excess of water was concentrated with a spatula into a single round spot. Water was removed under vacuum and the hydration carried out as usual by leaving the sample 24 hr in a water-saturated atmosphere of argon. The unlabeled lecithin was added carefully on the same plate by very closely surrounding the central patch with hydrated lecithin without covering it. Finally the mixture was pressed between two quartz plates. With this procedure the radius of the spin-label circle could be measured reasonably accurately by noting the pale orange color due to the nitroxide group of the spin labels concentrated in one region. The smallest diameter obtained was 0.8 mm.

The two quartz plates were held in a Teflon cell and the spectra recorded on a Varian E-12 spectrometer at 9.35 GHz, at room temperature (25°).

After each measurement the samples were kept in a humid atmosphere. Without this precaution the samples began to dry and show characteristic strongly immobilized nitroxide spectra during periods of the order of a few hours.

Results

Figure 2 gives a typical set of spectra for two different orientations of the magnetic field (sample prepared according to procedure 2). The vertical line drawn on these fixed microwave frequency spectra indicates the same reference field (3345 G) for both orientations. The shift of the spectra and the differences of the splittings clearly demonstrate that the molecules have a preferential anisotropic orientation. For a highly dilute sample the splittings are 35 G (26 G) when the magnetic field is parallel (perpendicular) to the planes of the bilayers.

An advantage of procedure 1 is that it generally leads to spectra showing the highest degree of molecular orientation. A disadvantage of procedure 1 is that some mixing of PC I and DPC (or egg lecithin)

(8) W. S. Singleton, M. S. Gray, M. L. Brown, and J. L. White, *J. Amer. Oil Chem. Soc.*, **42**, 53 (1965).

occurs during preparation of the sample, so that the appropriate values of R_0 and/or t_0 are accordingly uncertain. In procedure 1 where experiments involve small values of R_0 (0.2–0.4 mm), the time to reach the equilibrium state is between 10 and 20 hr. This equilibrium state exhibits spectra with the same narrow lines that are shown by a uniformly dilute sample (0.01 mole fraction of PC I).

In contrast, the larger patches of spin label made according to procedure 2, with an initial radius of up to 0.8 mm, generally start spreading as expected theoretically but frequently slow down their expansion after a certain time and even seem to get stuck (after 2 or 3 days). This departure from ideal behavior is clearly demonstrated when plotting a typical feature of the spectra *vs.* time and comparing with the theoretical expectation (see later discussion). In order to obtain a dilute solution of spin labels by diffusion from an initially concentrated spot, diffusion must occur over a large area relative to the initial area. In fact, the increase in area must be more than a factor of the order 20. For the large spots used in procedure 2, the required increase in diameter of the spots is of the order of magnitude of the average distance between defects detected by the polarizing microscope in the multilayer system. These defects may be air bubbles and/or folding of the bilayers. Whatever these defects are, they do not appear to limit seriously lateral diffusion from an initial spot within times of the order of 24–48 hr.

Simple considerations enable one to obtain a crude estimate of the diffusion constant D, using spectra such as those in Figure 2. For instance, when the mole fraction of spin label is about 0.1 or less, the *main* features of the infinitely dilute resonance spectra are seen. Thus, if these main features are present at time t after the start of an experiment with a spot having a radius R_0, we can say that the average molecule must have diffused a distance of the order of $2R_0$. Thus $(2R_0)^2 \simeq 4Dt$. The data in Figure 2 show that for an initial spot with radius $R_0 \simeq 0.8$ mm, the main features of the dilute resonance spectra are seen after 40–50 hr. This leads to an estimate of D which is of the order of magnitude of 10^{-8} cm^2/sec.

The same kind of experiments were performed with equimolar mixtures of cholesterol–egg lecithin and cholesterol–PC I. Although the shape of the spectra are slightly modified by the cholesterol molecules, it appears that the time to reach the same stage of evolution with a given R_0 has increased by a factor somewhere between 1.5 and 2.0. Consequently, the difference, while not striking, seems nevertheless to indicate a decrease of D. This is what one would expect from the previous studies of the influence of the cholesterol molecules on molecular motion in lecithin bilayers.[2-4]

Quantitative Analysis

The following analysis was used to determine the lateral diffusion coefficient for phospholipids in bilayers. Our starting point is an infinite two-dimensional plane (monolayer) of phospholipid molecules such that at time $t = 0$ all the spin-labeled molecules are present at a uniform concentration within a circle of radius R_0. The lateral diffusion of these molecules away from the center of the circle is described by the

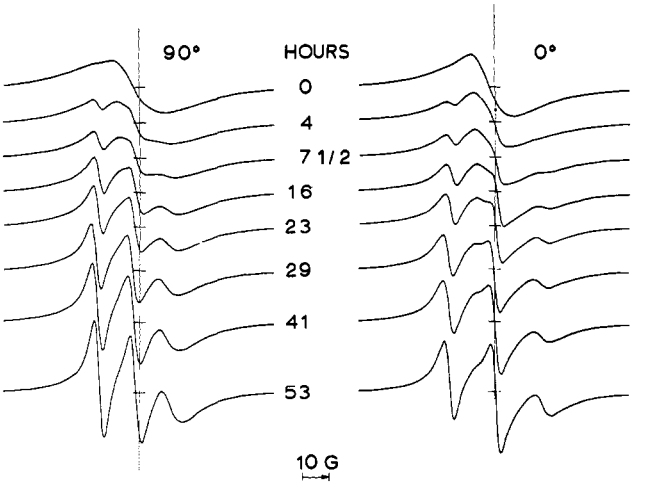


Figure 2. Observed paramagnetic resonance spectra of spin label PC I diffusing in bilayers of the host lipid DPC. Spectra were taken at different times with the applied field directions perpendicular (90°) and parallel (0°) to the plane of the bilayers. $R_0 \approx 0.8$ mm. See text for definitions of PC I, DPC, and R_0.

two-dimensional differential diffusion equation

$$D\nabla^2 P(r,t) = \partial P(r,t)/\partial t \tag{1}$$

Here $P(r,t)$ gives the concentration of spin-labeled molecules at a point r away from the center of the circle, at time t. In writing eq 1 it is assumed that D is independent of P. The concentration P may be expressed conveniently as the mole fraction of spin-label phospholipid. The solution of this equation, which is discussed in elementary textbooks,[9] can be written in the following form.

$$P(X,u) = \sum_{\nu=0}^{\infty}\left\{\sum_{\mu=0}^{\mu=\nu}(-1)^{\nu}\frac{\nu!}{((\nu-\mu)!)^2(\mu!)^2}\times\left(\frac{1}{u}\right)^{2(\nu+1)}X^{2\mu}\frac{1}{2^{\nu}(2(\nu-\mu)+2)}\right\} \tag{2}$$

Here the dimensionless parameters u and X are

$$u = \sqrt{2Dt}/R_0 \tag{3}$$

$$X = r/R_0 \tag{4}$$

The observed spectra can be related to the solution of the diffusion equation, as follows. Let $F(X,u)\mathrm{d}X$ be the fraction of the spin labels that lie between "radius" X and $X + \mathrm{d}X$ in the plane of diffusion.

$$F(X,u)\mathrm{d}X = 2XP(X,u)\mathrm{d}X \tag{5}$$

At one given time, or a given u, P and X are single valued functions of one another. Thus, eq 5 can also be expressed in terms of the variable P.

$$F(P,u)\mathrm{d}P = 2X(P,u)P(\mathrm{d}X/\mathrm{d}P)\mathrm{d}P \tag{6}$$

Here $F(P,u)\mathrm{d}P$ is the fraction of all the spin-label molecules that have a concentration between P and $P + \mathrm{d}P$ for a given u. If $s(P,H)$ is the (normalized) resonance absorption corresponding to a concentration P of spin label, for an applied field H, the shape of the

(9) H. Margenau and G. M. Murphy, "The Mathematics of Physics and Chemistry," Van Nostrand, New York, N. Y., 1943, p 109 and pp 235–236.

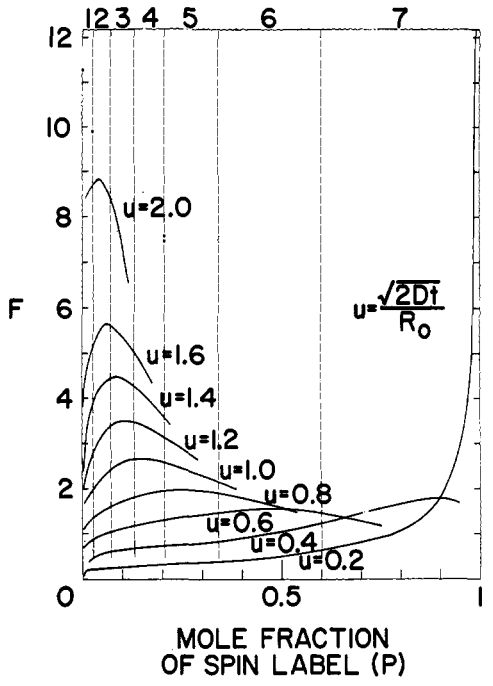


Figure 3. Plot of the distribution functions $F(P,u)$ as a function of the concentration (mole fraction) of PC I in DPC for selected values of the time parameter u. The vertical lines delineate the seven concentration domains discussed in the text.

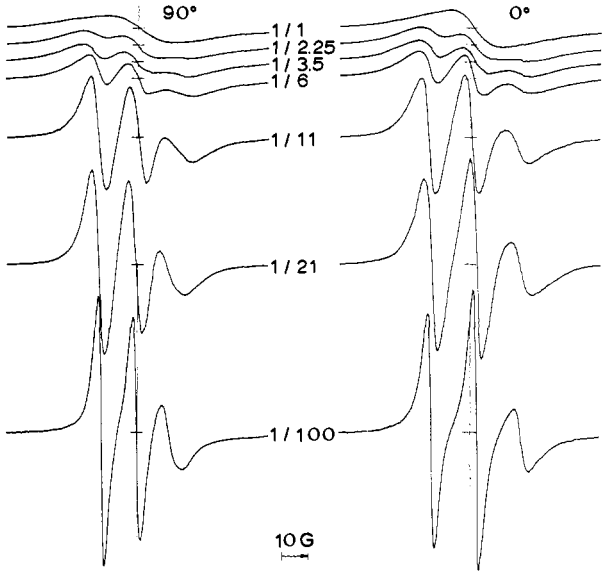


Figure 4. Normalized reference spectra corresponding to different homogeneous mixtures of PC I and DPC. The applied field is perpendicular (90°) to the planes of the bilayers in one set of spectra and is parallel (0°) in the other.

resonance spectrum for a diffusing mixture is then

$$S(u,H) = \int_0^1 F(P,u)s(P,H)\mathrm{d}P \qquad (7)$$

Figure 3 gives $F(P,u)$ as a function of P for selected values of u. The areas under the curves are constant, in accordance with the condition

$$\int_0^1 F(P,u)\mathrm{d}P = 1 \qquad (8)$$

As discussed above, when $u = 2$ most of the spin-label molecules are in dilute regions where $P \lesssim 0.1$.

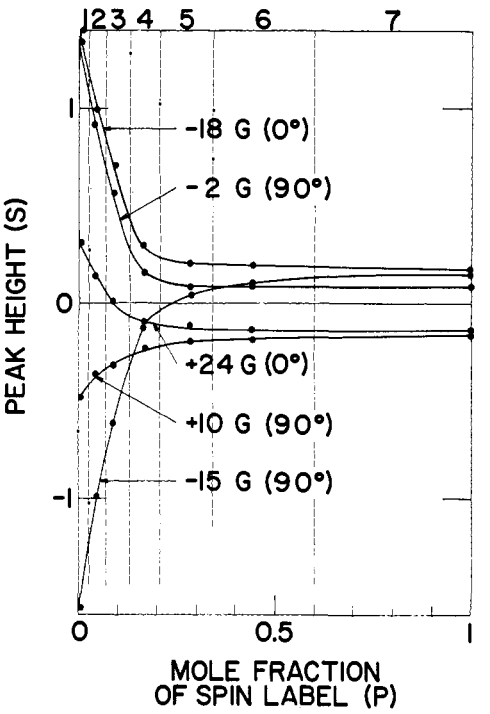


Figure 5. Paramagnetic resonance amplitudes (peak heights) for various values of the applied field (relative to 3345 G) and two orientations as a function of the concentration P. The limits of the seven concentration domains are shown.

In order to use eq 7 in the present analysis, we have recorded a set of reference spectra corresponding to different homogeneous mixtures of PC I in either egg lecithin or in DPC multilayers for two different orientations of the applied field. The spectra in the two host lipids were identical except for changes that can be attributed to loss of label by reaction with oxidation products in the egg lecithin. For this reason we only report spectra in DPC, as illustrated in Figure 4. All spectra were normalized with a computer in order to obtain the functions $s(P,H)$ used in eq 7. Since the analysis requires the quantitative summation of experimental spectra, it was necessary to record spectra at exactly the same cavity frequency. Seven reference spectra were used, corresponding to concentrations P_i, $i = 1$–7, where $P_1 = 1$, $P_2 = 0.44$, $P_3 = 0.29$, $P_4 = 0.091$, $P_6 = 0.048$, $P_7 = 0.01$. The whole range of concentrations $0 \leq P \leq 1$ was then divided in a rather arbitrary way into a limited number of domains such that each included a reference spectrum concentration and such that the resonance signal for a given field could be considered to be reasonably constant within the domain. Equation 7 was then approximated by the following expression.

$$S(u,H) \simeq \sum_{i=1}^{7} \bar{F}_i(u)s(P_i,H) \qquad (9)$$

Here $\bar{F}_i(u)$ is the integral of $F(P,u)$ over the chosen domain of P that includes P_i, and $s(P_i,H)$ is the ith reference spectrum. These domains are indicated in Figures 3 and 5. Illustrative values of $s(P_i,H)$ are given in Figure 5. The addition of the spectra was carried out using a computer and the results for selected values of u are illustrated in Figure 6.

The comparison between the experimental and calculated spectra in Figures 2 and 6 is satisfactory from

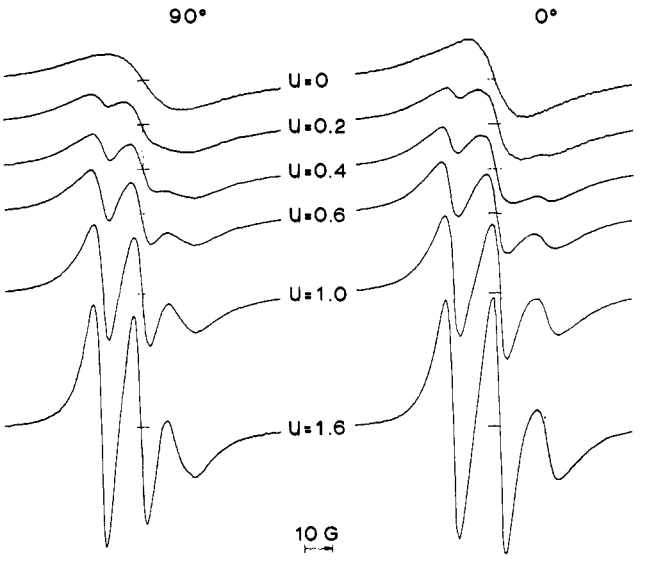


Figure 6. Calculated spectra obtained by appropriate addition of reference spectra.

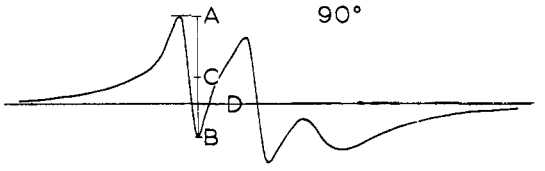


Figure 7. The spectral parameter α is defined by the ratio of the amplitudes, $\alpha = {}^1/_2(AB)/(CD)$. See text.

the point of view of the overall shape and time evolution of the spectra. The deviations between the two spectra can probably be attributed to imperfections in the initial state of the label spots such as deviations from circular shape, misorientation, some initial mixing, and different values of R_0 in the different layers. (Each multilayer consisted of about 300 layers of bilayers.) It is not believed that the use of more calibrated spectra would increase the agreement.

The following procedure was used to extract the best value of D from the experimental and calculated spectra. The empirical parameter α can be used to characterize each spectrum

$$\alpha = {}^1/_2(AB)/(CD) \qquad (10)$$

Here (AB) is the vertical distance (amplitude) between points A and B on a resonance spectrum, as illustrated in Figure 7. The point C is the midpoint of the vertical line AB, and D is on the base line. The quantity (CD) is the vertical distance (amplitude) between C and D. From the observed as well as calculated spectra it can be seen that $\alpha = 0$ when $u = 0$ and $\alpha \to \infty$ when $u \to \infty$. Since α only depends on the *shape* of the resonance spectrum, its use does not require normalization of the spectra.

By measuring α for ten different values of u on the calculated spectra, a smooth curve of α as a function of $u^2 = 2Dt/R_0^2$ was established (broken line in Figure 8). The experimental spectra give values of α rather well distributed on this curve. The points on Figure 8 represent experimental data.

Since R_0 is known experimentally, D can be chosen so that the experimental and calculated α *vs.* u^2 curves

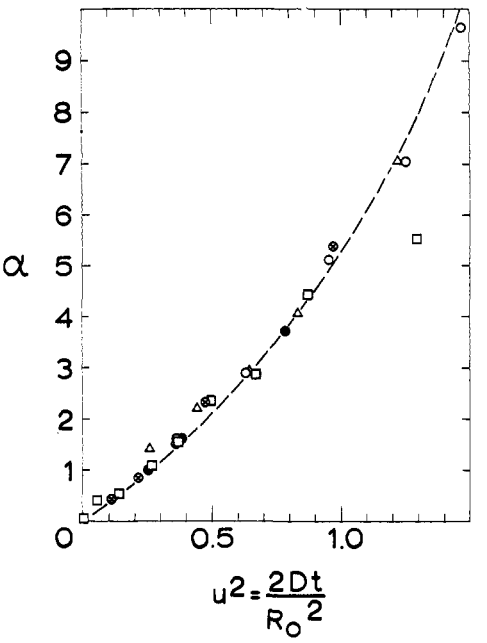


Figure 8. Comparison of calculated and experimental values of the spectral parameter α as a function of $u^2 = 2Dt/R_0^2$. Data refer to five different experiments (□, ○, ⊗, ●, △) with different values of R_0.

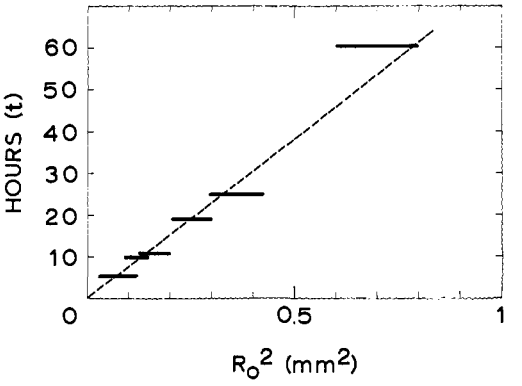


Figure 9. The time t required for a spectrum to develop to the condition $u = 1$, as a function of R_0^2.

match one another. The results then lead to a lateral diffusion coefficient

$$D = (1.8 \pm 0.6) \times 10^{-8}\ \text{cm}^2/\text{sec} \qquad (11)$$

We believe that most of the scatter in the fit of the experimental points to the calculated curve is due to errors in R_0. Errors in R_0 of the order of 20% correspond to errors in D of the order of $|\Delta D/D| \sim 2 \cdot |\Delta R_0/R_0| \sim 0.4$. This in turn corresponds to an uncertainty in D of $\sim \pm 0.7 \times 10^{-8}\ \text{cm}^2/\text{sec}$.

Values of R_0 in the range 0.2–0.8 mm lead to pronounced differences in the time required to reach a dilute spectrum. Even so, one expects from the theory used in the present work that the spectral parameter α is a unique function of u. Thus, a plot of the *time* required for a spectrum to develop to the condition $u = 1$ as a function of R_0^2 should be a straight line. This is found to be true, to within the experimental error, as shown in Figure 9.

Discussion

Physical Significance. The present work shows that the lateral diffusion coefficient D for spin label PC I in DPC multilayers is $(1.8 \pm 0.6) \times 10^{-8}$ cm^2/sec. This result is in excellent order-of-magnitude agreement with the lateral diffusion coefficient of a spin-labeled steroid in dipalmitoylphosphatidylcholine monolayers determined by Sackmann and Traüble, by an entirely different method of analysis.[7]

It is of interest to discuss our diffusion coefficient in terms of a two-dimensional lattice of PC phospholipids. For the sake of discussion, we assume that this lattice is hexagonal. For two-dimension diffusion

$$\overline{x^2} + \overline{y^2} = 4Dt \qquad (12)$$

where $\overline{x^2}$ and $\overline{y^2}$ are the mean square displacements in the x and y directions. If τ^{-1} is the probability per unit time that a lipid molecule jumps from one site to any one of the six neighboring sites, then

$$\tau = a^2/4D \qquad (13)$$

In DPC bilayers (at full hydration), the area per lipid molecule, $\sqrt{3}/2a^2$, can be set equal to 70 Å^2.[10] Thus the jumping rate τ^{-1} is of the order of magnitude of 10^7 sec^{-1}.

In considering the above jumping rate, it is known that the hydrocarbon chain isomerization takes place at this rate, or faster,[2] Also a number of amphiphilic labels undergo rotational diffusion about their long axes in PC bilayers at rates that are of this order of magnitude.[2] Thus, the above rate is consistent with what is known about the rates of other *in-plane* molecular motions in bilayers.

Biological Significance Since many biological membranes are thought to contain phospholipid bilayers, it is of interest to compare the lipid diffusion coefficient determined in the present work with what is known about lateral motion in intact biological membranes. Frye and Edidin have used Sendai virus to induce fusion of cells from tissue culture lines of human cells and mouse cells.[11] The heterokaryons produced in this way have both mouse and human surface antigens whose distribution over the fused cell surface can be followed by means of a fluorescent antibody method. It was found that within 40 min following fusion there was essentially complete mixing of both types of antigens in over 90% of the heterokaryon surface. Evidence was also obtained that this intermixing is due to physical diffusion and does not require short term cellular ATP production or protein synthesis. The distance over which these surface antigens must diffuse in ~20 min is of the order of 10 μm, leading to a diffusion coefficient of the order of 10^{-10} cm^2/sec at 37°. The surface antigens are thought to be protein or glycoprotein.[11] One may question the point of any semiquantitative comparison between the diffusion of a membrane glycoprotein and a spin-label phospholipid. However, according to the Stokes–Einstein equation, diffusion coefficients depend rather weakly on molecular size, being inversely proportional to the radius in the case of a sphere. (By contrast, rotational diffusion of a sphere depends on the cube of the radius.) Moreover, the surface antigen may well be "anchored" to a membrane lipid, in which case one could expect a close quantitative similarity.

The above results indicate the possibility of relatively rapid lateral diffusional motions of components of biological membranes. This picture is consistent with a number of other studies[2] that indicate rapid anisotropic rotational motions of the lipid components of biological membranes. Also, for example, low-angle X-ray scattering studies by Blasie and Worthington have suggested that molecules of the protein rhodopsin have a liquid-like in-plane distribution in photoreceptor membranes.[12]

Although the above discussion indicates the possibility of relatively rapid lateral motions of membrane components in some biological membranes, this conclusion certainly need not apply to all membranes, nor to all parts of any one membrane. In fact, if the diffusion observed by Frye and Edidin were due to the effect of a virus-induced lesion in a membrane component (*e.g.*, viral neuriminidase activity), then there would be no direct evidence for lateral diffusion in any "normal" biological membrane. Indeed in certain cases there is evidence against lateral diffusion of membrane components. For example, Morrison and Morowitz have carried out pulse-label autoradiographic studies of exponentially growing cells of *Bacillus megaterium* and have observed highly nonuniform distributions of radioactivity.[13] The cells were pulse labeled with radioactive palmitic acid, and it was demonstrated that the incorporated label was primarily in the membrane phospholipid. This result is clearly inconsistent with a lateral diffusion constant of the order of 10^{-8} cm^2/sec, since in this case it would take only a time of the order of 1 sec for lipids to distribute uniformly throughout the cell membranes.

A second example of a membrane where there is very probably little or no lateral diffusion is the purple membrane of the halophilic bacterium *Halobacterium halobium*. This purple membrane develops in patches that are part of the bacterial membrane. The patches are only found in stationary phase and can be separated from the remainder of this membrane.[14] Spin labels that bind to this membrane are "strongly immobilized" and show none of the "fluidity" so characteristic of many other biological membranes.[15] Moreover, X-ray diffraction studies of this purple membrane show very sharp reflections characteristic of a crystal-like hexagonal lattice.[16] Under these circumstances it is hard to imagine any significant lateral diffusion. Since the protein of the purple membrane is thought to be closely related to the cone pigment iodopsin, it is of interest to contrast this purple membrane with the frog photoreceptor membrane, which is highly fluid as judged both by low angle X-ray scattering[12] as well as the resonance spectra of spin labels.[17]

In an interesting theoretical paper, Adam and Del-

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(14) D. Oesterhelt and W. D. Stoeckenius, *Nature (London)*, **233**, 149 (1971).
(15) W. L. Hubbell and W. L. Stoeckenius, private communication.
(16) A. E. Blaurock and W. D. Stoeckenius, *Nature (London)*, **233**, 152 (1971).
(17) W. L. Hubbell, private communication.

brück have suggested that certain "messenger" molecules (*e.g.*, hormones or pheromones) may reach their targets (receptors) in cells by two-dimensional diffusion along membranes, rather than by a less efficient three-dimensional diffusion.[18] For receptors whose sizes are of the order of magnitude of the active sites of enzymes, it is found that the two-dimensional surface diffusion is the most efficient if the ratio of the coefficients of diffusion $D^{(2)}/D^{(3)}$ in two and three dimensions is not too small. For example, if the source–receptor distance is 1 μm, then the surface diffusion is more efficient if $D^{(2)}/D^{(3)} > 10^{-2}$. If the source–receptor distance is 10 μ, then the surface diffusion is more efficient if $D^{(2)}/D^{(3)} > 10^{-3}$. From the present study, and that of Sackmann and Traüble,[7] it is clear that a specialized membrane having a diffusion coefficient for steroids, lipids, or other small molecules similar to that of a PC bilayer could easily satisfy these conditions for enhanced two-dimensional diffusion.

In another interesting theoretical paper, Crick has pointed out that an important condition for chemical gradients to be involved in embryological development is that the diffusion be fast enough.[19] (For example, diffusion must be fast enough to cover distances of the order of 50–100 cell diameters in times of a few hours.) The chemical messenger might be water soluble (*e.g.*, cyclic AMP) or might bind to the membrane (as a steroid could). In the latter case lateral diffusion of small molecules in the surface of membranes having properties similar to the PC bilayers considered here might be involved in embryogenesis.

The above theoretical papers are of course highly speculative, and it will be of considerable interest to learn what the rates of lateral diffusion of the components of intact biological membranes are.[20]

Acknowledgments. We are greatly indebted to Dr. Roger Kornberg for the samples of didihydrosterculoyl phosphatidylcholine and spin-labeled phosphatidylcholine used in this work, along with many helpful discussions. It is also a pleasure to acknowledge that Dr. Betty McFarland suggested this use of multilayers to measure lateral diffusion. We thank Carol McNamee for some of the computer programs used in this work. Computer support for this research was obtained from ACME. ACME is supported by the NIH under Grant No. RR00311.

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(19) F. Crick, *Nature (London)*, **225**, 420 (1970).

(20) NOTE ADDED IN PROOF. The lateral diffusion constant of a spin-labeled phospholipid in sarcoplasmic reticulum from rabbit skeletal muscle has been found to be equal to 6×10^{-8} cm^2/sec at the physiological temperature, 37°: C. J. Scandella, P. Devaux, and H. M. McConnell, submitted for publication.