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Phospholipid/cholesterol membranes containing *n*-alkanols: a ^{2}H-NMR study

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The influences of 1-octanol and 1-decanol on aqueous multilamellar dispersions of 1-hexadecanoyl(octadecanoyl)-2-[$^2H_{31}$]hexadecanoyl-*sn*-glycero-3-phosphorylcholine (PC-d_{31})/cholesterol (3:1) have been examined using ^{2}H-NMR. The gel to liquid crystalline phase transition of the PC-d_{31}/cholesterol dispersion is modulated by the addition of 1-alkanol, which reduces the onset temperature and increases the width of the transition. 1-Octanol has a greater effect on the transition onset and completion temperatures than does 1-decanol, as determined from analysis of the temperature-dependent ^{2}H-NMR spectra. ^{2}H-NMR C-^{2}H bond order parameters as a function of phospholipid acyl chain position at 60 °C, where all dispersions are fully liquid crystalline, have been calculated from the depaked spectra. 1-Decanol reduces the phospholipid order by only 2%. This can be attributed to the lower effective cholesterol concentration in the 1-alkanol/PC-d_{31}/cholesterol dispersions. 1-Octanol, however, reduces the phospholipid order by 10% at 60 °C. Correlations between the effects of 1-octanol and 1-decanol on phospholipid order parameters and phospholipid/cholesterol phase transitions are discussed.

Introduction

Examination of the behavior of hydrated phospholipid membranes, either pure, or with added lipid-soluble components, is fruitful for gaining insight into the physical chemistry of phospholipid interactions in natural membranes. In particular, the *n*-alkanol class of lipid-soluble compounds has been studied in an attempt to discover whether an *n*-alkanol induced perturbation of the phospholipid membrane was responsible for the anesthetic properties of these compounds since the anesthetic potencies of the *n*-alkanols correlate well with their membrane solubilities [1]. Recent progress in the field of anesthetic interactions with membranes has been summarized in several publications [2–4].

Two of the more potent anesthetic *n*-alkanols, 1-octanol and 1-decanol, are the subjects of this investigation. Previous ^{2}H-NMR studies employing deuterated alkanols in multilamellar phospholipid environments have shown that both 1-octanol and 1-decanol exhibit similar variations in orientational order from their hydroxyl ends to their methyl groups as do the phospholipid acyl chains [5,6], indicating that these *n*-alkanols pack parallel to the acyl chains in liquid crystalline bilayers, with their hydroxyl groups near the lipid/water interface. We have previously reported ^{2}H-NMR studies showing no significant change in the orientational order parameters measured in fully hydrated membranes composed of

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acyl chain deuterated phospholipids upon incorporation of either 1-octanol or 1-decanol [7]. Thus, there is no apparent disruption of the phospholipid acyl chain packing in the presence of 1-octanol or 1-decanol. In contrast, the results of ESR measurements on phospholipid/cholesterol membranes indicate that *n*-alkanols reduce the membrane order [1].

Cholesterol is a major constituent of eukaryotic neural membranes. The cortical synaptosomal plasma membranes of rats, for example, have a cholesterol to phospholipid ratio of 0.8 [8]. It is likely that the discrepancy between the ESR and ^{2}H-NMR determinations of the influence of *n*-alkanols on phospholipid membrane structure is in large part due to the presence or absence of cholesterol in the membrane preparation. Indeed, egg phosphatidylcholine/cholesterol bilayers exhibit a 4-fold greater reduction in ESR order parameter upon 1-octanol incorporation than bilayers lacking cholesterol [9]. As well, it has been reported that cholesterol is a requirement for the expression of ethanol tolerance in bilayers made from mouse brain lipid [10].

To clarify the nature of the effect of *n*-alkanols on membranes, we present ^{2}H-NMR data on aqueous multilamellar dispersions of deuterated phosphatidylcholine, cholesterol and either 1-octanol or 1-decanol. Deuterium is a non-perturbing probe and problems associated with the bulky probes often used in ESR or fluorescence studies are avoided in ^{2}H-NMR. By using a saturated phospholipid it is possible to investigate the temperature dependence of the ^{2}H-NMR spectra as the dispersions change from gel to liquid crystalline. The phase behaviour of phosphatidylcholine/cholesterol systems is complex [11] and the inclusion of an *n*-alkanol is expected to complicate it further. In this report we correlate the observed differences in the orientational order along the phospholipid's deuterated acyl chain with changes in the phase transition temperature in order to further understand the interaction of the *n*-alkanols with bilayer lipids.

Materials and Methods

[$^2H_{31}$]Hexadecanoic acid was a gift from June Yue, and was 97% pure, with the remaining 3% composed of perdeuterated pentadecanoic or heptadecanoic acids. Egg yolk lysophosphatidylcholine and deuterium depleted water were purchased from Sigma Chemical Co. Sigma lysophosphatidylcholine contains 66–68% hexadecanoic acid, 24–26% octadecanoic acid, and 6–10% others. 1-Octanol was obtained from Matheson, Coleman and Bell and 1-decanol from Aldrich Chemical Co. Cholesterol was obtained from Fisher Scientific Co. and was recrystallized from benzene before use.

1-Hexadecanoyl(octadecanoyl)-2-[$^2H_{31}$]hexadecanoyl-*sn*-glycero-3-phosphorylcholine (PC-d_{31}) was synthesized by condensation of egg yolk lysophosphatidylcholine and [$^3H_{31}$]hexadecanoic acid using 1,1′-carbonyldiimidazole as previously reported [7].

Sample preparation

Aqueous multilamellar dispersions were prepared by codissolving PC-d_{31}, cholesterol and 1-decanol, as appropriate, in $CHCl_3$ and evaporating to near dryness with dry N_2, with further drying under high vacuum overnight. The mixtures were hydrated with a quantity of deuterium-depleted water equal in weight to the combined lipid weights. For the samples containing 1-octanol, 1-octanol was added at the hydration step. The final sample size was approx. 0.5 g. After hydration, the samples were vigorously mixed using a spatula and a Vortex mixer at a temperature of approx. 50 °C, the mole ratio of PC-d_{31} to cholesterol was 3 : 1 in all cases. Where an *n*-alkanol was included, the molar proportions of PC-d_{31}, cholesterol and *n*-alkanol were 3, 1 and 1, respectively. Samples were stored in NMR tubes at −18 °C until needed.

NMR spectroscopy

^{2}H-NMR spectra were recorded using the quadrupolar echo pulse sequence [12] as previously reported [7]. Unless otherwise indicated in the figure captions. The spectral parameters used were: pulse width = 6.5 μs (flip angle = 90 °); sweep width = ±250 kHz; data set size = 2 K; inter-pulse delay = 50 μs; scan repeat time = 1 s; line broadening = 50 Hz; number of acquisitions = 1000.

First moments (M_1) of the ^{2}H-NMR spectra

were calculated using a Nicolet BNC-12 computer from the equation

$$M_1 = \int_0^\infty \omega f(\omega)\, d\omega \Big/ \int_0^\infty f(\omega)\, d\omega \quad (1)$$

where $f(\omega)$ is the intensity at a given frequency displacement ω from the Larmor frequency and the integrals become sums in practice. M_1 is directly proportional to the average quadrupolar splitting in the axially symmetric spectra obtained in liquid crystalline lipid systems [13]. At low temperatures, where spectral shapes deviate from axial symmetry, the M_1 is a quantitative measure of spectral width. The M_1 values were extrapolated to zero inter-pulse delay to minimize effects due to distortions [7]. This extrapolation is expected to result in, at most, a 5% uncertainty in M_1.

Order parameters S_{C^2H} were calculated from the splitting of the quadrupolar doublet in the depaked spectra [14]. Depaking is a deconvolution procedure whereby the spectrum characteristic of oriented lipid bilayers is calculated from the spectrum obtained from random multilamellar dispersions. The splitting $\Delta\nu$ of the quadrupolar doublet obtained from depaking is characteristic of an oriented membrane whose surface is perpendicular to the applied magnetic field and is related to S_{C^2H} by [15]

$$\Delta\nu = (3/2)(e^2qQ/h)|S_{C^2H}| \quad (2)$$

where e^2qQ/h is the static quadrupolar coupling constant (168 kHz for a C-^{2}H bond in an alkane [16]). S_{C^2H} is a measure of the extent of the angular excursions of the C-^{2}H bond with respect to the normal to the bilayer surface, averaged over the NMR time scale (usually a few μs).

Differential scanning calorimetry

DSC traces were measured using a DuPont Instruments Series 99 thermal analyzer fitted with a 910 DSC accessory. Samples (2–5 mg) in sealed pans were heated from −25 °C to +75 °C at a rate of 5 degrees per minute (Cdeg/min). The onset (completion) temperature of the main transition was determined from the intersection of the tangent to the low (high) temperature side of the endothermic peak and the baseline [6].

Results

The phospholipid used in this study, PC-d_{31}, has a perdeuterated hexadecanoyl *sn*-2 acyl chain. Hexadecanoyl acyl chains predominant at the *sn*-1 position (66–68%) with the remaining chains composed of octadecanoyl (24–26%) and others (6–10%). The thermal behaviour of aqueous dispersions of PC-d_{31} has been shown to be closely analogous to that of 1,2-dihexadecanoyl-*sn*-glycero-3-phosphorylcholine [7].

Figs. 1 to 4 present ^{2}H-NMR spectra of PC-d_{31}, PC-d_{31}/cholesterol (3 : 1), PC-d_{31}/cholesterol/1-decanol (3 : 1 : 1) and PC-d_{31}/cholesterol/1-octanol (3 : 1 : 1) 50 wt% aqueous multilamellar dispersions, respectively. The temperature range studied was 0 °C to 60 °C in all cases. The intermediate temperatures shown in Figs. 1 to 4 vary to illustrate the regions where the spectra undergo the greatest changes. Spectra were obtained at 5 Cdeg intervals, except where large spectral changes occurred. In the latter case spectra were taken at 1 Cdeg intervals.

Fig. 1 shows the temperature dependence of the

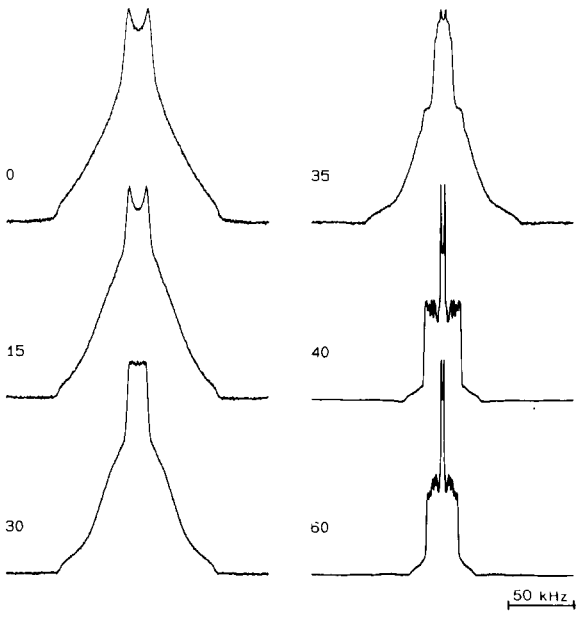

Fig. 1. ^{2}H-NMR spectra as a function of temperature (°C) for an aqueous multilamellar dispersion of PC-d_{31}. ±100 kHz is plotted in all cases. Spectral parameters are as described in Materials and Methods, except that the sweep width at 40 °C and 60 °C = ±100 kHz.

pure PC-d_{31}/water multilamellar dispersion. The spectra at 0 °C and 15 °C are very similar, and illustrate the broad, relatively featureless appearance characteristic of gel phase phosphatidylcholine. Close-packed acyl chains and slow motions on the NMR time scale cause the spectral shape to depart from the axially symmetric liquid crystalline 'powder pattern' seen above the main transition temperature. At 30 °C the spectrum has changed only slightly. The additional intensity near the resonance frequency reflects increased methyl group mobility. At 35 °C the spectrum reveals that the phase transition is incipient and sharp edges at ±16 kHz are beginning to emerge. Above the gel to liquid crystalline transition, the spectrum changes dramatically to one characteristic of uniaxial symmetry. The 40 °C and 60 °C spectra are a superposition of powder patterns of different splittings reflecting the variation in order from the middle of the bilayer to the lipid/water interface. At 40 °C the sharp edges at ±15 kHz are due to the superposition of signals from deuterons displaying nearly constant order. This is known as the 'plateau', with deuterons attached at C-3 to C-10 contributing to the signal. At 60 °C the spectrum has narrowed slightly and the intensity of the edges (at ±12 kHz) is reduced. The reduction in intensity of the edges is a consequence of the shortening of the plateau region, i.e. only deuterons attached at C-3 to C-8 contribute at this temperature.

Fig. 2 illustrates the ^{2}H-NMR spectra of a 3:1 PC-d_{31}/cholesterol dispersion. At 0 °C the spectrum has a shape similar to a typical gel phase spectrum, although the intensity of the sloping region between 10 and 60 kHz from the resonance frequency is reduced compared to the 0 °C spectrum of PC-d_{31}. This is attributed to spectral distortion: the quadrupolar echo height decayed rapidly as the inter-pulse delay was increased, indicating a short T_{2e} which is often accompanied by distortions since the second pulse of the quadrupolar echo only partially refocusses the signal [17]. Warming the sample to 15 °C does not change the spectral width, but does cause shoulders at ±28 kHz to become more prominent. These shoulders are squarer at 20 °C, and at 25 °C the spectrum is a powder pattern with sharp edges at ±28 kHz but without clear definition of peaks

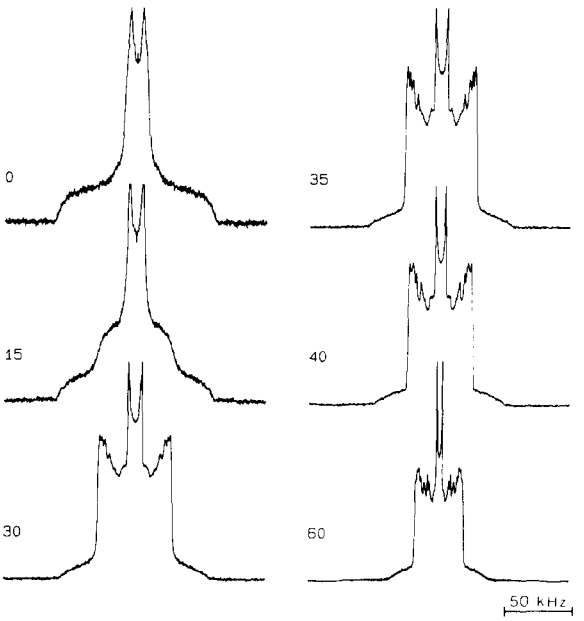

Fig. 2. ^{2}H-NMR spectra as a function of temperature (°C) for an aqueous multilamellar dispersion of PC-d_{31}/cholesterol (3:1). ±100 kHz is plotted in all cases. 60 °C sweep width = ±100 kHz.

from deuterons between the plateau region and the methyl group (spectra not shown). At 30 °C the inner peaks become resolved. Close examination of this spectrum reveals that the methyl group, responsible for the narrow splitting, gives rise to two signals: one more intense, with a splitting of 10.5 kHz and the other less intense, with a splitting of 8.2 kHz. These two signals are visible at 25 °C and 35 °C as well, and may be due to phase separation of the lipids into cholesterol-rich and cholesterol-poor phases [18] or alternatively due to microscopic heterogeneity as observed in the intermediate phase of dispersions of 1,2-ditetradecanoyl-*sn*-glycero-3-phosphorylcholine [19]. At 40 °C only the narrow methyl signal remains, indicating that the dispersion is fully homogeneous and liquid crystalline at this temperature. Further heating, to 60 °C, is accompanied by a slight reduction in the splittings. Note that the 60 °C spectrum in Fig. 2 is much wider (edge 36.3 kHz) than the 60 °C spectrum in Fig. 1 (edge 23.6 kHz), reflecting the well-known ordering effect of cholesterol on phosphatidylcholine bilayers.

Figs. 3 and 4 show the effects of added *n*-alkanol on the temperature dependence of the ^{2}H-

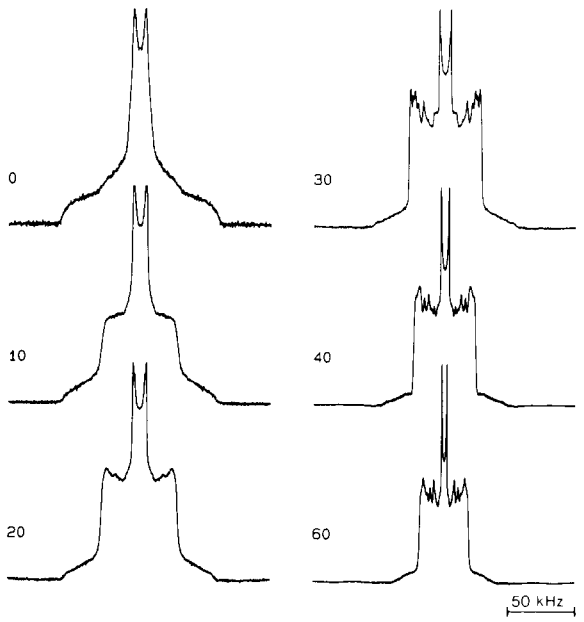

Fig. 3. ^{2}H-NMR spectra as a function of temperature (°C) for an aqueous multilamellar dispersion of PC-d_{31}/cholesterol/1-decanol (3:1:1). ±100 kHz is plotted in all cases. 40°C and 60°C sweep widths = ±100 kHz.

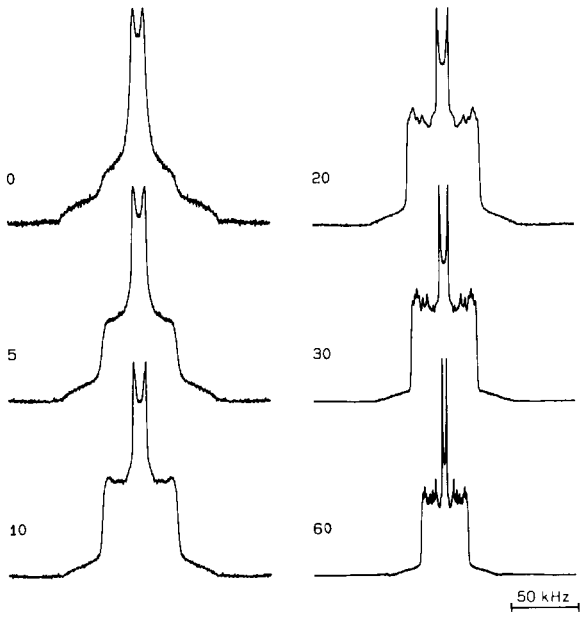

Fig. 4. ^{2}H-NMR spectra as a function of temperature (°C) for an aqueous multilamellar dispersion of PC-d_{31}/cholesterol/1-octanol (3:1:1). ±100 kHz is plotted in all cases. 30°C and 60°C sweep widths = ±100 kHz.

NMR spectra of PC-d_{31}/cholesterol. The spectral changes are similar to those shown in Fig. 2, but occur at lower temperatures. In Fig. 3 the multilamellar dispersions are made up of PC-d_{31}/cholesterol/1-decanol (3:1:1). At 0°C this system gives a gel phase spectrum. At 10°C the ±28 kHz shoulders are square and the spectrum resembles that of the PC-d_{31}/cholesterol (3:1) mixture at 20°C. At 20°C the powder pattern has been established, but without definition of the signals from deuterons between the plateau region and the methyl group, similar to the PC-d_{31}/cholesterol (3:1) sample's 25°C spectrum. The second methyl group signal is visible in Fig. 3 at 20°C and 30°C, and the spectrum at the latter temperature reveals sharp resonances. By 40°C only a single methyl group signal is evident, and the spectrum narrows gradually as the sample is heated to 60°C. The 60°C spectral width in Fig. 3 is not much different than the corresponding spectral width in Fig. 2.

In Fig. 4 the sample consists of PC-d_{31}/cholesterol/1-octanol (3:1:1). At 0°C the spectrum looks similar to the 15°C spectrum in Fig. 2. At 5°C the ±28 kHz edges are already prominent, and at 10°C the spectrum is roughly analogous to the 20°C PC-d_{31}/cholesterol (3:1) system. The second methyl group signal is visible at 10°C and 20°C in Fig. 4, and at 20°C the component resonances are beginning to sharpen. A single methyl resonance is observed by 30°C indicating that the sample has achieved liquid crystalline homogeneity at a temperature ten degrees (10 Cdeg) below the other cholesterol-containing samples. From 30°C to 60°C the spectrum narrows, and, at 60°C, the spectrum is visibly narrower than the 60°C spectra in Figs. 2 and 3.

Table I lists the quadrupolar splittings of component peaks in the 60°C spectra in Figs. 1–4, together with the assignment of the deuteron resonances to particular positions along the phospholipid's acyl chain. Assignment was accomplished as previously described [7], except that no attempt was made to pinpoint the location of the C-2 deuteron splittings. The C-2 resonances have reduced splittings compared to the plateau value due to the orientation of this segment of the phosphatidylcholine's *sn*-2 chain, and there is some

TABLE I

QUADRUPOLAR SPLITTINGS FOR THE *sn*-2 CHAIN SEGMENTS OF PC – d_{31} AQUEOUS DISPERSIONS, SHOWING THE EFFECT OF ADDED CHOLESTEROL (Chol) AND *n*-ALKANOL, AT 60 °C

Acyl chain position	Quadrupolar splitting (kHz) [a]			
	PC-d_{31}	PC-d_{31}/ Chol (3:1)	PC-d_{31}/ Chol/ 1-decanol (3:1:1)	PC-d_{31}/ Chol/ 1-octanol (3:1:1)
C3–C8	23.6	36.3	36.7	34.9
C9	22.0	36.3	36.7	33.4
C10	19.9	36.3	34.5	30.8
C11	18.5	33.6	32.2	28.9
C12	16.4	31.2	29.3	25.5
C13	15.0	27.9	26.3	22.6
C14	12.3	23.0	21.3	18.3
C15	9.5	17.9	16.2	13.8
C16	2.10	4.05	3.66	3.08

[a] Quadrupole powder pattern splittings are reported, i.e. 1/2 of the depaked spectrum splittings given by Eqn. 2.

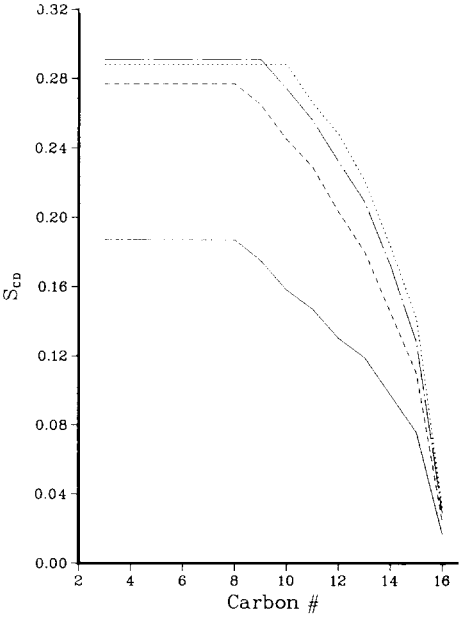

Fig. 5. Order parameter profiles at 60 °C. The S_{C^2H} values were calculated using quadrupolar splittings measured from the 60 °C powder patterns shown in Figs. 1–4, after depaking. PC-d_{31}/cholesterol (3:1), ·····; PC-d_{31}/cholesterol/1-octanol (3:1:1), — — —, PC-d_{31}/cholesterol/1-decanol (3:1:1), ·–·–·; PC-d_{31}, ———.

uncertainty in the values of the splittings when selectively deuterated analogues are not used. For all other positions increasing splittings are assigned to methylene deuterons in a monotonic fashion starting from the methyl group (the narrowest splitting) until the composite plateau peak, which exhibits the maximum splitting, is reached. The remaining deuterons are assigned to the plateau, which at 60 °C contains contributions from six to eight methylene groups depending on the sample.

Fig. 5 presents order parameter profiles for the four multilamellar dispersions at 60 °C. By comparing the profile of PC-d_{31} with that of PC-d_{31}/cholesterol it is clear that cholesterol greatly increases the order parameter, i.e. restricts the extent of motion of the phospholipid acyl chain at all positions. Inclusion of 1-decanol does not appreciably change the plateau order parameter but does reduce the S_{C^2H} along the rest of the chain by 5–10%. The plateau for PC-d_{31}/cholesterol extends from C-3 to C-10 at 60 °C, with a quadrupolar splitting of 36.3 kHz (Table I), while the average splitting for the same methylenes in the PC-d_{31}/cholesterol/1-decanol dispersion at 60 °C is almost identical at 36.4 kHz. From C-11 to C-16 the ratios of the order parameters in PC-d_{31}/cholesterol/1-decanol to those in PC-d_{31}/cholesterol decrease monotonically from 0.96 to 0.90. 1-Octanol reduces the plateau S_{C^2H} of PC-d_{31}/cholesterol by about 5% but towards the methyl group the reduction exceeds 20%. The average quadrupolar splitting for C-3 to C-10 in PC-d_{31}/cholesterol/1-octanol at 60 °C is 34.2 kHz. From C-11 to C-16 the ratios of the order parameters in PC-d_{31}/cholesterol/1-octanol to PC-d_{31}/cholesterol decline from 0.86 to 0.76, monotonically.

It is useful to document the spectral narrowing that takes place as the sample is warmed by plotting M_1 vs. temperature, as shown in Fig. 6. The PC-d_{31} sample has a sharply sigmoidal transition with a midpoint at 37 °C. This is 2.5 °C lower than the PC-d_{31} preparation used in Ref. 7. The difference is attributed to the presence of 3% pentadecanoic and heptadecanoic acids in the [$^2H_{31}$]hexadecanoic acid sample used to synthesize the present PC-d_{31}. Upon addition of cholesterol, Fig. 6 shows that the reduction in M_1, reflecting the spectral narrowing accompanying

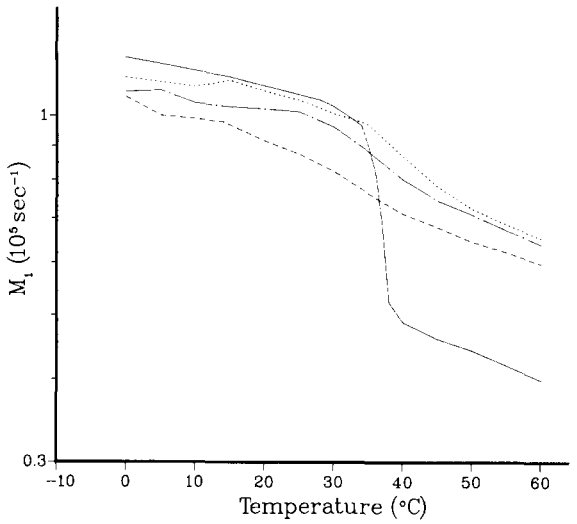

Fig. 6. M_1 vs. temperature plots for the four samples illustrated in Figs. 1–4. PC-d_{31}/cholesterol (3:1), ·····; PC-d_{31}/cholesterol/1-octanol (3:1:1), — — —; PC-d_{31}/cholesterol/1-decanol (3:1), ·–·–·; PC-d_{31}, ———.

the phase transition, is much decreased. A slight inflection at 35 °C in the PC-d_{31}/cholesterol (3:1) M_1 curve is visible, and a gradual reduction in slope occurs between 45 °C and 50 °C. At 60 °C the PC-d_{31}/cholesterol sample's M_1 value is 1.6-times that of PC-d_{31}. Below 35 °C the M_1 value for PC-d_{31}/cholesterol (3:1) is slightly less than that for pure PC-d_{31}, indicating that gel-phase acyl chain packing has been disturbed somewhat. Upon addition of 1-decanol, the M_1 vs. temperature plot has an inflection in the 25–30 °C range, levelling out again at 40–45 °C. At all temperatures the M_1 is slightly reduced from the value in PC-d_{31}/cholesterol. The reduction is small for temperatures of 50 °C or higher; at 60 °C the ratio of the M_1 values of PC-d_{31}/cholesterol/1-decanol (3:1:1) and PC-d_{31}/cholesterol (3:1) is 0.98. 1-Octanol has a greater effect on the M_1 vs. temperature plot. There appears to be an inflection at 15 °C, but there is no observable levelling off of the curve at higher temperatures. The M_1 of PC-d_{31}/cholesterol/1-octanol is reduced at all temperatures compared to the M_1 values for PC-d_{31}/cholesterol (3:1) and PC-d_{31}/cholesterol/1-decanol (3:1:1). At 60 °C the ratio of the M_1's of PC-d_{31}/cholesterol/1-octanol (3:1:1) and PC-d_{31}/cholesterol (3:1) is 0.91. The low temperature nonlinearities in Fig. 6 occur at temperatures where it was necessary to extrapolate to zero inter-pulse delay to measure M_1, and so these values are subject to greater uncertainties than the remaining M_1 values. The extrapolations expected to result in significant (5%) error occurred at $T = 0$ °C and 5 °C for PC-d_{31}/cholesterol/1-octanol, $T = 5$ °C, 10 °C and 15 °C for PC-d_{31}/cholesterol/1-decanol and $T = 10$ °C, 15 °C and 20 °C for PC-d_{31}/cholesterol.

The DSC results for the four model membrane systems are described in Table II. DSC serves to monitor the phase behaviour of the bulk dispersion. PC-d_{31} aqueous multilamellar dispersions have, in addition to the main transition described in the table, a pretransition which is eliminated by cholesterol and/or *n*-alkanol [7]. Cholesterol reduces the onset temperature and broadens the main phase transition. 1-Decanol and 1-octanol reduce the onset temperature even further, although the completion temperature appears to remain relatively constant. The enthalpy of the transitions in the presence of cholesterol is low, and conseqently the error in measuring the transi-

TABLE II

TRANSITION TEMPERATURES FOR AQUEOUS MULTILAMELLAR DISPERSIONS OF PC-d_{31}, PC-d_{31}/CHOLESTEROL (3:1), PC-d_{31}/CHOLESTEROL/1-DECANOL (3:1:1) AND PC-d_{31}/CHOLESTEROL/1-OCTANOL (3:1:1), OBTAINED FROM DSC THERMOGRAMS

Sample	Pretransition onset (°C)	Main transition (°C)	
		onset	completion
PC-d_{31}	30.5 ± 0.5	37.8 ± 0.5	40.6 ± 0.6
PC-d_{31}/cholesterol (3:1)	–	29 ± 2	45.0 ± 0.3
PC-d_{31}/cholesterol/1-decanol (3:1:1)	–	27 ± 2	46 ± 2
PC-d_{31}/cholesterol/1-octanol (3:1:1)	–	23 ± 2	47 ± 1

tion temperatures is appreciable. This is particularly true in the case of the completion temperature, where sample size and heating rate can influence the measurement considerably. The values reported in Table II are the means of three repetitions of the DSC heating trace in each case, at a scan rate of 5 Cdeg/min.

Discussion

The major findings of this work are that 1-octanol and 1-decanol reduce the gel to liquid crystalline phase transition temperature in fully hydrated PC-d_{31}/cholesterol dispersions and also reduce the order of the phospholipid acyl chains in these dispersions at temperatures above the PC-d_{31}/cholesterol gel to liquid crystalline melting point. These two effects are related. 1-Decanol, which has only a small effect on the transition temperature, similarly has a small effect on the PC-d_{31} acyl chain order at 60 °C. 1-Octanol has a greater effect on both phase behaviour and order. The difference between the two alkanols is likely due to the chain length disparity between the alkanol and the phospholipid acyl chains. We have shown previously [5,7] that 1-decanol intercalates between 1,2-dihexadecanoyl-*sn*-glycero-3-phosphorylcholine molecules in lamellar dispersions, causing negligible disruption. 1-Octanol, having a shorter chain length, is less able to intercalate in gel phase dispersions without disrupting the phospholipid acyl chain packing and hence the phase transition temperature is lowered considerably.

When comparing the order of the acyl chains of a phospholipid in a bilayer it is important to consider the phase behaviour if one is interested in the actual physical disruption to the membrane due to an added perturbant. Seelig and Browning [20] applied the reduced temperature concept to membrane systems and found fair agreement between the order parameter profiles of different phospholipids with a wide range of transition temperatures when compared at a fixed reduced temperature. For broad transitions, the onset temperature was taken as the transition temperature in Ref. 20. More recently, ^{2}H-NMR order parameters have been referenced to the completion temperature of the gel to liquid crystalline transition [6,21]. The completion temperature is more accurately measured by ^{2}H-NMR than by DSC due to the variability in the DSC results caused by sample size or heating rate, especially when low-enthalpy transitions are being monitored. The ^{2}H-NMR data indicate that both PC-d_{31}/cholesterol (3 : 1) and PC-d_{31}/cholesterol/1-decanol (3 : 1 : 1) have the same completion temperature, being entirely in the liquid crystalline phase by 40 °C. The 40 °C spectra (see Figs. 2 and 3) no longer contain any broad component nor is there any evidence of coexisting phases. Thus, the observation of similar order parameter profiles at 60 °C in these two lamellar systems is not surprising.

In contrast, PC-d_{31}/cholesterol/1-octanol (3 : 1 : 1) is fully melted by 30 °C (see Fig. 4). Therefore, this sample's behaviour should correspond closely to the behaviour of PC-d_{31}/cholesterol when the temperatures at which their respective phase transitions are complete are taken into account. For temperatures close to the transition, the difference between a reduced temperature T_r comparison ($T_r = (T - T_{mf})/T_{mf}$, where T_{mf} is the completion temperature of the transition) and a simple ΔT comparison is negligible ($\Delta T = T - T_{mf}$), so we have chosen the latter method, which is also used in Ref. 6. To illustrate the result of a shift of 10 °C in the data for PC-d_{31}/cholesterol/1-octanol (3 : 1 : 1), Fig. 7 compares M_1 vs. $(T - T_{mf})$ for PC-d_{31}/cholesterol and PC-d_{31}/cholesterol/1-octanol. The result of the comparison is that the two systems now correspond very closely at a fixed temperature above T_{mf}.

The remaining slight differences in order between those dispersions that contain 1-alkanol and those that do not, arise from the different effective cholesterol concentrations in the two sample categories. Since both 1-decanol and 1-octanol are approximately half the length of the PC-d_{31} acyl chains, the increase in the volume of the hydrophobic region of the membrane upon incorporation of one mole of 1-alkanol for each three moles of phospholipid is approximately 8.3%. The ratio of 'phospholipid equivalents' (four 1-alkanol molecules corresponding to 1 phospholipid) to cholesterol in the PC-d_{31}/cholesterol/1-alkanol (3 : 1 : 1) dispersions is 3.25, giving an overall cholesterol concentration of 23.5%. The M_1 values for PC-d_{31}/cholesterol/1-decanol (3 : 1 : 1) and

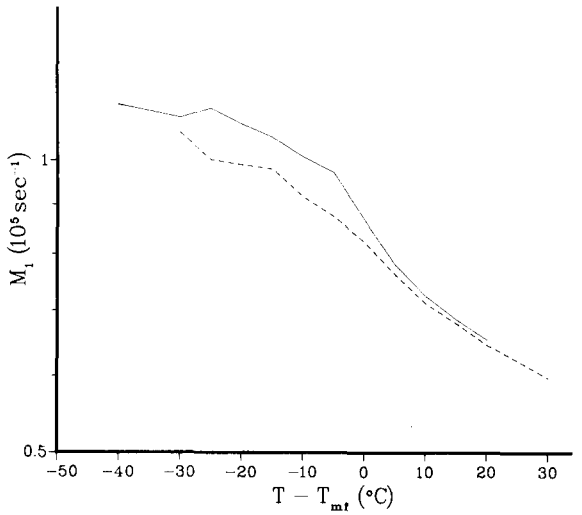

Fig. 7. M_1 vs. $T - T_{mf}$, where T_{mf} is the temperature at which the gel to liquid crystalline transition is complete, for PC-d_{31}/cholesterol (3:1) and PC-d_{31}/cholesterol/1-octanol (3:1:1). PC-d_{31}/cholesterol (3:1), _____; PC-d_{31}/cholesterol/1-octanol (3:1:1), — — —.

PC-d_{31}/cholesterol/1-octanol (3:1:1) at a temperature 20 Cdeg above their transition completion temperatures agree to within 1%, with an average value of $(0.642 \pm 0.003) \cdot 10^5$ s^{-1}. This value is 2% below $0.6536 \cdot 10^5$ s^{-1}, the M_1 of PC-d_{31}/cholesterol (3:1) at the equivalent temperature. Assuming that the effect of added cholesterol on phospholipid acyl chain order is linear to a cholesterol concentration of at least 25%, the M_1 value of a 23.5% cholesterol content is calculated to be $0.638 \cdot 10^5$ s^{-1}, which is in close agreement with the value measured in the alkanol-containing dispersions.

It is also interesting to compare the isothermal effects of added 1-alkanols on the PC-d_{31} acyl chain order in the presence of cholesterol in liquid crystalline multilamellar dispersions. Fig. 5 shows that there is a significant reduction in order parameter at 60 °C for all positions of the chain when 1-octanol is incorporated. The reduction in order in the plateau region is small, about 6%, and increases toward the methyl group, whose splitting is reduced by 25%. In the case of 1-decanol, the plateau region does not experience reduced order, but the other C^2H_2 positions and the methyl group do have order parameter reductions of 4–10%. It is clear, then, that the constant temperature perturbation due to added 1-alkanol is maximal for those acyl chain positions in the middle of the bilayer, since the plateau order is affected little even with a large quantity of added 1-octanol or 1-decanol. This is intuitively reasonable given that the 1-alkanols are known to have a time-averaged orientation parallel to the phospholipid acyl chains with their hydroxyl groups near the glycerol backbone [5,6], giving the C-12 to C-16 region of the phospholipid acyl chain potentially greater motional freedom. A possible explanation for the observed isothermal disordering effect of 1-alkanols on phospholipid acyl chains in PC-d_{31}/cholesterol dispersions versus PC-d_{31} dispersions is that 1-alkanols interact with cholesterol alone. If such a preferential interaction occurred, the 1-alkanol could serve to partially shield the phospholipid chains from the ordering effect of cholesterol. However, since membrane bilayers are fluid above the main transition it is difficult to imagine such a preferential interaction between 1-alkanols and cholesterol to the exclusion of 1-alkanols and PC. As well, differences in disordering capabilities of 1-octanol and 1-decanol on the PC-d_{31}/cholesterol membrane dispersion could not be explained by 1-alkanol/cholesterol interactions unless 1-octanol associated more strongly than 1-decanol with cholesterol.

Our observation of reduced order in liquid crystalline PC-d_{31}/cholesterol (3:1) dispersions containing 1-alkanols is consistent with previous ESR findings in egg-yolk PC/cholesterol (2:1) dispersions [1,9]. Due to differences between the membrane compositions in the present work and the previous ESR studies quantitative comparisons are not possible. Also, thermal phase transition effects were not considered in Refs. 1 and 9. Our previous study of PC-d_{31}/1-alkanol dispersions [7] showed no significant change in liquid crystalline order parameters due to the presence of 1-octanol or 1-decanol at a phospholipid:1-alkanol ratio of 3:1. The effect on order parameters we observe in the current study can therefore be unambiguously assigned to the incorporation of cholesterol in the membrane.

In conclusion, we have shown that 1-octanol or 1-decanol causes a reduction in the ^{2}H-NMR order parameter of a deuterated phosphatidylcholine/cholesterol bilayer, with 1-octanol being more ef-

fective. In the absence of cholesterol, these two alkanols do not reduce the phospholipid acyl chain order [7]. Expression of the alkanols' disordering effect is directly related to the influence of cholesterol on the phase behaviour of the dispersions. Two of the theories of anesthetic mechanism which have received considerable attention are the disordered lipid hypothesis, which states that anesthetics disorder or 'fluidize' the membrane, and the phase transition hypothesis, which states that anesthetics alter the phase behavior of a critical membrane region. With regard to the latter hypothesis the heterogeneity of neural membranes usually prevents the observation of a phase transition, although there has recently been a report [22] of a transition in rat synaptic plasma membranes centered at 37 °C. As cholesterol is a major component of neural membranes our results indicate that the disordered lipid and the phase transition hypotheses are likely to be interrelated to the point of being inseparable.

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