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Lateral Diffusion of Phospholipids in a Vesicle Membrane

(spin-labeled phosphatidylcholine/nuclear resonance)

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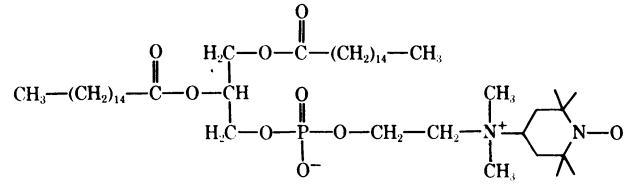
ABSTRACT **Nuclear-resonance spectra of phosphatidylcholine (PC) vesicles are broadened by spin-labeled PC. The broadening of the *N*-methyl proton line is proportional to spin-labeled PC concentration; the broadening by 1 molecule in 100 of spin-labeled PC is 9.7 Hz at 35°C; the broadening is not due to collisions between vesicles, exchange of spin label between vesicles, or fusion of vesicles. These findings are proof of rapid diffusion in the plane of the PC bilayer (lateral diffusion). A further study of the broadening reveals the molecular frequency of the translation step for lateral diffusion to be much greater than 3×10^3 sec^{-1} at 0°C.**

Two kinds of self-diffusion are possible for phospholipids in a bilayer structure: phospholipid molecules may pass from one monolayer to the other (flip-flop) and they may move about within each monolayer alone (lateral diffusion). We have found (1) that phosphatidylcholine (PC) flip-flop in a bilayer free of oxidized lipids is less frequent than 2×10^{-5}/sec at 30°C. We now report that the translation step for lateral diffusion in PC bilayers is at least eight orders of magnitude more frequent.

MATERIALS AND METHODS

Didihydrosterculoyl PC. Dihydrosterculic acid is a derivative of oleic acid in which a cyclopropane ring replaces the double bond; it was prepared as follows (2, 3). Anhydrous diethyl ether (120 ml) was added to a three-necked 500-ml flask containing 51.8 g of Zn dust and 78.3 g of CuCl. The mixture was refluxed with mechanical stirring for 30 min under dry nitrogen. Methyl oleate (30 g) and then CH_2I_2 (27 ml) were introduced and the mixture was stirred at reflux for 90 min more. The mixture was allowed to cool, filtered by gravity, and centrifuged. The supernatant was diluted with diethyl ether to a total volume of 300 ml, extracted twice with 300 ml of 5% (w/v) HCl, twice with 300 ml of water, and twice with 300 ml of 5% (w/v) sodium sulfite, then dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to a clear yellow oil (23 g). Part of the oil (20 g) was dissolved in 200 ml of toluene, applied to a column of 500 g of Silica Gel (Grace) in toluene, eluted with toluene, concentrated under reduced pressure to a clear colorless oil (18 g), and dissolved in 75 ml of absolute ethanol containing 4.3 g of KOH. The mixture was heated at reflux for 5 hr, allowed to cool, and diluted with water to a total volume of 500 ml. The pH of the solution was adjusted to 1.5 by dropwise addition of concentrated HCl with magnetic stirring. A white precipitate was collected by suction filtration, dissolved in absolute ethanol, concentrated under reduced pressure, dissolved in toluene, and concentrated again under reduced pressure. The residual oil was dissolved in 150 ml of pentane and filtered by gravity. White crystals (11 g) of dihydrosterculic acid were collected from the filtrate at −20°C: mp(uncorr) 39–41°C; IR(KBr) band at 1020 cm^{-1} (cyclopropane ring), identical with published spectra (4) in all respects.

Anal. Calcd. for $C_{19}H_{36}O_2$: C, 76.97; H, 12.24. Found: C, 76.96; H. 12.18.



spin-labeled phosphatidylcholine

Glycerylphosphorylcholine from egg PC was acylated with dihydrosterculic anhydride in the presence of sodium dihydrosterculate (5).

Miscellaneous. The preparations of spin-labeled PC and egg PC, and the preparation of PC vesicles were as previously described (1) with three exceptions: the egg PC was further purified by chromatography on SilicAR CC-4, 100-200 mesh (Mallinckrodt) in mixtures of $CHCl_3$ and CH_3OH; the buffered salt solution for PC vesicle preparation was 0.1 M NaCl–0.05 M Na Borate in D_2O, pD 8.0 [pH meter reading +0.4 (6)] in all cases; the concentrations of phospholipid phosphorus in PC vesicle preparations were 25 or 50 mM in all cases. Spin label concentrations in the various spin-labeled didihydrosterculoyl PC vesicle preparations were determined from the amplitudes of paramagnetic resonance spectra with reference to the paramagnetic resonance spectrum of an unsonicated dispersion of spin-labeled PC and didihydrosterculoyl PC. (There was about 0.25 mol % loss of spin-label paramagnetism during sonication in all cases.) Proton resonance measurements at 60 and 100 MHz were performed on Varian A-60 and HA-100 spectrometers with variable temperature accessories and a Varian C-1024 time-averaging computer. Proton-resonance measurements at 220 MHz and phosphorus-resonance measurements at 24.3 MHz were performed by a Fourier transform method (Horwitz, A. F., W. J. Horsley, and M. P. Klein, to be published). Phosphorus determinations were as previously described (1). Egg lyso PC was prepared

Abbreviation: PC, phosphatidylcholine.

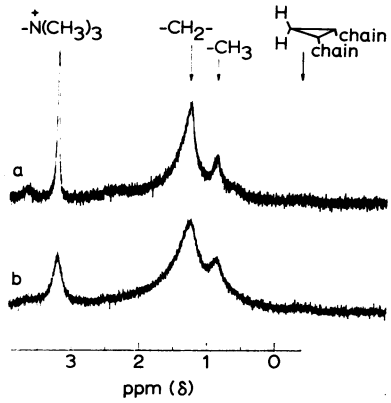


FIG. 1. Effect of spin-labeled PC on the proton resonance spectrum of PC vesicles at 100 MHz, 35°C. (*a*) Pure didihydrosterculoyl PC vesicles, relative gain 1.0. (*b*) 1.1 mol % spin-labeled didihydrosterculoyl PC vesicles, relative gain 1.4. The assignments of peaks to *N*-methyl, hydrocarbon chain methylene, hydrocarbon chain terminal methyl, and cyclopropane methylene protons (see also Fig. 4*a*), and the chemical shift of the terminal methyl-proton peak (in parts per million with respect to tetramethylsilane at zero) are based on data from the literature (10, 11).

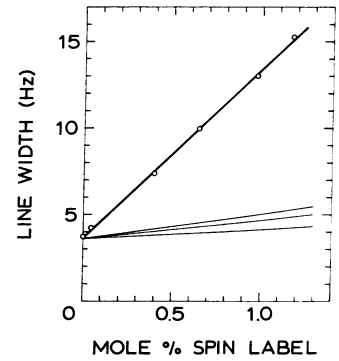


FIG. 2. Dependence of the *N*-methyl proton linewidth on the spin label content of spin-labeled PC vesicles. *Circles:* data on pure and spin-labeled didihydrosterculoyl PC vesicles at 100 MHz, 35° C. *Heavy line:* a straight line with slope to fit the data. *Thin lines:* calculated curves for the case of no diffusion and three values of the strength of the interaction between *N*-methyl protons and spin label; the upper curve is the closest possible fit of these calculations to the data; details of the calculations are given in the *Appendix*. The linewidths were measured at half-height in all cases.

from egg PC with snake venom phospholipase (*Crotalus adamanteus*, Pierce Chemical Co.).

RESULTS

Spin-Labeled PC Is Stable in Didihydrosterculoyl PC Vesicles. Throughout this work we have used the spin-labeled PC vesicles that result from prolonged sonication of PC with spin-labeled PC in salt solution. Attwood and Saunders (7) and Huang (8) have found that prolonged sonication of egg PC produces particles of 2×10^6 weight-average weight and 12.5 nm (125 Å) hydrodynamic radius. We found in previous work (1) that a 5-mol % admixture of spin-labeled PC does not alter these features of egg PC particles. We concluded from the contained volume for disaccharides and ratio of internal to total spin-labeled PC molecules that each particle is a vesicle with one bilayer membrane.

Spin-labeled PC is unstable in egg PC vesicles: the amplitude of the paramagnetic resonance spectrum[a] of egg PC vesicles containing 3.3 mol % of spin-labeled PC is diminished by 5% after 2.5 hr at 30°C and 99% after 24 hr at 40°C. Spin-labeled PC is, by contrast, indefinitely stable in didihydrosterculoyl PC vesicles: the amplitude of the paramagnetic resonance spectrum[a] of didihydrosterculoyl PC vesicles containing 3.3 mol % of spin-labeled PC does not decrease during 24 hr at 40°C. We think that the structures of the egg PC and didihydrosterculoyl PC vesicles in these experiments were similar, because the ratios of internal to total spin-labeled PC molecules were the same (the ratio of amplitudes of paramagnetic resonance spectra[a] before and after addition of sodium ascorbate was 4.0 for the egg PC vesicles and 3.9 for the didihydrosterculoyl PC vesicles). So we attribute the instability of spin-labeled PC in egg PC vesicles to occasional coupling of spin labels with free-radical intermediates in the peroxidation of unsaturated fatty acids.

We have used didihydrosterculoyl PC not only because it is resistant to peroxidation, but also because we think a didihydrosterculoyl PC vesicle must closely resemble in its physical properties the vesicles of PC from natural sources [we expect didihydrosterculoyl PC vesicles and dioleoyl PC vesicles to be very much alike, and dioleoyl PC vesicles are known to resemble egg PC vesicles in such physical properties as the temperature and cholesterol dependencies of their glycerol permeabilities (9)].

Lateral Diffusion is Rapid; Intervesicle Exchange and Fusion Are Slow. The effect of spin-labeled PC on a proton-resonance spectrum of didihydrosterculoyl PC vesicles is shown in Fig. 1. The broadening of the *N*-methyl proton line is striking. It is possible to determine from a plot of *N*-methyl proton linewidth versus mol % spin-labeled PC whether lateral diffusion occurs (case 1) or not (case 2). For case 1 and small values of the mol % spin label, the plot may be linear (13). For case 2, the plot will vary with the strength of the interaction between *N*-methyl protons and spin label; we can specify a range of possible values of the strength of the interaction, so we can specify a family of possible plots. These predictions are compared with data on six PC vesicle preparations in Fig. 2. Evidently case 1 applies.

Whatever is the mechanism of lateral diffusion, its rate can be expressed in terms of the length of time two molecules are nearest neighbors. Let τ_M be the length of time a PC molecule and spin-labeled PC molecule are nearest neighbors. The slope of the linear plot in Fig. 2 is related to τ_M by

$$S_2^H = q/[314(\tau_M + T_{2M}^H)] \quad (1)$$

where S is the slope (the subscript 2 refers to a plot of linewidth versus mol % spin label; the superscript H denotes *N*-methyl proton resonance), q is the number of PC molecules that are nearest neighbors of a spin-labeled PC, and T_{2M}^H is a linewidth parameter for the *N*-methyl proton resonance of PC molecules nearest a spin-labeled PC (13). The limiting

[a] We refer to peak-to-peak amplitudes of the low-field lines in spectra recorded at 0°C.

forms of Eq. 1 are:

slow exchange case

$$\tau_M \gg T_{2M}^H \qquad S_2^H = q/314\ \tau_M \tag{2a}$$

fast exchange case

$$T_{2M}^H \gg \tau_M \qquad S_2^H = q/314\ T_{2M}^H \tag{2b}$$

The slow and fast exchange cases can be distinguished by at least three kinds of experiments. First, S_2^H should increase with increasing temperature for slow exchange and may decrease with increasing temperature for fast exchange[b]. Second, S_1^H (the subscript *1* refers to a plot of π times the resonance lifetime T_1 versus mol % spin label) may be equal to S_2^H for slow exchange and must be different from S_2^H for fast exchange[c]. Third, S_1^H and S_2^H may be equal to S_1^P for slow exchange and should be different from S_1^P for fast exchange (the superscript *P* denotes phosphorus resonance).[c] The results of these experiments are shown in Fig. 3 and Table 1. The temperature study excludes a slow exchange case. All of the data are consistent with a fast exchange case[d]. We therefore proceed with statements **2b**, which imply $\tau_M \ll q/(314\ S_2^H)$. Taking q to be 6 and substituting the largest value of S_2^H from Fig. 3 yields $\tau_M \ll 3.4 \times 10^{-4}$ sec.

An exchange of spin-labeled PC between vesicles would give the appearance of lateral diffusion in our experiments; the observations we have attributed to lateral diffusion would be due entirely to the exchange process if exchange were rapid enough. Put differently, the duration (τ_M) of an encounter between PC and spin-labeled PC is limited by the residence time, τ_R, of spin-labeled PC in a vesicle; the observations we have attributed to lateral diffusion would be due entirely to exchange between vesicles if τ_M were equal to τ_R. We have found τ_M to be much shorter than 3.4×10^{-4} sec. We can estimate τ_R from time-dependent features of the *N*-methyl proton resonance of a mixture of spin-labeled PC vesicles and pure PC vesicles. The exchange of spin-labeled PC between vesicles leads to a broadening and diminution of peak height of the *N*-methyl proton line of the pure PC vesicles. It is easy to monitor the *N*-methyl proton line of pure PC vesicles in a mixture with 15 mol % spin-labeled vesicles because the *N*-methyl proton line of 15 mol % spin-labeled vesicles is too broad to be observed. The results of mixing equal quantities of 15 mol % spin-labeled vesicles and pure PC vesicles are shown in Table 2. It follows from the peak heights at 125 min of samples *A* and *B* that[e] $\tau_R \geq 28$ hr. So, τ_R is many orders of magnitude longer than τ_M, and we conclude that the observations we have attributed to lateral diffusion are not due in any part to exchange between vesicles.

The fusion of spin-labeled vesicles with pure PC vesicles would also cause a diminution of the peak height of sample *B* in Table 2[f]. Small amounts of lyso PC induce the fusion of various animal cells (15), so we thought the addition of 10 mol % of lyso PC might enhance the diminution of peak height of sample *B*. Instead we found no diminution at all (compare peak heights of sample *C* at 5 min and 125 min[g]). We know the lyso PC we added was taken up by the vesicles because the *N*-methyl proton line of lyso PC alone (2.5 mM; buffered salt solution and nuclear-resonance measurements as in Table 2) is 2 Hz wide and 36 units high and the *N*-methyl

[b] These are consequences of statements 2 and the following remarks. $1/\tau_M$ should increase with increasing temperature (the rates of most diffusion processes increase with increasing temperature). $1/T_{2M}$ may decrease with increasing temperature because it is proportional (see *Appendix*) to a monotone increasing function of τ_c, the electron-nuclear correlation time, which is generally a decreasing function of the temperature.

[c] $S_1^{H,P}$ is related to τ_M by an analog of Eq. 1,

$$S_1^{H,P} = q/[314(\tau_M + T_{1M}^{H,P})] \tag{i}$$

where $T_{1M}^{H,P}$ is the resonance lifetime T_1 of nuclei (*N*-methyl protons or phosphorus) nearest a spin-labeled PC (14). The limiting forms of Eq. *i* are

$$\tau_M \gg T_{1M}^{H,P} \qquad S_1^{H,P} = q/(314\ \tau_M) \tag{iia}$$

$$T_{1M}^{H,P} \gg \tau_M \qquad S_1^{H,P} = q/(314\ T_{1M}^{H,P}) \tag{iib}$$

The statements in the text all follow from statements 2 and *ii* and the relative magnitudes of T_{1M}^H, T_{1M}^P, and T_{2M}^H[$T_{1M}^H \geq 1.17 \times T_{2M}^H$; T_{1M}^H and T_{1M}^P may be comparable but they are not likely to be equal (see *Appendix*)].

[d] The data on S_1^H and S_2^H are consistent with a fast exchange case, not only because they are different, but also because they lead in fast exchange to reasonable values of τ_c, the electron-nuclear correlation time, and r, the distance of closest approach of *N*-methyl protons and unpaired electron [the data on S_1^H and S_2^H lead in fast exchange (Eqs. **2b** and *iib*) to 73 sec^{-1} and 510 sec^{-1} for $1/T_{1M}^H$ and $1/T_{2M}^H$ and hence (see *Appendix*, Eqs. **3** and **4**) to 4.7×10^{-9} sec and 8.9Å for τ_c and r].

[e] τ_R may be different for molecules on the two sides of a vesicle membrane. Consider the limiting cases of equal rates of exchange for molecules on both sides of the membrane and exchange of the molecules on one side only. In either case, the exchange process involves a population of molecules with 15 mol % of spin label at zero time (class 1) and a population with no spin label at zero time (class 2). Classes 1 and 2 have equal numbers of molecules, so both have 7.5 mol % of spin label at equilibrium. It follows from simple exchange kinetics that

$$\tau_R = 2t/\ln[7.5/(7.5 - C)] \tag{iii}$$

where C is the mol % of spin label in class 2 at time t. The peak height of the *N*-methyl proton line is a measure of C (the *N*-methyl proton lines of 7.5–15 mol % spin-labeled vesicles are so broad that the contribution to the peak height from class 1 is never detectable). The peak height when C is zero should be 4 times the peak height when C is 1 mol % (the peak height of pure PC vesicles is 4 times that of 1 mol % spin-labeled vesicles). Let t_1 be the time when C is 1 mol %. If the exchange rates of molecules on both sides of the membrane are equal, then the peak height at time t_1 of sample *A* in Table 2 should be 4 times the peak height of sample *B*. If exchange involves the molecules on the outside only [65% of all the molecules (1)], then the peak height at time t_1 of sample *A* should be 2 times the peak height of sample *B*. In either case, we find that $t_1 \geq 2$ hr which, together with Eq. *iii*, yields the result in the text.

[f] Neglecting exchange, the fractional diminution of peak height is equivalent to the fraction of pure PC vesicles that have fused with spin-labeled vesicles (the fusion product is a 7.5 mol % spin-labeled vesicle, which makes no detectable contribution to the peak height). The extent of fusion of all the vesicles in the mixture may be twice as great as the extent of fusion of pure PC vesicles with spin-labeled vesicles (if all fusion products are equally probable, then the fusion of like with like vesicles, which does not affect the peak height, is as frequent as the fusion of like with unlike, which does affect the height).

[g] We attribute the broadening and diminution of peak height at zero time (compare data at 5 min on samples *A*, *B*, and *C*) to collisions of spin-labeled and pure PC vesicles without fusion or exchange.

TABLE 1. *Slopes of plots of linewidth and T_1 versus mol % spin label for N-methyl protons and phosphorus*

Slope (Hz/mol %)	N-Methyl protons	Phosphorus
S_1	4.4 ± 0.7	5.0 ± 0.4
S_2	9.8 ± 0.8	—

Each slope is taken from a plot of measurements on 0, 0.38, 0.65, 0.81, and 1.11 mol % spin-labeled didihydrosterculoyl PC vesicles. The measurements for *N*-methyl protons were at 220 MHz and 35°C. The measurements for phosphorus were at 24.3 MHz and 35°C.

proton line of a mixture of lyso PC and 15 mol % spin-labeled PC vesicles [2.5 mM lyso PC–25 mM (PC plus spin-labeled PC); buffered salt solution and nuclear resonance measurements (5 min after mixing) as in Table 2] is too broad to be observed. We conclude that 10 mol % of lyso PC does not induce the fusion of spin-labeled and pure PC vesicles.

A Nuclear Resonance Linewidth Is Evidence of Internal Motion in PC Vesicles. Internal motions of phospholipid molecules may be a factor in the lateral diffusion rate. Motions of the hydrocarbon chains of didihydrosterculoyl PC are revealed by the cyclopropane methylene proton resonance. This resonance is sufficiently well resolved (Figs. 1*a*, 4*a*) for its width to be determined. The width is field-dependent: it is 15.9 Hz at 60°C and 60 MHz, and 18.3 Hz at 60°C and 100 MHz. By a linear extrapolation we find the field-independent part of the width to be 12 Hz at 60°C[h]. About 9 Hz of this is due to unresolved multiplet structure (Fig. 4*b*). The residual width, 3 Hz, must be largely due to dipolar coupling of the cyclopropane methylene protons. If there were no motion of the cyclopropane ring, this width would be 3×10^4 Hz[i]. So, there must be motion of the cyclopropane ring, which may arise from conformational isomerizations of the hydrocarbon chain or rotation of the PC vesicle. The more rapid of these motions will account for the small residual width that we observe. We find no dependence on the viscosity of the aqueous phase and conclude that hydrocarbon chain isomerizations and not vesicle rotation account for the small residual width[j]. Thus, the frequency of hydrocarbon chain isomerizations must be greater than the frequency of vesicle rotation, which is 1.2×10^6 sec^{-1} at 60°C (see *Appendix*).

[h] The *N*-methyl and hydrocarbon-chain methylene proton linewidths of egg PC vesicle are proportionals to field strength (12). We assume this is true of the cyclopropane methylene proton linewidth as well.

[i] This is $3\ \mu/r^3$, the separation of the lines in a powder spectrum of two coupled protons. Here μ is the magnetic moment of a proton and r is the interproton distance [1.85 Å in cyclopropane (16)].

[j] The frequency of hydrocarbon chain isomerizations should not be much affected by the viscosity of the aqueous phase. The frequency of vesicle rotation should, by contrast, be inversely proportional to the viscosity of the aqueous phase (Stokes–Einstein relation). We find the cyclopropane methylene proton linewidth at 100 MHz and 35°C to be 28 Hz for a pure didihydrosterculoyl PC vesicle preparation and 29 Hz for a mixture of 0.20 ml of the same vesicle preparation with 0.04 ml of 0.1 M NaCl–0.05 M Na Borate, pD 8.0 and 160 mg of glycerol.

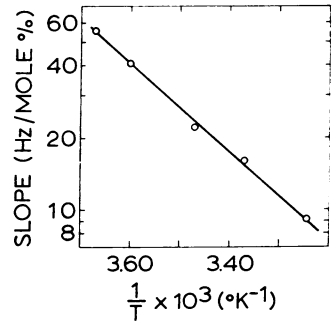


FIG. 3. Temperature dependence of the slope of a plot of *N*-methyl proton linewidth versus mole % spin label. Each data point is taken from measurements at 100 MHz on 0, 0.045, and 0.81 mol % spin-labeled didihydrosterculoyl PC vesicles.

DISCUSSION

The mechanism of lateral diffusion is not known. The translation step may be a pairwise exchange of nearest neighbors. The length of the step would be the distance between nearest neighbors; this is 0.9 nm (9.1 Å) in a PC bilayer (17)[k]. The molecular frequency of a step would be the reciprocal of the duration of an encounter between nearest neighbors[k]; if this is the same for a PC–PC pair as a PC-spin-labeled PC pair, then the frequency of a step in a PC bilayer is $f = 1/\tau_M$. Since we have found τ_M to be much less than 3.4×10^{-4} sec, we have $f \gg 3 \times 10^3$ sec^{-1}. A PC molecule moves the root mean square distance $(9.1 \times 10^{-4})\ (ft)^{1/2}\ \mu$m in t sec, or more than 0.05 μm in 1 sec.

We indicate the possible biological significance of this result with an example. A membrane lipid would diffuse from one end of a 1 μm long bacterium to the other in much less than 5 min if the lateral diffusion were as rapid as for PC and unobstructed by other membrane constituents. So it may be that the lateral distribution of lipids in the membrane of a 1 μm long bacterium is always at equilibrium. (This would not require that the lateral distributions of all classes of lipids in the membrane be the same. Unsaturated lipids might be concentrated near transport sites due to a special affinity for the proteins there. The lipids with net negative charge might be clustered with polyvalent cations.) Of course, it is also possible that the lipid bilayer in a membrane is divided up by barriers to lateral diffusion. The lateral distribution of lipids within each region of the bilayer would equilibrate by diffusion, but the lipids in one region might not equilibrate with the lipids in another.

APPENDIX

The expressions for $1/T_{1M}$ and $1/T_{2M}$ in limits appropriate to our work are

$$\frac{1}{T_{1M}} = [(3.44 \times 10^7\ \gamma^2)/r^6][3\tau_c/(1 + 3.94 \times 10^{17}\ \tau_c^2)] \quad (3)$$

$$\frac{1}{T_{2M}} = [(1.72 \times 10^7\ \gamma^2)/r^6][4\tau_c + 3\tau_c/(1 + 3.94 \times 10^{17}\tau_c^2)] \quad (4)$$

[k] Here we have assumed a hexagonal lattice. Had we assumed a cubic lattice, the nearest neighbor distance would have been 0.84 nm (8.4 Å) and the frequency per molecule of a step would have been 1.5 times the reciprocal of the duration of an encounter between nearest neighbors.

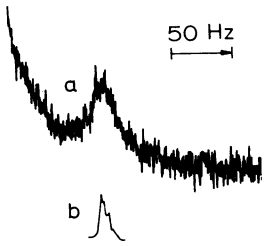


FIG. 4. Cyclopropane methylene proton lines at 100 MHz and 35°C of (*a*) pure didihydrosterculoyl PC vesicles (the average of 4 scans) and (*b*) 1 M dihydrosterculic acid in CCl_4. The chemical shift is given in Fig. 1.

where γ is the nuclear magnetogyric ratio, r (in Å) is the distance of closest approach of the nucleus and unpaired electron, and τ_c is the electron-nuclear correlation time (18). In experiments on vesicles, τ_c cannot exceed the correlation time for vesicle rotation, which we calculate (from the Stokes–Einstein relation for a sphere of 12.5 nm (125 Å) radius in water) to be 1.4×10^{-6} sec at 35°C and 0.84×10^{-6} sec at 60°C. We have used Eq. 4 to write an *N*-methyl proton line shape function, $g(\nu)$, for spin-labeled PC vesicles in the case of no diffusion:

$$g(\nu) = [(1-c)^N/A]/[1+4\pi^2(\nu-\nu_0)^2/A^2] + [Nc(1-c)^{N-1}]/\pi r_1^2 \int_{r_0}^{r_1} [2\pi r dr/(A+B/r^6)]/[1+4\pi^2 \times (\nu-\nu_0)^2/(A+B/r^6)^2] + [N(N-1)c^2(1-c)^{N-1}]/2\pi^2 r_1^4 \times \int_{r_0}^{r_1}\int_{r_0}^{r_1} [4\pi^2 r r' dr dr'/(A+B/r^6+B/r'^6)]/[1+4\pi^2 \times (\nu-\nu_0)^2/(A+B/r^6+B/r'^6)^2] + [N(N-1)(N-2)c^3 \times (1-c)^{N-3}]/6\pi^3 r_1^6 \int_{r_0}^{r_1}\int_{r_0}^{r_1}\int_{r_0}^{r_1} [8\pi^3 r r' r'' dr dr' dr''/(A+B/r^6 + B/r'^6 + B/r''^6)]/[1+4\pi^2(\nu-\nu_0)^2/(A+B/r^6+B/r'^6 + B/r''^6)^2] + \ldots \quad (5)$$

Here A is π times the linewidth for pure PC vesicles, B is $1.25 \times 10^{16}[4\tau_c + 3\tau_c/(1 + 3.94 \times 10^{17}\,\tau_c^2)]$, r_0 is some short distance, r_1 is a value of r in Eq. 4 for which $1/T_{2M}$ is much smaller than A, N is $\pi r_1^2/71.2$ [taking the surface area per molecule in a PC bilayer to be 0.712 nm² (71.2 Å²) (17)], and c is the mol fraction of spin-labeled molecules. The three thin-line curves in Fig. 2 were obtained from Eq. 5. The values of τ_c and r_1 were 10^{-6} sec and 6.9 nm (69 Å) for the *upper curve*, 5×10^{-7} sec and 6.0 nm (60 Å) for the *middle curve*, and 10^{-7} sec and 4.6 nm (46 Å) for the *lower curve*. The curves for smaller values of τ_c would lie between the lower curve and the horizontal.

TABLE 2. *Intervesicle exchange and fusion*

Sample	Peak height (units)		Linewidth (Hz)	
	5 min	125 min	5 min	125 min
A (0 mol % spin label)	145	144	4.0	4.1
B (0 + 15 mol % spin label)	100	72	5.7	6.8
C (B + 10 mol % lyso PC)	102	109	5.6	5.4

Sample *A:* Pure egg PC vesicles (25 mM PC). Sample *B:* 0.2 ml of pure egg PC vesicles (50 mM PC) plus 0.2 ml of 15 mol % spin-labeled egg PC vesicles [50 mM (PC plus spin-labeled PC)]. Sample *C:* 0.40 ml of sample *B* plus 0.04 ml of egg lyso PC (0.05 M in 0.1 M NaCl–0.05 M Na Borate, pD 8.0). Each sample was kept at 35°C after mixing. Measurements at the designated times after mixing were at 100 MHz and 35°C.

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