Rydström, J., Hoek, J. B., & Hundal, T. (1974) *Biochem. Biophys. Res. Commun. 60*, 448–455.
Rydström, J., Kanner, N., & Racker, E. (1975) *Biochem. Biophys. Res. Commun. 67*, 831–839.
Rydström, J., Hoek, J. B., Ericson, B. G., & Hundal, T. (1976) *Biochim. Biophys. Acta 430*, 419–425.
Van de Stadt, R. J., Nieuwenhuis, F. J. R. M., & Van Dam, K. (1971) *Biochim. Biophys. Acta 234*, 173–176.
Wikström, M. K. F., & Sarri, H. T. (1977) *Biochim. Biophys. Acta 462*, 347–361.

Dynamic Properties of Binary Mixtures of Phosphatidylcholines and Cholesterol†

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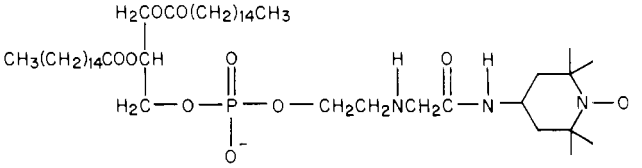
ABSTRACT: We have observed the paramagnetic resonance spectra of a head group spin-labeled phosphatidylethanolamine (L-α-dipalmitoylphosphatidyl-*N*-ethanolamine) as a function of temperature and cholesterol concentration in binary mixtures of cholesterol and dimyristoylphosphatidylcholine. These spectra bear on two interrelated topics involving mixtures of phosphatidylcholine and cholesterol: (1) lipid phase equilibria and the lateral ordering and diffusion of lipid molecules and (2) model membrane immunochemistry using spin-label lipid haptens.

There are three interesting, interrelated problems involving model membranes composed primarily of binary mixtures of phosphatidylcholine and cholesterol. One problem concerns the lipid phase equilibria and the lateral ordering and motion of lipid molecules in the plane of the membrane. The second problem concerns the lateral motion and distribution of membrane proteins included in these binary mixtures. The third problem concerns the immunochemical (or, more generally, biochemical) properties of these model membranes. In the present paper we describe the paramagnetic resonance spectra of the head group spin-label I in binary mixtures of



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cholesterol and dimyristoylphosphatidylcholine as a function of composition and temperature. We show how these spectral data are related to the first and last of the three problems mentioned above and how they are related to earlier studies that bear on these three problems. The effects of lipid composition on the distribution and motion of membrane proteins have been described earlier (Kleemann & McConnell, 1976; Grant & McConnell, 1974). The effects of lipid composition on protein diffusion will be described in a subsequent publication.

Materials and Methods

Lipids. Dimyristoylphosphatidylcholine was obtained from Sigma and stored in ethanol. Concentrations were determined by phosphate assay (McClare, 1971). Cholesterol was recrystallized twice from ethanol and stored in an ethanolic solution under argon. Concentrations were determined as described by Solow & Freeman (1970). All lipids were stored at −20 °C.

Spin-Labeled Lipids. Spin-labeled dipalmitoylphosphatidylethanolamine (I) was prepared by the method of Brûlet & McConnell (1976a,b). A cholestane spin-label was synthesized as described by Keana & Dinerstein (1971).

Liposomes. Liposomes were formed by mixing 5 μmol of various combinations of dimyristoylphosphatidylcholine and cholesterol with 0.005 μmol of spin-labeled lipid. These samples were rotoevaporated to remove the ethanol solvent and resuspended in 0.1 mL of redistilled chloroform. This solution was rotoevaporated to form a thin homogeneous film. The samples were then placed in a vacuum desiccator for >8 h to remove any residual organic solvent. A 0.5-mL amount of 0.010 M $NaPO_4$ and 0.15 M NaCl, pH 7.0 (PBS),[1] degassed and stored under argon, was added to the lipid film. The samples were then incubated at 50 °C for 2 min and vigorously vortexed for 10 s. A 1.5-mL amount of PBS was added to the samples, which were transferred to centrifuge tubes. The liposomes were collected as a pellet, after spinning at 500*g* for 5 min, and transferred to a 50-μL Corning glass micropipet. The pipet was sealed at one end by melting the glass and sealed at the other end with a Teflon septum cap. Attempts were made to keep the volume of the resuspended pellet as low as 25 μL to obtain as many of the liposomes as possible in the ESR cavity.

Electron Spin Resonance. Spectra of the spin-labeled phospholipid and steroid were recorded on a Varian E-12 spectrometer with temperature control to ±0.5 °C. The spectra were stored in a computer where they were integrated and normalized to the same number of spins. Typical spectra of the spin-labeled phospholipid in mixtures containing phosphatidylcholine and cholesterol are shown in Brûlet & McConnell (1976a).

Peak heights in normalized derivative curve paramagnetic resonance spectra are quite sensitive to line shapes; peak heights are inversely proportional to the square of the line width for signals of a specific form (i.e., Gaussian, Lorentzian, etc.). The particular base line correction used for the present

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[1] Abbreviation used: PBS, 0.5 mol of 0.010 M $NaPO_4$ and 0.15 M NaCl, pH 7.0.

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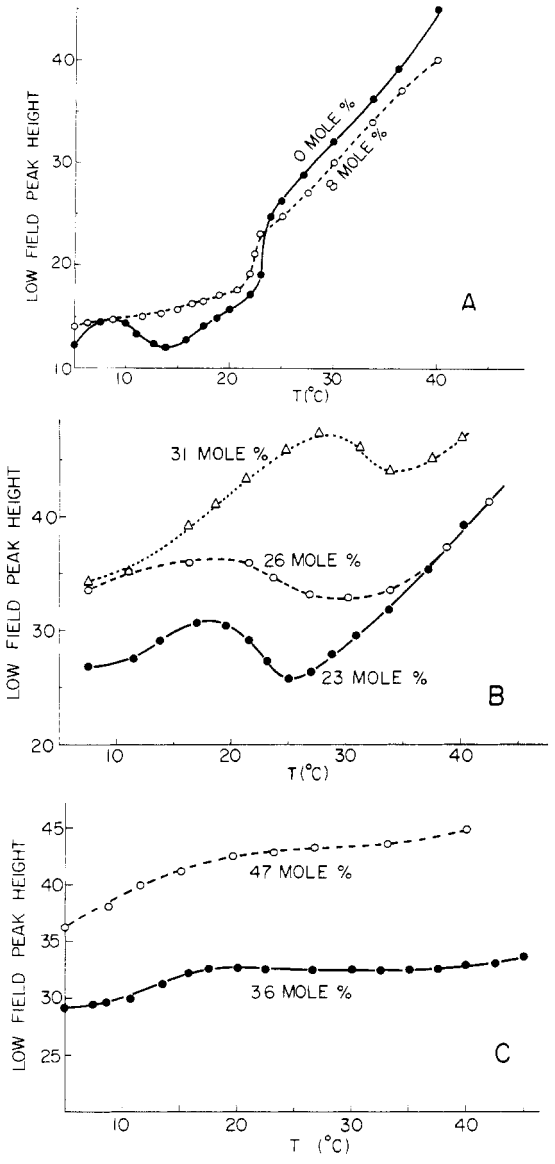

FIGURE 1: Change of the normalized peak height of the lowest magnetic field absorption for spin-label I with increasing temperature in liposomes containing a variety of dimyristoylphosphatidylcholine and cholesterol compositions and 0.1 mol % spin-label I. (A) 0 mol % cholesterol (●), 8 mol % cholesterol (○); (B) 23 mol % cholesterol (●), 26 mol % cholesterol (○), 30 mol % cholesterol (△); (C) 36 mol % cholesterol (●), 47 mol % cholesterol (○). The probable error in the compositions is 1 mol % cholesterol. The probable error in the temperatures is ±0.5 °C.

work was applied systematically to all spectra, and inaccuracies in this correction could not give rise to nonmonotonic variations of line shape with host lipid composition.

Results

Measurements of the signal intensity of the low-field peak (low-field peak height) of spin-label I, shown in Figure 1, demonstrate that the addition of more than 20 mol % cholesterol removes all signs of the cooperative chain-melting transition observed in the pure phospholipid at 23.8 °C. Similar observations with a cholestane spin-label also showed the disappearance of the transition in the presence of more than 20 mol % cholesterol.

Note in Figure 1A that the low-field peak height has a maximum at 9 °C and a minimum at 14 °C. These temperatures are in the vicinity of the $L_{\beta'}$–$P_{\beta'}$ transition. (The transition shows considerable hysteresis, so it is difficult to

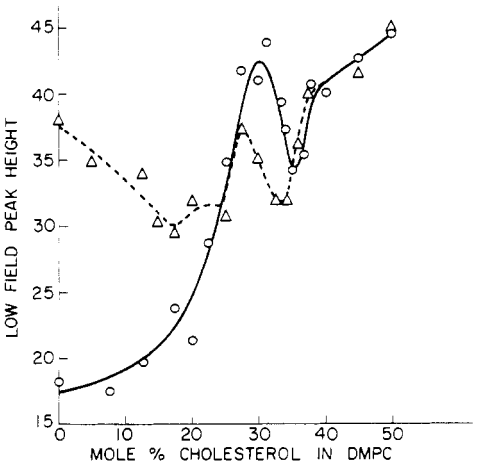

FIGURE 2: Change of the normalized peak height of the lowest magnetic field absorption for spin-label I at 33 (△--△) and 22 °C (○—○) in liposomes made from various mixtures of dimyristoylphosphatidylcholine and cholesterol.

compare the above temperatures with "the" transition temperature.) The significance of peak height maxima and minima for phase transitions and phase separations will be discussed later. The addition of 8 mol % cholesterol destroys this minimum in the spin-label I paramagnetic resonance signal.

The decrease in peak height from 0 to 20 mol % cholesterol in the fluid state (33 °C) and the gradual increase in peak height in the solid state (22 °C) are shown in Figure 2.

Between 20 and 33 mol % cholesterol, we observed that increasing the temperature did not always increase the peak height. In fact, minima in peak height were observed in this composition range (Figure 1B). These minima are not due to a large increase in spin exchange, caused by spin-label probe partitioning into a new phase, because a 10-fold increase in the probe concentration did not increase the magnitude of the minimum (it decreased it).

The position of the minima increased in temperature from ~25 °C for 23 mol % cholesterol to ~34 °C for 31 mol % cholesterol. The minimum first widened and then contracted as cholesterol was added, so that the widths were approximately the same at 23 and 31 mol % cholesterol. These minima are reminiscent of the one seen at the $L_{\beta'}$–$P_{\beta'}$ phase transition in pure dimyristoylphosphatidylcholine (Figure 1A).

The next prominent result is that 34 ± 2 mol % cholesterol has a minimum in peak height with respect to neighboring compositions for all temperatures investigated. Measurements of peak height at different temperatures demonstrate that the motion of the head group spin-label is virtually insensitive to changes in temperature in the composition region of 34–39 mol % cholesterol (Figure 1C). We also measured the change in hyperfine splitting from 12 to 33 °C at a variety of compositions (Figure 3). This plot reveals that 34 ± 2 mol % cholesterol produces a membrane which is minimally sensitive to temperature changes in its polar region. Similar results, based on the increase of the peak height of a cholestane spin-label with temperature, reveal that the hydrocarbon region is less temperature sensitive at 35 mol % than at 31 or 39 mol % cholesterol.

Between 38 and 50 mol % cholesterol, the peak height is not very temperature sensitive. The amplitude of the signal suggests relatively rapid motion of the probe.

All of these data are compiled in Figure 4. The dark line represents the minimum in peak height at 34 ± 2 mol % cholesterol. The dashed line separates the regions of fast and

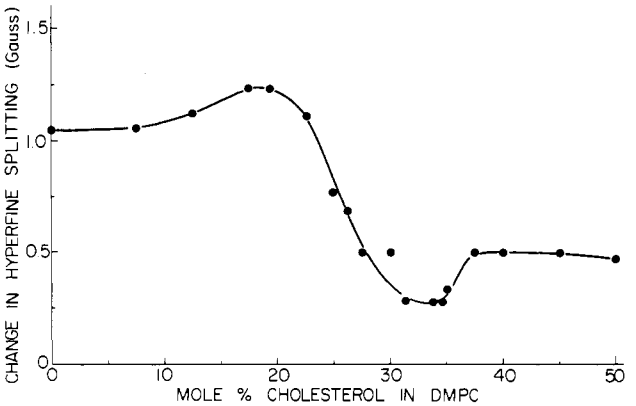

FIGURE 3: Change in the hyperfine splitting of the low- and mid-field peaks of spin-label I between 33 and 12 °C in liposomes containing dimyristoylphosphatidylcholine and cholesterol. Error of the change in hyperfine splitting is ±0.1 G. These data show that 34 mol % represents a composition where the hyperfine splitting changes the least with temperature.

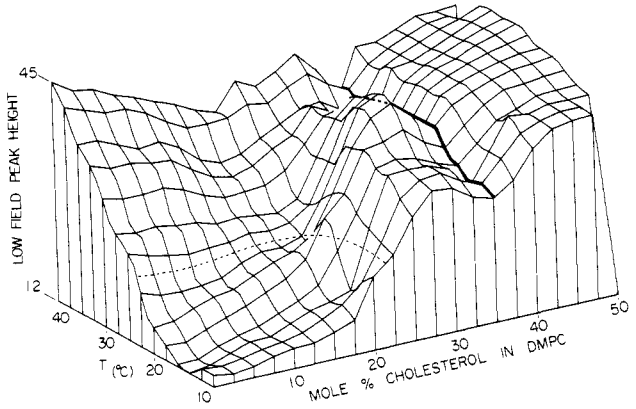

FIGURE 4: Perspective three-dimensional plot (transect plot) of the normalized low-field absorption peak height of spin-label I vs. cholesterol concentration in dimyristoylphosphatidylcholine and vs. temperature. Data at 33, 27, 22, and 12 °C were determined experimentally by varying the cholesterol concentration. Other data were obtained from the peak height vs. temperature dependence determinations for various compositions of cholesterol and dimyristoylphosphatidylcholine (as shown in Figure 1A–C). The dark solid line points out the minimum in peak height at 34 ● 2 mol % cholesterol. The dashed line blocks out the temperature–composition domain of slow phospholipid diffusion over distances of the order of microns (Rubenstein et al., 1979).

slow phospholipid diffusion which we reported earlier (Rubenstein et al., 1979).

Some of the results obtained in the present work, and also in our freeze–fracture studies of binary mixtures of phosphatidylcholines and cholesterol, appear to differ from results obtained in other laboratories. We suggest that some of these discrepancies may arise in the method of sample preparation. Our lipid mixtures are obtained by evaporation from chloroform prior to hydration. Other investigators have prepared samples by the hydration of cholesterol–phosphatidylcholine mixtures obtained by evaporation from methanol or ethanol or methanol– or ethanol–chloroform mixtures. In unpublished work in this laboratory, J. Wallace Parce has observed that mixtures of cholesterol and dimyristoylphosphatidylcholine, evaporated from methanol and hydrated, form heterogeneous liposomes having different densities. Such liposomes may well have different cholesterol concentrations.

The results we obtained with the cholestane spin-label (not presented here in any detail) differ significantly from those observed by Shimoyama et al. (1978); this difference may be due to the solvent effect mentioned above or the fact that these authors used a DL mixture of dipalmitoylphosphatidylcholine.

Discussion

The normalized paramagnetic resonance peak heights for spin-label I shown in Figure 2 exhibit a strong, nonmonotonic dependence on cholesterol concentration in the range 0–50 mol %. These results are in marked contrast to several earlier studies of the effects of cholesterol on phospholipids, using other spin-labels, which have generally reported more gradual changes in label spectra as a function of cholesterol concentration (Schreier-Muccillo et al., 1973; Marsh & Smith, 1973; Hemminga, 1975). Exceptions are to be found in the work of Shimshick & McConnell (1973), who noted abrupt changes in the paramagnetic resonance spectrum of a phospholipid spin-label in binary mixtures of cholesterol and dipalmitoylphosphatidylcholine at cholesterol concentrations on the order of 20 mol % and temperatures below the chain-melting transition temperature of dipalmitoylphosphatidylcholine. A second exception is the study of Neal et al. (1976), who found an abrupt change in the paramagnetic resonance spectrum of a cholestane spin-label in brain lipids as a function of cholesterol concentration.

Our experimental results are most easily discussed by referring to Figure 4. Throughout the temperature–composition region shown, the paramagnetic resonance low-field peak height is determined primarily by the motional freedom (frequency as well as amplitude) of the nitroxide group in I [see, e.g., Gaffney & McConnell (1974)]; peak heights may be slightly affected by spin–spin interactions between labels, but this is not a dominant effect in the present experiments [see, e.g., Figure 3 in Humphries & McConnell (1977) for the concentration dependence of resonance line widths of a lipid hapten in bilayers].

As mentioned earlier, the dashed temperature–composition line in Figure 4 denotes a region within which (temperature less than 23 °C, cholesterol concentration less than 20 mol %) the observed rate of lateral diffusion of a fluorescent lipid probe is at least an order of magnitude less than outside this region (Rubenstein et al., 1979). The diffusion results and the low-field peak heights in Figure 4 are completely consistent with one another in that at low cholesterol concentrations there is a rapid increase in head group motional freedom near the transition temperature of ~23.8 °C (Figure 1A) and a corresponding increase in the lateral diffusion coefficient of fluorescein-labeled and head group spin-labeled phospholipid molecules (Rubenstein et al., 1979; Sheats & McConnell, 1978). On the other hand, at lower temperatures, 10–20 °C, increasing cholesterol concentration brings about an abrupt increase in the rate of translational diffusion at 20 mol %, and no such abrupt change can be seen in Figure 1B; there is a rapid increase in peak height between 20 and 30 mol % at 22 °C but certainly no sharp break at 20 mol %. We believe this difference in behavior can be understood as follows.

Recent (Copeland & McConnell, 1980) as well as earlier (Kleeman & McConnell, 1976) freeze–fracture electron microscopic studies of the temperature–composition region *within* the dashed line in Figure 4 indicate that this should be regarded as a single phase in a macroscopic thermodynamic sense, but from a microscopic point of view (on the distance scale of hundreds of angstroms) the bilayer may consist of alternating, ordered domains of "fluid" (fast diffusion, 1:4 cholesterol–phospholipid complex) and "solid" (slow diffusion, essentially pure phospholipid) bands. Our freeze–fracture data showing the dependence of band spacing on cholesterol concentration agree with the early results of Verkleij et al. (1974) but not with those of Gebhardt et al. (1977). The spacing

between the solid, dimyristoylphosphatidylcholine bands increases with increasing cholesterol concentration, and the spacing appears to approach infinity asymptotically at 20 mol % cholesterol. The thermally induced melting of these bands then accounts for the sharp spike in the calorimetric data of Estep et al. (1978) and Mabrey et al. (1978) that vanishes at 20 mol % cholesterol. A quantitative analysis of the dependence of band spacing on cholesterol concentration indicates that the fraction of the phospholipids in the fluid domains is $4.0x/(1.0 - x)$ and the fraction of the phospholipids in the solid bands is $(1.0 - 5.0x)/(1.0 - x)$, where x is the mole fraction of cholesterol in the bilayer, and the fluid domains are comprised of 20 mol % cholesterol and the solid bands are pure dimyristoylphosphatidylcholine. Except when x is very close to 0.20, the spacing between the solid bands and the fluid domains, 150–800 Å, is small compared to the spatial repeat periods used in the pattern photobleaching experiments, 5–10 μm. For a fluorescent probe that partitions between solid bands and fluid domains in proportion to the number of phospholipid molecules in each region, the results of experimental measurements of apparent diffusion coefficients will in general depend in a complicated way on the diffusion coefficients within the solid and fluid domains, the time and distance scales of the measurements, and also the method of data analysis. Under the conditions of most photobleach–recovery experiments, it has been shown by theoretical calculations that one should observe a strong dependence of apparent diffusion coefficient on composition in the neighborhood of 20 mol % cholesterol (Owicki & McConnell, 1980).

Although this theoretical calculation readily accounts for the above-described apparent discrepancy of the peak height data and the diffusion data, two notes of caution are in order. Our "model" of the microphases consisting of solid bands and fluid domains within the dashed region of Figure 4 is obviously incomplete, since it does not explain the long-range order exhibited by the alternating solid bands and fluid domains. A more comprehensive theory might reveal a relation between the change in peak heights in the 20–30 mol % cholesterol concentration range and the abrupt change in diffusion rates at 20 mol % cholesterol. Also, we cannot rule out the possibility that the effects under discussion could be accounted for by other models of cholesterol–phospholipid interactions (Owicki & McConnell, 1979; Pink & Chapman, 1979).

It is also clear from the above discussion that any interpretation of the deuterium-labeled phospholipid nuclear resonance spectra in the temperature–composition region within the dashed region in Figure 4 must take into account phospholipid diffusion between the solid bands and fluid domains (Haberkorn et al., 1977; Oldfield et al., 1978). It has already been shown by Brûlet & McConnell (1976b) that lipid lateral diffusion between fluid and solid domains can affect nuclear resonance spectra.

At all temperatures and compositions outside the dashed line in Figure 4, the system is "fluid" in the sense that the lateral diffusion coefficients of fluorescent lipid probes (over macroscopic distances, 5–10 μm) are large, 10^{-8}–10^{-7} cm^2 s^{-1}. In the fluid temperature–composition region there are evidentally a number of variations of peak height with temperature and composition, but one pronounced feature in the data is the sharp minimum in peak height seen at ~34 mol % cholesterol (with varying cholesterol concentration) at all temperatures in the range 8–40 °C. See also Figure 2.

It can be seen in Figure 4, and more clearly in Figure 2, that *above* the chain-melting transition temperature, the low-field peak also has a minimum in the vicinity of 20 mol % cholesterol. According to our interpretation of the paramagnetic resonance spectra, this corresponds to a minimum in the motional freedom of the nitroxide group.

It is particularly interesting to note that Oldfield et al. (1978) have observed a *maximum* in the deuterium CD_3 nuclear quadrupole splitting at 20 mol % cholesterol in binary mixtures of deuterium-labeled dimyristoylphosphatidylcholine at the choline methyl and cholesterol at 45 and 55 °C and a break in slope at 20 mol % cholesterol (and 30 °C), when deuterium quadrupole splitting is plotted as a function of cholesterol concentration. The deuterium resonance data show no indication of a change in splitting that would mark 35 mol % as a special composition. Samples used in the work of Oldfield et al. and samples used in the present work were prepared in the same way. The maximum in the nuclear quadrupole splitting data at 20 mol % cholesterol (and above the chain-melting transition) indicates a minimal mobility of the choline methyl group, in perfect accord with the data from spin-label I. Note that both the spin-label data and the deuterium resonance quadrupole data reflect the dynamical (and to some degree, structural) properties of the bilayer system. Other dynamics-sensitive physical properties also show significant changes in the 15–25 mol % cholesterol range at temperatures above the chain-melting transition temperature. These include lateral diffusion (Rubenstein et al., 1979), ultrasonic velocity (Sakanishi et al., 1979), and water permeation (Blok et al., 1977).

Other types of experiments show no evidence of any special effects at ~20 mol % cholesterol but rather at ~33–35 mol %. These include density–composition isotherms (Gershfeld, 1978), viscometric and diffusion data for vesicles (Newman & Huang, 1975), the X-ray diffraction and model building studies of Engleman & Rothman (1972), and the ^{13}C-labeled choline methyl *chemical shift* data of Brûlet & McConnell (1976b).

Thus, the two peak height minima seen in the "fluid state" in Figures 2 and 4 occur at compositions of 20 ± 5 and 34 ± 2 mol % cholesterol where other physical properties of this binary system undergo changes.

We suggest that the broad minimum in peak height seen in Figures 2 and 4 in the fluid state at ~20 mol % cholesterol is due to two compensating effects. As cholesterol concentration is increased at low cholesterol concentration the condensing effect of a single cholesterol molecule is sensed by a sizable number (e.g., 10) of surrounding phospholipids, inhibiting the head group motional freedom and decreasing the peak height. With further increases of cholesterol concentration this effect is reversed due to the increased average spacing of the choline head groups due to the intervening cholesterol molecules. Thus, the peak height again increases above 20 mol % cholesterol.

The variation of peak height in the fluid phase in the range 25–40 mol % cholesterol is so sharp as to suggest that there must be a two-phase region (macroscopic or microscopic—see below) somewhere in this composition range. That is, as cholesterol is increased above 26–28 mol % the peak height begins to drop because a second phase with lower peak height begins to form. The phase with the higher cholesterol concentration is presumably the more ordered phase. It is difficult to say precisely where the phase boundaries are because of the strong cholesterol dependence of peak height in both phases. As a minimal statement we can say that the region 28–32 mol % cholesterol is a two-phase region for most temperatures, but the two-phase region may have a broader composition range. Note that our conclusions are consistent with the formation

of a "compound" containing, say, 34 mol % cholesterol. In this connection, see Figure 3. See also Engleman & Rothman (1972).

The deuterium resonance data referred to above give little or no hint of the spectral variation seen in Figures 2 and 4. [But see the 45 °C plot in Figure 9 in Oldfield et al. (1978).] It should be noted that the distinct "phases" referred to in our above discussion may not be thermodynamic, macroscopic phases of the sort observed in many binary mixtures of phospholipids [see, for example, McConnell (1978)] but may be microscopic in the sense of that discussed above and elsewhere in connection with the cholesterol-rich region within the dashed region of Figure 4 (Copeland & McConnell, 1980). In this case phospholipid exchange between these microphases may be fast on the NMR time scale and thus could elude detection. On the other hand, ^{13}C-labeled choline methyl NMR studies of binary mixtures of cholesterol and phosphatidylcholines are entirely consistent with the spin-label data in Figure 4 and our earlier interpretation of these data (Brûlet & McConnell, 1977).

Our discussion of the cholesterol dependence of the peak heights as it relates to phase separations can also be extended to the temperature dependence of peak heights. For example, the $L_{\beta'}$–$P_{\beta'}$ phase transition is quite pronounced in Figure 1A because of the different peak heights that label I has in the two phases. In a similar way, the maxima and minima seen in Figure 1B suggest a temperature-dependent phase separation.

The present work bears on two aspects of membrane function. It may be recalled that spin-label I (as well as a number of related lipid spin-labels) can serve as a lipid hapten for antibody binding (Brûlet & McConnell, 1977), complement activation (Esser et al., 1979), and cellular activation (Hafeman et al., 1979) by phosphatidylcholine–cholesterol liposomes containing this hapten. The effect of increasing cholesterol concentration in enhancing these functions is understandable in view of the enhanced rotational and perhaps lateral mobility of the nitroxide group. There is also a dramatic effect of cholesterol in enhancing the free-radical reactivity of head group nitroxide spin-labels similar to spin-label I in lipid bilayers (Sheats & McConnell, 1979).

The second point that warrants comment is that the interactions giving rise to peak height minima in the fluid-phase regions of Figures 1B and 4 may be present in membranes containing cholesterol and complex mixtures of phosphatidylcholines as well as other phospholipids. (We have in fact observed similar peak height maxima and minima in mixtures of cholesterol and bovine brain sphingomyelin.) Thus, modest changes in cholesterol concentration may lead to marked and nonmonotonic changes in the biophysical properties of membranes.

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References

Blok, M. C., van Deenen, L. L. M., & de Gier, J. (1977) *Biochim. Biophys. Acta 464*, 509–518.

Brûlet, P., & McConnell, H. M. (1976a) *Proc. Natl. Acad. Sci. U.S.A. 73*, 2977–2981.

Brûlet, P., & McConnell, H. M. (1976b) *J. Am. Chem. Soc. 98*, 1314–1318.

Brûlet, P., & McConnell, H. M. (1977) *Biochemistry 16*, 1209–1217.

Copeland, B. R., & McConnell, H. M. (1980) *Biochim. Biophys. Acta* (in press).

Engleman, D., & Rothman, J. (1972) *J. Biol. Chem. 247*, 3694–3697.

Esser, A. F., Bartholomew, R. M., Parce, J. W., & McConnell, H. M. (1979) *J. Biol. Chem. 254*, 1768–1770.

Estep, T. N., Mountcastle, D. B., Biltonen, R. L., & Thompson, T. E. (1978) *Biochemistry 17*, 1984–1989.

Gaffney, B. J., & McConnell, H. M. (1974) *J. Magn. Reson. 16*, 1–28.

Gebhardt, C., Grulet, H., & Sackmann, E. (1977) *Z. Naturforsch. C 32*, 581–596.

Gershfeld, N. L. (1978) *Biophys. J. 22*, 469–488.

Grant, C. W. M., & McConnell, H. M. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 4653–4657.

Haberkorn, R. A., Griffin, R. G., Meadows, M. D., & Oldfield, E. (1977) *J. Am. Chem. Soc. 99*, 7535–7537.

Hafeman, D. G., Parce, J. W., & McConnell, H. M. (1979) *Biochem. Biophys. Res. Commun. 86*, 522–528.

Hemminga, M. A. (1975) *Chem. Phys. Lipids 14*, 151–173.

Humphries, G. M. K., & McConnell, H. M. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 3537–3541.

Keana, J. F. W., & Dinerstein, R. J. (1971) *J. Am. Chem. Soc. 93*, 2808–2810.

Kleeman, W., & McConnell, H. M. (1976) *Biochim. Biophys. Acta 419*, 206–222.

Mabrey, S., Mateo, P. L., & Sturtevant, J. M. (1978) *Biochemistry 17*, 2464–2468.

Marsh, D., & Smith, I. C. P. (1973) *Biochim. Biophys. Acta 298*, 133–144.

McClare, C. W. F. (1971) *Anal. Biochem. 39*, 527–530.

McConnell, H. M. (1978) *Adv. Chem. Phys. 39*, 240–285.

Neal, M. J., Butler, K. W., Polnaszek, C. F., & Smith, I. C. P. (1976) *Mol. Pharmacol. 12*, 144–155.

Newman, G. C., & Huang, C. (1975) *Biochemistry 14*, 3363–3370.

Oldfield, E., Meadows, M., Rice, D., & Jacobs, R. (1978) *Biochemistry 17*, 2727–2740.

Owicki, J. C., & McConnell, H. M. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4750–4754.

Owicki, J. C., & McConnell, H. M. (1980) *Biophys. J.* (in press).

Pink, D., & Chapman, D. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 1542–1546.

Rubenstein, J. L. R., Smith, B. A., & McConnell, H. M. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 15–18.

Sakanishi, A., Mitaku, S., & Ikegami, A. (1979) *Biochemistry 18*, 2636–2642.

Schreier-Muccillo, S., Marsh, D., Dugas, H., Schneider, H., & Smith, I. C. P. (1973) *Chem. Phys. Lipids 10*, 11–27.

Sheats, J. R., & McConnell, H. M. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 4661–4663.

Sheats, J. R., & McConnell, H. M. (1979) *J. Am. Chem. Soc. 101*, 3272–3277.

Shimoyama, Y., Eriksson, L. E. G., & Ehrenberg, A. (1978) *Biochim. Biophys. Acta 508*, 213–235.

Shimshick, E. J., & McConnell, H. M. (1973) *Biochem. Biophys. Res. Commun. 53*, 446–451.

Solow, E. B., & Freeman, L. W. (1970) *Clin. Chem. (Winston-Salem, N.C.) 16*, 472–476.

Verkleij, A. J., Ververgaert, P. H. J. Th., de Kruyff, B., & van Deenen, L. L. M. (1974) *Biochim. Biophys. Acta 373*, 495–501.