Deuterium Nuclear Magnetic Resonance Investigation of Dimyristoyllecithin–Dipalmitoyllecithin and Dimyristoyllecithin–Cholesterol Mixtures†

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ABSTRACT: Deuterium nuclear magnetic resonance (NMR) spectra of 1,2-dimyristoyl-3-*sn*-phosphatidylcholines (DMPCs) specifically deuterated in the 2-chain at one of positions 2′, 3′, 6′, or 14′ have been obtained by the quadrupole-echo Fourier transform method at 34.1 MHz (corresponding to a magnetic field strength of 5.2 T) or the pure material as a function of temperature, and in the presence of either 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine (DPPC) or cholesterol as a function of temperature and composition. The results with pure DMPC and DMPC–DPPC mixtures indicate that a sharp, intense deuterium resonance is characteristic of fluid-phase lipids, whereas a broad resonance is characteristic of solid-phase lipids. There is shown to be good agreement between the deuterium NMR derived DMPC–DPPC phase diagram and that derived by using other techniques. The deuterium NMR results obtained with the DMPC–cholesterol system are not interpreted in terms of a phase diagram. They do indicate, however, that the transition breadth is increased considerably and the temperature at which the lipid chains "solidify" is depressed by the addition of cholesterol to the DMPC bilayer. The particular nature of the increase and the depression is found to be dependent on where the label is located on the lipid.

The examination of simple, well-characterized model membrane systems constitutes an essential step toward elucidation of the processes that occur within, and are influenced by, the vastly more complex membrane systems found in living organisms. The phospholipid bilayer is one such simple system. It has received much attention (Hubbell & McConnell, 1971; Sklar et al., 1975; Trauble & Eibl, 1974; Ranck et al., 1974; Engelman, 1970; Ladbrooke & Chapman, 1969; Hinz & Sturtevant, 1972). A large degree of complexity can be attained in this system by introducing known amounts of other species into the bilayer. Mixtures of different chain-length saturated chain phospholipids have been studied in some detail from both experimental (Mabrey & Sturtevant, 1976; Wu & McConnell, 1975; Brulet & McConnell, 1976; Shimshick & McConnell, 1973a) and theoretical (McCammon & Deutch, 1975; Jacobs et al., 1977) viewpoints. Mixtures of lipids with different head groups (Wu & McConnell, 1975; Blume & Ackermann, 1974) and with unsaturated hydrocarbon chains have also been examined (Brulet & McConnell, 1976). The phase diagrams of many of these systems are reasonably simple (e.g., DMPC–DPPC mixtures)[1] while some are quite elaborate (Wu & McConnell, 1975).

The lipid–cholesterol mixture has also been subject to examination by many investigators using a variety of techniques (Chapman & Penkett, 1966; Lippert & Peticolas, 1971; Haberkorn et al., 1977; Oldfield et al., 1971, 1978a; Shimshick & McConnell, 1973b; Mabrey et al., 1978; Tsong, 1975; Marsh, 1974; Hemminga, 1975). Nevertheless, no unambiguous picture of this system has emerged.

Deuterium nuclear magnetic resonance (NMR) studies using specifically ^{2}H-labeled lipids have provided useful and interesting information about hydrocarbon chain motions in lipid bilayers (Oldfield et al., 1978c; Seelig, 1977), including the lecithin–cholesterol system (Oldfield et al., 1978a; Stockton et al., 1976). Until recently it has not been possible to reproduce faithfully the deuterium NMR line shape, even in the fluid-crystalline phase, by using pulsed FT methods. However, the recent introduction of the quadrupole-echo Fourier transform NMR method (Davis et al., 1976) now permits essentially undistorted ^{2}H NMR spectra to be recorded. We show that this technique can be used to obtain phase diagrams for multicomponent bilayers by deriving the phase diagram for the DMPC–DPPC system from the ^{2}H NMR spectra of 2-[6′,6′-$^2H_2$]DMPC–DPPC mixtures. We find that the ^{2}H NMR line shapes and intensities are very different for the fluid and solid lipid regions. Results obtained with cholesterol-containing bilayers are more complex than those obtained with pure lipid mixtures and do not permit determination of the corresponding lecithin–cholesterol phase diagram.

Experimental Section

Nuclear Magnetic Resonance Spectroscopy. Deuterium NMR spectra were obtained on a widebore superconducting magnet spectrometer in the Fourier transform mode at 34.1 MHz (corresponding to a magnetic field strength of 5.2 T). The construction of this instrument is described elsewhere (Oldfield et al., 1978a). All spectra were obtained by using the quadrupole-echo technique (Solomon, 1958; Abragam, 1961; Davis et al., 1976) with $\tau_1 = \tau_2 = 60$ μs. Typically, 10000 scans were taken for each spectrum and the subsequent free induction decay was processed with an exponential multiplication factor of 150 Hz. The pulse sequence repetition period for the methyl-labeled compound was 0.24 s, while that for all other labeled compounds was 0.054 s. Due to a limited data acquisition rate in our present system, we recorded half of the ^{2}H NMR spectrum while operating exactly on the ^{2}H resonance frequency. For convenience of interpretation, these half spectra *plus* their mirror images are presented in all figures.

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[1] Abbreviations used: CHOL, cholesterol; DMPC, 1,2-dimyristoyl-3-*sn*-phosphatidylcholine; DPPC, 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine; NMR, nuclear magnetic resonance; ESR, electron spin resonance; DSC, differential scanning calorimetry; HWHH, half-width at half-height; T_c, gel-to-liquid crystal phase transition temperature.

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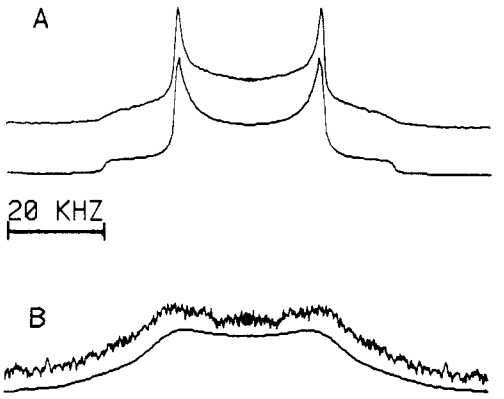

FIGURE 1: Experimental and simulated spectra of pure 2-[6′,6′-$^2H_2$]DMPC above and below its phase transition temperature. Simulations are the lower traces in each pair. (A) 25 °C, for the simulation HWHH = 600 Hz and $\Delta\nu$ = 30 kHz. (B) 20 °C, for the simulation HWHH = 6000 Hz and $\Delta\nu$ = 34 kHz. The vertical scale changes by a factor of 8 on going from the 25 °C spectrum to the 20 °C spectrum. Spectral width is 100 kHz in both cases.

Sample Preparation. Samples were prepared and checked for purity as described previously (Oldfield et al., 1978a), except that lipid plus cholesterol was initially dissolved in benzene–MeOH (90:10, v/v), which was removed by lyophilization followed by pumping under vacuum ($\lesssim$1 mmHg) for 24–48 h at ~50 °C. The DMPC–CHOL samples were then dispersed above the pure lipid T_c in an approximately equal weight of ^2H-depleted water (Aldrich, Milwaukee, WI; ^2H content = natural abundance × 0.011) and sealed. Several other methods of sample preparation did not effect complete mixing of the CHOL with the lipid [see, for example, Oldfield et al. (1978b)]. All samples contained 30–50 mg of deuterium-labeled DMPC in a 200-μL sample volume.

Theoretical Background. Derivations and discussions of the quadrupole Hamiltonian, order parameter (S_{CD}), and the "powder pattern" line shape are all found elsewhere (Oldfield et al., 1978a; Seelig, 1977; Abragam, 1961; Slichter, 1978; Petersen & Chan, 1977). In a powder pattern (spin I = 1 system) one finds that the separation between peak maxima (the residual quadrupole splitting $\Delta\nu$) is related to S_{CD} as shown in eq 1

$$\Delta\nu = \frac{3}{4}\frac{e^2qQ}{h} S_{CD} \qquad (1)$$

where e^2qQ/h is the static quadrupole coupling content (~170 kHz for the C-D bond; Derbyshire et al., 1969; Burnett & Muller, 1971).

In the simplest case this powder pattern can be described as an envelope of Lorentzian functions. The line shape then becomes

$$G(\nu) = g_+(\nu) + g_-(\nu) \qquad (2)$$

where

$$g_\pm(\nu) = C\int_0^{\pi/2} (\sin\theta\, d\theta)(\delta/\pi)/[\delta^2 + \{\nu \pm (\Delta\nu/2)(3\cos^2\theta - 1)\}^2] \qquad (3)$$

The theoretical line shape is completely determined by three parameters: C, a constant determining the integrated intensity of the powder pattern; δ, the half-width at half-height (HWHH) of the Lorentzian functions; and $\Delta\nu$, the quadrupole splitting. Comparison of an experimentally measured spectrum with one simulated by using eq 2 gives a method of determining the line width δ of the resonance. Since $\Delta\nu$ can be measured accurately and C is a simple scaling constant, we need vary only one parameter (δ) to obtain a "best fit" between the experimental and simulated spectra. A spectrum of pure 2-[6′,6′-$^2H_2$]DMPC is shown in Figure 1A along with its simulation. An acceptable fit is observed with a HWHH of 600 Hz, a $\Delta\nu$ of 30 kHz, and a value of C which equates the intensities of the two spectra. Because the experimental spectra are subject to distortions from such sources as errors in phasing and pulse power falloff (Vega & Pines, 1977), we cite an uncertainty in the calculated HWHH's of ca. ±10%.

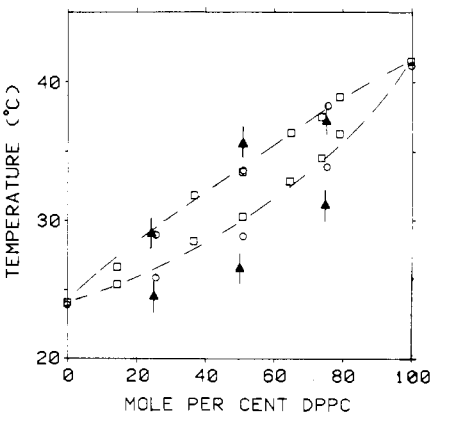

FIGURE 2: Phase diagram for the DMPC–DPPC binary mixture derived from DSC (□) (Mabrey & Sturtevant, 1976), ESR (○) (Shimshick & McConnell, 1973a), and this work (▲).

The liquid-crystalline and gel phases of the pure phospholipid bilayer display very different ^2H NMR spectra. In the liquid-crystalline state a sharp powder pattern is observed with a quadrupole splitting dependent on the position of the ^2H label and the temperature. The "sharpness" of the spectra is measured by the HWHH in eq 2. It is relatively insensitive to both label position and temperature above T_c (600–800 Hz in all cases examined). On cooling to just below T_c, the spectral intensity decreases abruptly and the line shape resembles a single very broad hump. When these spectra are simulated, HWHH's an order of magnitude larger than those found just above T_c are necessary to obtain reasonable fits (see Figure 1). The decrease in intensity is brought about principally by our still rather limited spectral width (100 kHz), which enables us to see only a portion of the broad resonance.

In situations where both phases exist simultaneously, one would hope to be able to distinguish two types of resonance: one from the fluid phase and one from the solid phase. The relative intensities of the two types would then be a measure of their concentrations. In fact, what is observed under our experimental conditions is a decrease in intensity and a gradual broadening of the liquid-crystalline type powder pattern as one passes through the two-phase region into the gel state. The initial decrease in intensity signals the inception of solid-phase formation. The leveling off of signal intensity (Figures 2 and 3) indicates the disappearance of all of the fluid-phase lipids. A similar type of phenomenon has been shown to occur when ^{19}F NMR of labeled fatty acids is used to monitor lipid phase transitions (Gent & Ho, 1978). We have found that all of the spectra of Figure 3, which are from samples containing either pure liquid-crystalline bilayers, mixed gel plus liquid-crystalline bilayers, or entirely solid gel-state bilayers, may be quite accurately simulated by using only one powder pattern line shape (at each temperature). Total integrated signal intensity and HWHH line width information may be used to derive phase diagram information from this analysis. Notably, quadrupole splitting (i.e., order parameter) information, under our spectral conditions, has not yielded useful phase diagram information.

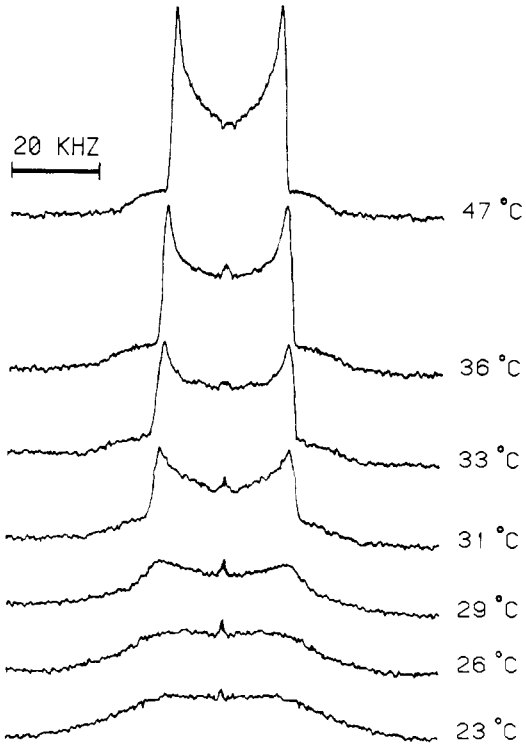

FIGURE 3: ^2H NMR spectra of 2-[6′,6′-$^2H_2$]DMPC mixed with 50 mol % DPPC at the temperatures indicated. The same spectrometer settings were used to obtain all spectra. Spectral width is 100 kHz at all temperatures.

Results and Discussion

Mixtures of DMPC and DPPC. DMPC and DPPC are miscible in all proportions. They form a simple binary mixture which has been examined with both DSC and ESR techniques (Shimshick & McConnell, 1973a; Mabrey & Sturtevant, 1976). Both of these techniques are in general agreement as to the shape and position of the two-phase region in the phase diagram of this mixture (see Figure 2). We have employed the ^2H NMR technique to study this system in order to test its usefulness in the examination of multicomponent lipid bilayer systems.

The temperature dependence of the spectra of a 1:1 mol ratio sample of 2-[6′,6′-$^2H_2$]DMPC and DPPC is shown in Figure 3. As the temperature is lowered through the two-phase region, the integrated intensity of the resonance begins to diminish (at ~35 °C) and the HWHH begins to increase from its high-temperature value of 500 Hz. At about 27 °C the intensity reaches a minimum, the HWHH has increased to 6 kHz, and the spectrum appears as a single broad hump characteristic of completely immobilized lipids. Figure 4 shows intensity and HWHH data for pure 2-[6′,6′-$^2H_2$]DMPC and this lipid mixed with 25, 50, and 75 mol% DPPC. The phase diagram described by the onset and completion of our signal intensity changes is shown in Figure 2. There is good qualitative agreement between our results and those from other methods. We feel that this confirms the appropriateness of the ^2H NMR technique in studying multicomponent systems.

In terms of quantitative agreement, our data indicate that the disappearance of all lipid chain mobility occurs at temperatures somewhat lower than the solidus curve described by ESR and DSC results. Incipient chain motion or disorder in high-temperature solid lipids has been observed in several other studies: (1) a Raman investigation of pure DMPC detected a limited amount of gauche–trans isomerization just below T_c (Lippert & Peticolas, 1971, 1972); (2) a ^{19}F NMR study of DMPC–DSPC mixtures indicated that some disorder or chain

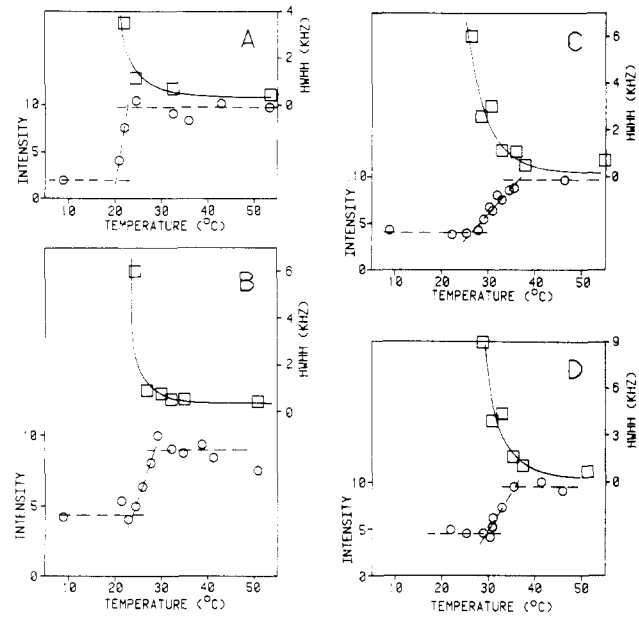

FIGURE 4: Integrated intensity (○) and HWHH's (□) of 2-[6′,-6′-$^2H_2$]DMPC–DPPC mixtures: (A) pure DMPC; (B) 25 mol % DPPC; (C) 50 mol % DPPC; (D) 75 mol % DPPC.

mobility is present in the solid phase immediately below the solidus curve in the phase diagram for this system (Gent & Ho, 1978); (3) a Tempo solubility study of DMPC indicated formation of defects some 20 °C below T_c (Lee, 1977).

Mixtures of DMPC and Cholesterol. We have examined DMPC–CHOL mixtures with three different specifically ^2H-labeled DMPCs: 2-[14′,14′,14′-$^2H_3$]DMPC, 2-[3′,3′-$^2H_2$]DMPC, and 2-[2′,2′-$^2H_2$]DMPC. Up to 50 mol % cholesterol may be incorporated into the DMPC bilayer (Ladbrooke et al., 1968). The temperature dependence of the ^2H NMR spectra of each of the labeled compounds in a 50 mol % cholesterol mixture is shown in Figure 5. These spectra point out the broad nature of the transition (Lippert & Peticolas, 1971; Oldfield et al., 1971; Mabrey et al., 1978). It is also apparent that the terminal methyl end of the lipid hydrocarbon chain is mobile to much lower temperatures than that portion of the chain near the bilayer surface. Plots of integrated intensity vs. temperature at several cholesterol concentrations for the 2′ and 3′ labels are given in Figure 6. These results give a more quantitative measure of the transition breadth. At the highest cholesterol concentrations, signal loss does not begin until ~10 °C, well below the T_c for pure DMPC (23.9 °C, Hinz & Sturtevant, 1972). At −5 °C the signal from 2-[3′,3′-$^2H_2$]DMPC in a 50 mol % cholesterol mixture is only just detectable. At intermediate cholesterol concentrations signal loss is observed over some 30 °C. Although intensity measurements were not performed on the methyl-labeled compound, we noted significant intensity in the 20–50 mol % cholesterol mixtures at −18 °C but very little intensity from the 0 and 10 mol % cholesterol samples below 10 °C.

As with the DMPC–DPPC mixtures, increases in HWHH tend to follow the loss of signal intensity, with one important exception. Above T_c the HWHH's of all of the spectra are quite insensitive to *both* cholesterol content and temperature changes. Below T_c the 14′ label spectra remain narrow to low temperatures. The 3′ spectra become broad even at 10 °C unless the cholesterol content is quite high.

Residual quadrupole splittings ($\Delta\nu$) are plotted as a function of cholesterol concentration in Figure 7. In a previous study (Oldfield et al., 1978a) we observed that the quadrupole

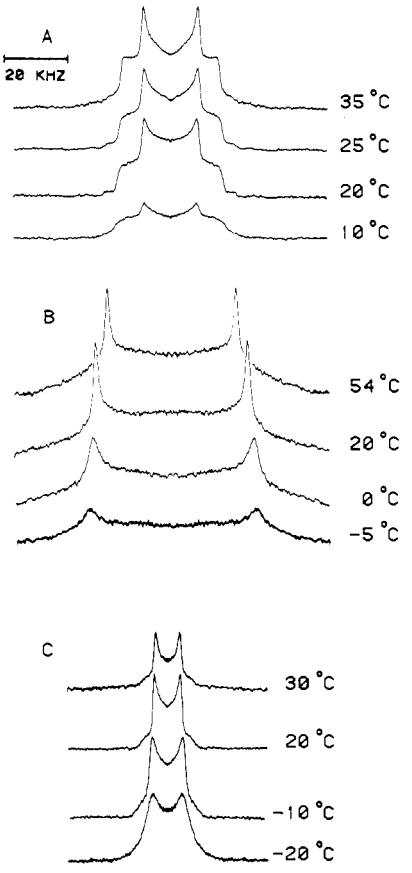

FIGURE 5: ^2H NMR spectra of three labeled DMPC's in the presence of 50 mol % cholesterol at several temperatures: (A) 2-[2′,2′-$^2H_2$]DMPC; (B) 2-[3′,3′-$^2H_2$]DMPC; (C) 2-[14′,14′,14′-$^2H_3$]DMPC.

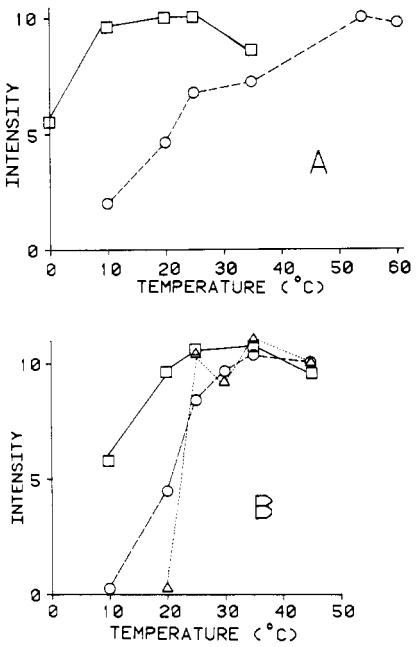

FIGURE 6: Integrated intensity as a function of temperature for (A) 2-[3′,3′-$^2H_2$]DMPC with 20 mol % cholesterol (○) and 40 mol % cholesterol (□); (B) 2-[2′,2′-$^2H_2$]DMPC with 0 mol % cholesterol (△), 20 mol % cholesterol (○), and 50 mol % cholesterol (□).

splitting of DMPC labeled as N–CD_3 in the choline head group went through a maximum at ~20 mol % cholesterol above 45 °C. As can be seen in Figure 7, no extrema are found in any of the $\Delta\nu$ vs. cholesterol content graphs for the chain-labeled compounds. Furthermore, no sharp breaks are displayed in these plots, although it is not possible to discount

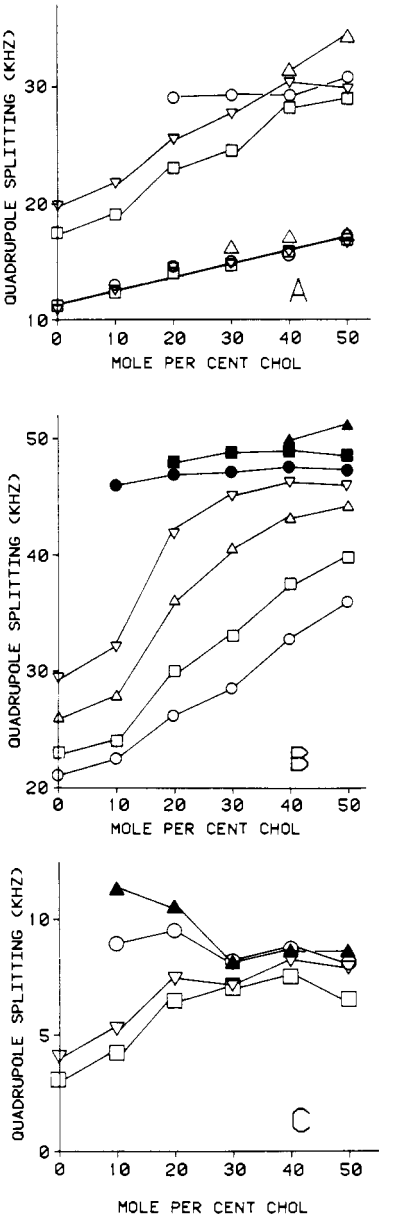

FIGURE 7: Quadrupole splitting for labeled compounds as a function of cholesterol content. (A) 2-[2′,2′-$^2H_2$]DMPC at 35 (□), 25 (▽), 20 (○), and 15 °C (△). (B) 2-[3′,3′-$^2H_2$]DMPC at 73 (○), 54 (□), 35 (△), 25 (▽), 20 (●), 10 (■), and 0 °C (▲). (C) 2-[14′,14′,-14′-$^2H_3$]DMPC at 35 (□), 25 (▽), 20 (○), and 15 °C (▲).

very shallow inflections as seen in the DPPC–CHOL system by Haberkorn et al. (1977).

Two quadrupole splittings are observed for 2-[2′,2′-$^2H_2$]-DMPC (Seelig & Seelig, 1975). Both the inner and outer quadrupole splittings increase monotonically and in a roughly linear fashion with increasing cholesterol concentration. At both high cholesterol concentration and low temperatures, the outer set of peaks in the spectra of the 2′ label is somewhat obscured. This leads to a larger uncertainty in the $\Delta\nu$'s for these situations.

Quadrupole splittings for 2-[3′,3′-$^2H_2$]DMPC plus cholesterol at temperatures well above T_c are also found to increase with increasing cholesterol concentration in a roughly linear fashion (Figure 7B). Quadrupole splittings vs. cholesterol content at 73, 60, and 54 °C are all nearly parallel. The curves are shifted to higher values at lower temperatures. Close to T_c the splittings tend to level off at high cholesterol concentrations.

The methyl-labeled $\Delta\nu$'s follow the same trend found for the 3′ label, except that below T_c the 14′ $\Delta\nu$'s clearly decrease as cholesterol is added. The opposite effects produced by increasing cholesterol content above and below T_c are particularly interesting. This is indicative of the tendency (Lippert & Peticolas, 1971; Oldfield & Chapman, 1972; Kleeman & McConnell, 1976) of cholesterol to disorder the solid phase while ordering the fluid phase of the lipid bilayer.

It is useful to compare the ^{2}H NMR results with those obtained on the lipid–cholesterol system by using other techniques. ESR studies using nitroxide-labeled lipids (Shimshick & McConnell, 1973b) and steroids (Marsh, 1974; Hemminga, 1975), ^{1}H NMR (Gent & Prestegard, 1974; Kroon et al., 1975), laser Raman (Lippert & Peticolas, 1971), X-ray and neutron diffraction (Franks, 1976; Worcester & Franks, 1976), and high-sensitivity DSC (Mabrey et al., 1978) have all been employed to study this system. There is general agreement among all of the techniques that the addition of cholesterol changes the sharp (<1 °C) gel-to-liquid crystalline phase transition of the pure lecithin to a diffuse phenomenon spanning several decades of °C. One would like to know the underlying cause of this diffuseness. Is it due to phase separation (Shimshick & McConnell, 1973b), a lack of cooperativity (Lippert & Peticolas, 1971; Mabrey et al., 1978), or some other as yet unsuspected cause (Mabrey et al., 1978)?

Several ESR studies employing nitroxide-labeled steroids (Marsh, 1974; Hemminga, 1975) agree with our ^{2}H NMR results, which indicate that the onset of fluidization of the bilayer decreases with increasing cholesterol concentration. Information obtained from Tempo partitioning (Shimshick & McConnell, 1973b) and freeze-fracture electron microscopy studies using protein markers (Kleeman & McConnell, 1976) argues that the onset temperature is relatively independent of cholesterol content at low concentrations and increases at higher (>20%) concentrations of cholesterol. It would therefore appear that the onset of different events is being measured.

A recent high-sensitivity study of DPPC and of DMPC mixed with cholesterol shows that these systems exhibit quite complicated behavior (Mabrey et al., 1978). The excess specific heat curves have multiple peaks in the low cholesterol concentration range. The curves between ~13.5 and 20 mol % cholesterol in DMPC appear to be composed of three peaks. Above 20 mol % a single broad peak is observed. Its maximum moves toward high temperatures, and it becomes broader as cholesterol content is increased. It becomes undetectable at 50 mol % cholesterol. It is conceivable that the ^{2}H NMR technique and those ESR techniques employing labeled steroids are sensitive to events occurring at the low-temperature extremes of the DSC endotherms, while Tempo solubility and the movement of protein markers are sensitive to events occurring near the maximum heat absorption in this system. Unfortunately, our ^{2}H NMR data offer no obvious explanation for the multiple peak behavior found in the heat absorption curves. One thing that is *not* indicated by our study is the formation of specific lipid–cholesterol complexes (Darke et al., 1971).

The most unusual aspect of our findings is the appearance of fluidlike powder patterns at very low temperatures. It has been tentatively suggested (Mabrey et al., 1978) that the lecithin–cholesterol system may form an "unusual" solid phase which exhibits a continuous transition. This continuity is presumably a reflection of lattice forces too weak to enforce the highly cooperative phase transition observed in the pure lipid. Such weak lattice forces might also lead to significant disorder and chain mobility down to low temperatures.

Summary

Deuterium NMR employing specifically ^{2}H-labeled DMPCs has been used to derive the phase diagram for a simple lipid mixture. It was found that the intensities and shapes of the spectra are indicators of phase changes within the bilayer. A spectral simulation routine was used to obtain a quantitative measure of spectral line shapes in terms of HWHH's. We were able to follow changes in intensity and HWHH as a function of temperature and composition in the DMPC–DPPC mixture. From these changes a phase diagram in agreement with previous measurements was obtained.

Similar measurements were taken on the DMPC–cholesterol system. These ^{2}H NMR results are not, however, amenable to interpretation in the form of a phase diagram. Very broad transitions were observed. Spectra characteristic of fluid-phase lipids were observed 20–40 °C below T_c when the cholesterol concentration was high. No indication of lipid–cholesterol compound formation was observed.

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Lateral Distribution of Negatively Charged Lipids in Lecithin Membranes. Clustering of Fatty Acids†

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ABSTRACT: From ζ-potential measurements on multilamellar liposomes, it is concluded that negatively charged phospholipids incorporated in liquid-crystalline phosphatidylcholine bilayers are randomly distributed in the plane of the bilayer. Furthermore, the distribution of the negatively charged phospholipid between the two halves of the bilayer and over the concentrically arranged bilayers of the multilamellar structure is uniform. The same is true for spin-labeled fatty acids. In contrast, long-chain fatty acids appear to be clustered at neutral pH; i.e., they segregate into patches within the plane of the bilayer. Their carboxyl groups are fully ionized only at a pH $\geq$ 11, and charge repulsion leads then to a random distribution. At pH <7 the binding of $^{45}Ca^{2+}$ to arachidic acid monolayers is insignificant, and only at pH $\sim$10 does the amount of Ca^{2+} bound per fatty acid become comparable to that bound to phosphatidylserine monolayers at neutral pH. Ca^{2+} binding obviously parallels the deprotonation of the fatty acid carboxyl group in phosphatidylcholine bilayers. The pH dependence of the Ca^{2+} binding to fatty acid monolayers is similar to the pH–electrophoretic mobility relationship observed with fatty acid containing phosphatidylcholine liposomes. From the similarity in behavior of fatty acid monolayers and fatty acids present in bilayers, it is proposed that in the latter case fatty acids occur as patches of monolayers (clusters). Fatty acids and spin-labeled fatty acids also differ in their effect on the gel to liquid-crystalline transition of dipalmitoylphosphatidylcholine bilayers. The former are stabilizing as evident from an increase in the transition temperature while the latter have a destabilizing effect. Deuterated fatty acids behave like ordinary fatty acids in terms of clustering. The use of probing the structure and dynamics of phospholipid bilayers and membranes with deuterated fatty acids is therefore subject to criticism, particularly when large quantities of fatty acids have to be utilized as in deuteron magnetic resonance experiments. The information thus derived is likely to be affected by probe–probe interactions.

There is an increasing body of evidence indicating that in addition to the asymmetric distribution of lipids and proteins between the two halves of the membrane there is also a nonrandom (asymmetric) distribution of components within half (one monolayer) of the membrane. This segregation or clustering of membrane components into highly specialized regions is related to the fast lateral diffusion allowing the various components to come in contact and to interact with each other. The lateral distribution of fatty acids in bilayers and biological membranes is interesting from two points of view: (1) fatty acids occur, though to a small extent, in biological membranes as the result of phospholipid turnover and have been shown to affect the permeability and various transport functions of the mitochondrial membrane (Wojtczak, 1976) and (2) spin-labeled fatty acids have been used ex-

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