Biochimica et Biophysica Acta 939 (1988) 64–78
Elsevier

BBA 73927

The interaction of *n*-alkanols with lipid bilayer membranes: a ^{2}H-NMR study

P.W. Westerman [a,b], J.M. Pope [c], N. Phonphok [a,b], J.W. Doane [a,b] and D.W. Dubro [c]

[a] *Department of Biochemistry, Northeastern Ohio Universities College of Medicine, Rootstown OH (U.S.A.),* and [b] *Liquid Crystal Institute and Department of Physics, Kent State University, Kent, OH (U.S.A.)* and [c] *School of Physics, The University of New South Wales, Kensington, N.S.W. (Australia)*

(Received 28 April 1987)

Key words: Alcohol–membrane interaction; *n*-Alkanol; Anesthesia; Cut-off effect; NMR, ^{2}H-

The interaction of eight *n*-alkanols with bilayers of dimyristoylphosphatidylcholine (DMPC) has been studied by deuterium nuclear magnetic resonance (^{2}H-NMR). At comparable temperatures and concentrations of solute in the bilayer, order parameters measured at the 1-methylene segment of the *n*-alkanols show a maximum for *n*-dodecanol. For both *n*-dodecanol and *n*-tetradecanol, orientational ordering shows a maximum at the C-4 to C-7 methylene segments, with labels at both ends of the *n*-alkanol exhibiting reduced order. These observations are consistent with earlier findings for *n*-octanol and *n*-decanol. Unlike the longer chain *n*-alkanols, ordering in *n*-butanol decreases from the hydroxyl group end to the methyl group end of the molecule. Orientational ordering at nine inequivalent sites in DMPC, has also been measured as a function of temperature, for bilayers containing *n*-butanol, *n*-octanol, *n*-dodecanol and *n*-tetradecanol. At the 3*R*,*S* sites on the glycerol backbone, for comparable temperatures and solute concentrations, *n*-butanol produces a larger disordering than the other *n*-alkanols. This result probably reflects the greater fraction of time spent by the hydroxyl group of *n*-butanol in the vicinity of the lipid polar head group compared with the hydroxyl group in longer chain *n*-alkanols. It was found that *n*-octanol orders the acyl chains of DMPC, unlike *n*-butanol which disorders them, and the longer chain *n*-alkanols which have little effect. Within experimental error, the effect of *n*-dodecanol on order at all sites in DMPC is the same as *n*-tetradecanol. The influence of *n*-alkanols on DMPC ordering at twelve sites has been compared with that of cholesterol which is shown to interact with DMPC bilayers in a distinctly different manner from the *n*-alkanols.

Introduction

It is still unclear how and where anesthetics exert their pharmacological effects on excitable tissues and produce the physiological phenomenon known as anesthesia [1–5]. Disagreement exists as to whether the primary site of action of anesthetics is lipid or protein [1,2,6,7]. Lipid-based unitary theories of anesthesia propose that general anesthetics first perturb the lipid bilayer, resulting in either a functional change in excitable membrane proteins or in a change of membrane permeability [8–10]. Those lipid-based unitary theories invoking ultimately an excitable membrane protein may be grouped into three broad categories, depending on whether they emphasize membrane expansion or thickening, an anesthetic-induced phase transition, or changes in fluidity as triggering the onset of anesthesia [1–5].

Abbreviations: DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine.

Correspondence: P.W. Westerman, Department of Biochemistry, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272, U.S.A.

As a first step in critically evaluating lipid-based unitary theories of anesthesia, it is necessary to determine the distribution of anesthetic molecules in lipid bilayers and their effect on bilayer structure. A systematic investigation of the bilayer disposition of *n*-alkanols in membranes provides a particularly useful approach for the study of the mechanism of anesthesia because of the so-called 'cut-off' effect observed in this homologous series [1]. The anesthetic potency (determined in tadpoles) of saturated *n*-alkanols increases with increasing chain length up to 1-dodecanol after which it decreases sharply. *n*-Tridecanol is a partial anesthetic, whereas *n*-tetradecanol and higher alcohols are totally inactive.

The changes induced by members of the *n*-alkanol family in lipid bilayer phase transitions and acyl chain order and mobility have been examined using ^{2}H-NMR [11–14], ESR [15–19], DSC [20–27], fluorescence [25,28] and turbidity measurements [29]. *n*-Alkanols can either raise or lower the main gel to liquid crystal phase transition of the phospholipid, depending on the length of the alcohol and its concentration [26] as well as the type of lipid used [27]. 1-Alkanols are found to decrease slightly the ESR order parameter of spin-labelled lipid and fatty acid probes in both model and biological membranes. The degree of disordering is proportional to the concentration of anesthetic in the membrane and also depends upon the lipid composition [30]. Changes in steady-state depolarization of fluorescent probes in several biological membranes, caused by 1-alkanols have been attributed to increased motion of the lipid acyl chains. ^{2}H-NMR has been used to study the interaction of *n*-butanol, *n*-octanol, and *n*-decanol with DMPC and dipalmitoylphosphatidylcholine (DPPC) bilayer membranes [11–14]. Unlike the nitroxide spin-label and fluorescent probes, the deuterium nucleus is a non-perturbing probe that can be attached to the *n*-alkanol and water components of the system as well as to the lipid. It has been found that the order profiles for both *n*-octanol and *n*-decanol exhibit a maximum at carbon segments 3–5, with reduced order being displayed at each end of the molecule [13,14]. The shape of the profile for *n*-octanol is dependent upon both solute concentration and extent of bilayer hydration [13]. 1-Alkanols have been found, at 25–35 mol% concentration, to have no significant effect on the profile on the C–^{2}H bond order parameter along the phospholipid acyl chain except in the upper or 'plateau' region, where *n*-octanol and *n*-decanol increase the order while *n*-butanol decreases it [13].

In order to examine further the interaction of *n*-alkanols with phospholipid bilayers we have undertaken a systematic study of the interaction in the L_α phase of *n*-butanol, *n*-octanol, *n*-dodecanol and *n*-tetradecanol with DMPC bilayer membranes. Both *n*-alkanol and lipid have been extensively deuterium labelled, especially in the glycerol backbone and choline headgroup regions of the phospholipid. Comparable data have been obtained for cholesterol, to determine whether different patterns of changes in lipid ordering can be found for this related solute. It is our aim to associate different patterns of changes in ordering to the time-averaged disposition of the solute in the bilayer. DSC measurements have been made on the same systems so that comparisons of ordering may be made at the same reduced temperatures.

Experimental procedures

Materials

Unlabeled DMPC was obtained from Avanti Polar Lipids, Inc., Birmingham, AL. Deuterated solutes and reagents (MeO^2H, ^2H_2O, $LiAl^2H_4$, NaB^2H_4) were purchased from Aldrich Chemical Co., Milwaukee, WI, KOR Isotopes, Cambridge, MA, and MSD Isotopes, Montreal, Canada.

Synthesis of ^{2}H-labelled fatty acids

Deuterium exchange at the α-position of tetradecanoic acid was accomplished by using a modification of the method of Aasen et al. [31]. Perdeuterated butanoic, dodecanoic and tetradecanoic acid were prepared by catalytic exchange at high temperature and pressure, of the alkanoic acid with ^2H_2O in a pressure vessel according to the procedure of Dinh-Nguyen and Stenhagen [32]. The purity of the ^{2}H-labelled alkanoic acid was confirmed by GLC of the methyl ester. GLC was performed on a Hewlett-Packard 5840 gas chromatograph with a column packed with 10% Silar-10C on 100–120 mesh gas Chrom Q. The

position and extent of isotopic incorporation of the ^{2}H-labels were determined by ^{1}H- and ^{13}C-NMR and mass spectrometry. Natural abundance ^{13}C-NMR and ^{1}H-NMR were obtained on Bruker WP80, Varian FT-80 and EM 360 spectrometers.

Synthesis of ^{2}H-labelled DMPC

DMPC, selectively ^{2}H-labelled on the 2′-positions of both *sn*-1 and *sn*-2 chains was prepared by acylation of GPC · $CdCl_2$ with activated derivatives of 2,2-[$^2H_2$]tetradecanoic acid [33,34]. Using the same procedures with perdeuterated tetradecanoic acid, DMPC, perdeuterated on both acyl chains, was prepared. In each of the above syntheses, the product was isolated by column chromatography on silica gel, eluting with chloroform/methanol (8 : 2 or 7 : 3, v/v). TLC analyses were carried out on Absorbosil Plus-1 silica gel plates (Applied Sciences), using chloroform/methanol/water (65 : 25 : 4, v/v) as the solvent system and Molybdenum blue and iodine vapor to visualize spots.

Selective deuteration of the glycerol moiety of DMPC was achieved by the following procedure. *rac*-Isopropylidene [1,3-$^2H_4$]glycerol was synthesized from diethylacetoxymalonate by reduction with $LiAl^2H_4$ [35]. *rac*-Isopropylidene [1,3-$^2H_4$]glycerol was used to prepare in several steps, 1,2-dimyristoyl-*rac*-[1,1,3,3-$^2H_4$]glycerol, which was then converted to DMPC, ^{2}H-labelled at the 1*R*,*S* and 3*R*,*S* sites in the glycerol backbone

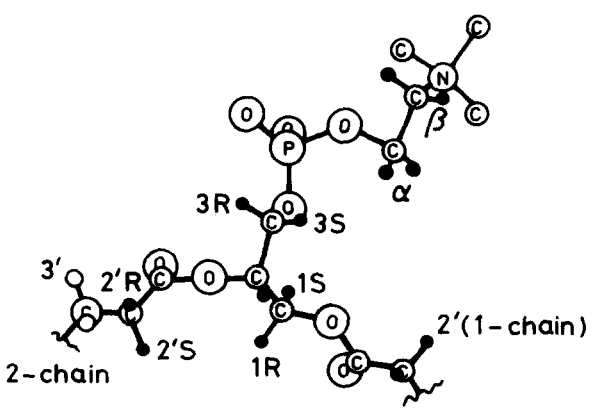

Fig. 1. Portion of the structure of dimyristoylphosphatidylcholine (DMPC) indicating the selectively ^{2}H-labelled sites in the region of the headgroup and glycerol backbone.

[36]. DMPC, selectively deuterated on the choline moiety, was prepared by the same procedure utilizing ^{2}H-labelled ethanolamine [37] at the appropriate step. If 1,2-dimyristoyl-*rac*-[1,1,3,3-$^2H_4$]glycerol is used as starting material, DMPC, ^{2}H-labelled at eight sites in both the glycerol backbone and choline headgroup is synthesized. The positions of the labelled sites in the glycerol backbone and headgroup region of the DMPC molecule are illustrated in Fig. 1.

Synthesis of ^{2}H-labelled n-alkanols

Methyl esters of specifically (2,2-$^2H_2$) deuterated or perdeuterated butanoic, dodecanoic or tetradecanoic acid were reduced with $LiAlH_4$ to give a quantitative yield of butanol, dodecanol or tetradecanol. The alcohol was ^{2}H-labelled at all sites except the 1-position if perdeuterated ester was starting material, or only at the 2-position if α-deuterated ester was the starting material. Butanol, pentanol, hexanol, octanol, decanol, dodecanol, tetradecanol and hexadecanol, specifically ^{2}H-labelled at their 1-positions, were prepared from the appropriate commercially available methyl (or ethyl) ester (Aldrich) by reduction with $LiAl^2H_4$. The crude product in each case was purified by vacuum distillation to give material with physical properties consistent with published data.

Sample preparation and data acquisition

The purity of phospholipid samples was checked by TLC both before and after recording NMR spectra. DMPC samples containing 33 mol% *n*-alkanol were prepared by adding the appropriate quantities of *n*-alkanol and ^{2}H-depleted water (Aldrich) to a weighed amount of vacuum-dried DMPC in a 5 mm or 10 mm NMR tube with an approx. 1 mm constriction. A lipid-to-water molar ratio of 1 : 25 was used except where indicated. Additional water has no observable effects on measured quadrupole splittings in either lipid or *n*-alkanol. The NMR tube was then sealed and the contents were mixed by repeated centrifugation through the constriction at a temperature above the phase transition. In samples containing ^{2}H-labelled DMPC, the labelled lipid was first diluted with a 2-fold excess of commercial unlabelled DMPC, in order to reduce any influence of minor

impurities in the synthetic material. NMR spectra of samples containing ^{2}H-labelled butanol, pentanol, hexanol, octanol and decanol were recorded at 30.87 MHz on a 'home-built' spectrometer. Samples containing perdeuterated and specifically labelled dodecanol, tetradecanol and hexadecanol were obtained with the same instrument. The $\pi/2$ pulse width for this spectrometer was 3–4.5 μs, which is adequate power for the ^{2}H-NMR spectra in all the systems investigated. Because the normal two-pulse quadrupole echo sequence [38] was affected by acoustic ringing, a modified quadrupole echo sequence was used for data acquisition [39]. Typically, the value of τ in the quadrupole echo sequence was 80 μs, and the pulse sequence was normally repeated three to five times per second. Spectra were recorded 'on-resonance' using quadrature detection. Sample temperature was controlled by a computer-controlled liquid (ethylene glycol/H_2O) flow system, to an accuracy of less than 0.05 C° in a single-coil probe head with a temperature gradient less than 0.1 C° across the sample. The sample tube and coil are encased in a boron nitride block which sits in a cavity of a larger block of the same material, through which the refrigerant circulates. The whole unit is enclosed in a vacuum-jacketed and water-cooled brass oven. Sample temperature is automatically read at the beginning, middle, and end of an accumulation, while the RF transmitter is off, by a 100 Ω platinum resistor (System 193, Keiphley) placed in close proximity to the sample coil.

Spectra from the specifically ^{2}H-labelled DMPC bilayers containing dodecanol or tetradecanol were recorded on a Bruker CXP300 NMR spectrometer operating at 46.063 MHz. Further details of this instrumentation and its temperature control are contained in Ref. 11.

DSC measurements were made on a Perkin-Elmer differential scanning calorimeter, normally at a heating rate of 5 C°/min. While this produced significant broadening of the transitions for pure lipid samples, it was found to be adequate for observing the transitions of the ternary systems under study here.

Interpretation of NMR spectral parameters

The theory of ^{2}H-NMR as applied to labelled sites in bilayer membranes is well established and a full discussion can be found elsewhere [40–43]. Of the several parameters that can be measured in the ^{2}H-NMR spectrum of an oriented bilayer sample perhaps the most informative is the splitting ($\Delta\nu_i$) between the 90° singularities of the spectral powder pattern. This quantity is given by:

$$\Delta\nu_i = 3/4\ \nu_q\ \langle 3/2 \cos^2\sigma_i - 1/2\rangle \quad (1)$$

where ν_q is the solid-state quadrupole coupling constant associated with a given C–^{2}H$_i$ bond and $\langle 3/2 \cos^2\sigma_i - 1/2\rangle$, or the order parameter S_{C-^2H} is the time average of the quantity in brackets, taken over all the angles (σ_i) that the C–^{2}H$_i$ bond direction assumes with respect to the time averaged principal axis of the electric-field-gradient tensor during the time course of the NMR measurement. For the C–^{2}H, C–^{2}H$_2$ and C–^{2}H$_3$ groups in these DMPC-solute samples, a value of 170 kHz for ν_q has been assumed [44]. A principal goal in the study of membrane structure by ^{2}H-NMR, is the interpretation of S in terms of the relative importance of the various motions that time average the quadrupole interaction for a particular C–^{2}H$_i$ segment. In these multilamellar samples, it is assumed that translational diffusion and vesicle tumbling are not significant in the time-averaging process. However, no attempt is made, at least in the conformationally flexible solute molecules and acyl chains of DMPC, to quantify the relative contributions to the time-averaging of ν_q, of segmental motions produced by rotational isomerization about C–C bonds, and of rigid-body reorientations produced by motion of the molecule as a whole. Such an analysis for DMPC/alkanol systems is currently being attempted using data from the more rigid glycerol backbone region of DMPC.

Results

A representative 30.87 MHz ^{2}H-NMR spectrum of an approximately 33 mol% solution of perdeuterated *n*-butanol in bilayers of DMPC is shown in Fig. 2(a). The assignment of the powder pattern with the largest splitting to the 1-methylene segment was made on the basis of similar results for *n*-butanol selectively deuterated at that

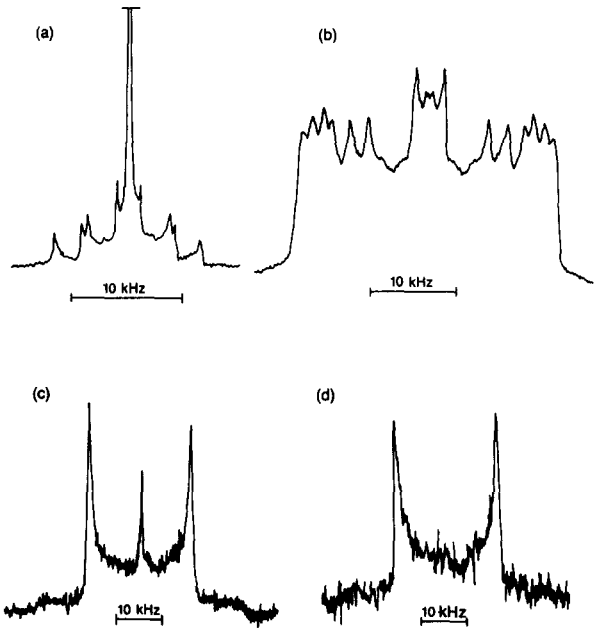

Fig. 2. ^{2}H-NMR spectra for 33 mol% specifically ^{2}H-labelled *n*-alkanols in DMPC bilayers in the liquid crystal (L_α) phase (A) DMPC/50 H_2O/0.5 *n*-butanol-d_9 at 43° C; (B) DMPC/25 H_2O/0.5 *n*-tetradecanol-d_{27} at 45° C; (C) DMPC/25 H_2O/0.5 *n*-tetradecanol-2-d_2 at 54° C; (D) DMPC/25 H_2O/0.5 *n*-dodecanol-1-d_2 at 47° C.

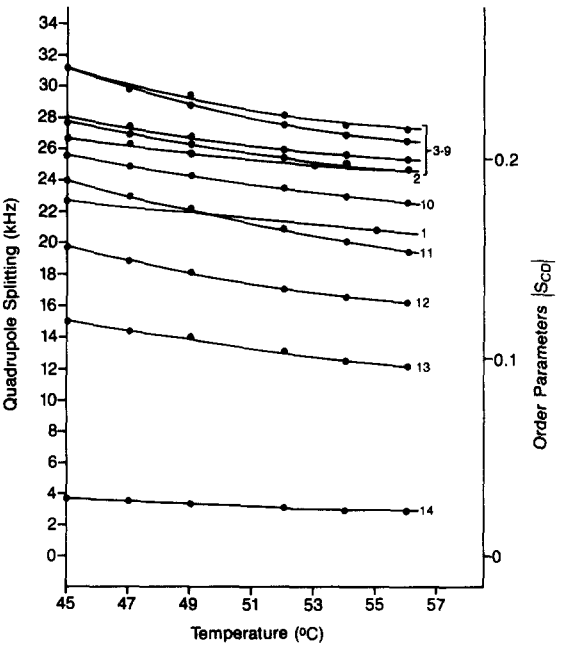

Fig. 3. Temperature dependence of ^{2}H-NMR splittings for 33 mol% deuterated *n*-tetradecanol in DMPC bilayers. Data for the 1- and 2-methylene segments were obtained using specifically labelled alcohols. Quadrupole splittings for other positions were measured with alcohol perdeuterated at all positions except 1.

position. Confirmation was obtained from an observed chemical shift of approximately 1–2 ppm between the mid-points of the 1-C^{2}H$_2$ component and that of the central isotropic signal. This latter signal arises from *n*-butanol dissolved in the aqueous phase. It is evident that the rate of exchange between *n*-butanol intercalated in the lipid bilayer and *n*-butanol dissolved in water, is slow on the time scale of the NMR measurement. The concentration of *n*-butanol in the bilayer is only approximate, being estimated from the known quantities of lipid and water in the sample and the reported partition coefficient [45].

The ^{2}H-NMR spectrum of 33 mol% *n*-tetradecanol, perdeuterated at all positions except C-1, in bilayers of DMPC is shown in Fig. 2(b). Several of the outer singularities present in this spectrum become resolved into two or three peaks at higher temperatures. The origin of the signals in the center of the spectrum probably arise from an ^{2}H-labelled impurity in the alcohol. The variation with temperature of the splittings between the principal peaks in the spectrum is shown in Fig. 3. Assignment of these splittings was only certain in the case of the 1,2 and methyl sites. The lowest temperature for which data are reported corresponds to T_{MF} which is defined as the finishing temperature of the main thermal transition determined from the DSC thermogram (vide infra). At temperatures immediately below T_{MF}, in the case of both multi-deuterated *n*-dodecanol and *n*-tetradecanol, two sets of spectral components were observed (data not shown). The narrower set of powder patterns arises from *n*-alkanol dissolved in more fluid regions of the bilayer, while the broader set of components arises from solute in gel-like regions. The splittings from the solute in the more fluid environment are relatively temperature invariant throughout the broad phase transition. These spectral components gradually decrease in intensity with decreasing temperature until they disappear at approximately T_{MS}, the starting temperature of the main thermal transi-

tion. Similar observations have been made previously for *n*-octanol [13].

On the basis of Fig. 3 and similar data for *n*-butanol and *n*-dodecanol order profiles were calculated for *n*-butanol, *n*-dodecanol and *n*-tetradecanol. These profiles are compared in Fig. 4, together with the order profile for *n*-octanol estimated from data in Ref. 13, after corrections had been made for differences in solute concentration and hydration. The order profiles were all determined at 10 C° above T_{MF}. The rationale for making the comparisons relative to T_{MF} rather than T_M, is provided in the same reference [13]. The most striking feature of Fig. 4 is the existence of an ordering maximum at $C_{3,4}$ in *n*-octanol, and C_{4-7} in *n*-dodecanol and *n*-tetradecanol, with labels at both ends of the alcohol molecules exhibiting reduced ordering. Similar effects have been noted previously for *n*-octanol in DMPC [13] and for *n*-decanol in soap bilayers [46,47] and DPPC [14]. Explanations for the reduced order towards the hydroxyl group of *n*-decanol in DPPC have been provided in terms of either increased amplitudes of motion or geometric effects due to hydrogen bonding [14]. In contrast, the ordering profile for *n*-butanol shows no ordering maximum in the center of the alkyl chain, an effect probably arising from the shortness and low mass of the butyl chain preventing it from being anchored in the hydrophobic portions of the bilayer for any significant fraction of time. Another feature of Fig. 4 is the increased ordering of the methyl group with decreasing alkanol chain length, reflecting the lower depth of bilayer penetration of the terminal methyl group in *n*-octanol compared with *n*-dodecanol and *n*-tetradecanol.

To further examine the dependence of solute ordering on solute chain length we have measured at comparable reduced temperatures and concentrations the quadrupole splittings at the 1-C^2H_2

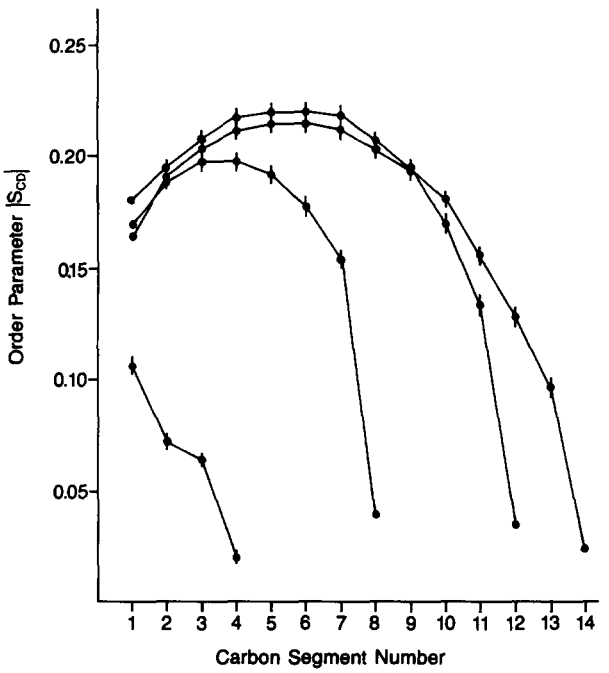

Fig. 4. Order parameter profiles for 33 mol% *n*-butanol, *n*-octanol, *n*-dodecanol and *n*-tetradecanol in DMPC bilayers at 10 C° above T_{MF}. (DMPC/25 H_2O/0.5 *n*-alkanol) Error bars indicate deviations of ±2 C° in the determination of T_{MF}. The S_{CD} values at C-1 were obtained using selectively ^2H-labelled alcohols. Except in the case of *n*-butanol S_{CD} values at C-2 were also measured with selectively labelled alcohols. The remaining data are from samples containing (C_2–C_N) perdeuterated alcohols so that uncertainty exists in assignments for several chain positions near the plateau. The data for *n*-octanol have been estimated from the order profiles reported in Ref. 13, after first correcting for the known dependence of order on hydration and solute concentration described therein.

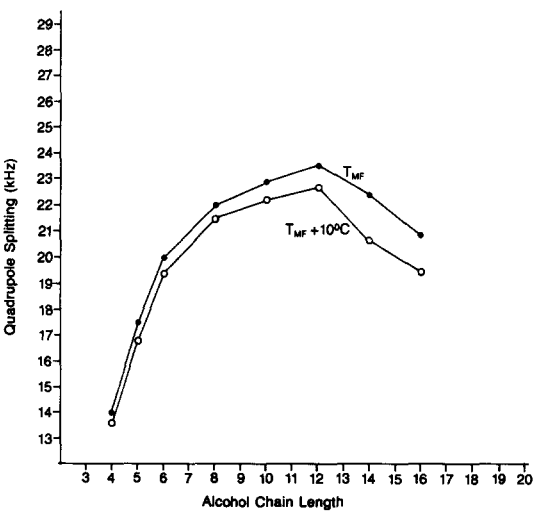

Fig. 5. Dependence of quadrupole splitting of the 1-segment of *n*-alkanols as a function of alkyl chain length for DMPC bilayers containing 33 mol% *n*-alkanol. Data are for equivalent reduced temperatures.

segment for *n*-alkanols varying in chain length from *n*-butanol to *n*-tetradecanol. The data (Fig. 5), for bilayers containing 33 mol% solute at T_{MF} and at 10 C° above T_{MF} shows maximal ordering for *n*-dodecanol. The existence of the maximum was confirmed by obtaining the equivalent data for *n*-hexadecanol, which showed a further reduction in splitting compared to *n*-tetradecanol (Fig. 5).

All the *n*-alkanols studied broaden the main gel to liquid-crystal thermal transition, T_M, as well as shifting its temperature. In the presence of excess water, short chain *n*-alkanols reduce T_M, while the longer-chain solutes give rise to an increase in T_M [21,25,26,48,49]. In comparing the effects of one solute with another on the order and mobility of both the solute molecules themselves and the bilayer in which they are dissolved, it is important to determine the magnitude of such changes in chain-melting temperature. While changes in T_M are apparent from the ^{2}H-NMR results themselves, such data are better obtained from DSC. Typical DSC thermograms are shown in Fig. 6, which also demonstrates our method for determining T_{MS} and T_{MF}.

The dependence of solute ordering on solute concentration in DMPC bilayers has been studied at the α-methylene site of *n*-tetradecanol. In Fig. 7 is shown a plot of the quadrupole splitting at this position as a function of temperature for bilayers containing 9, 16.7 and 33 mol% *n*-tetradecanol. In the same figure the width of the phase transition in each case as determined DSC is shown. The starting and final temperature of the transition are marked as T_{MS} and T_{MF}. For the highest concentration of *n*-tetradecanol the ^{2}H-NMR spectra at two temperatures between T_{MS} and T_{MF} are shown in insets. In both instances, two splittings are evident, indicating the existence of two populations of motionally averaged *n*-tetradecanol. The proportion of the more ordered component increases with decreasing temperature until at T_{MS} (33° C) it disappears. Below this temperature the spectrum is a broad featureless hump of approximate width 30 kHz. An interesting feature of Fig. 7 is that the ordering at T_{MF} is approximately the same (23 ± 0.5 kMz), regardless of the concentration of solute. The temperature dependence of the splitting above T_{MF}, however, varies with

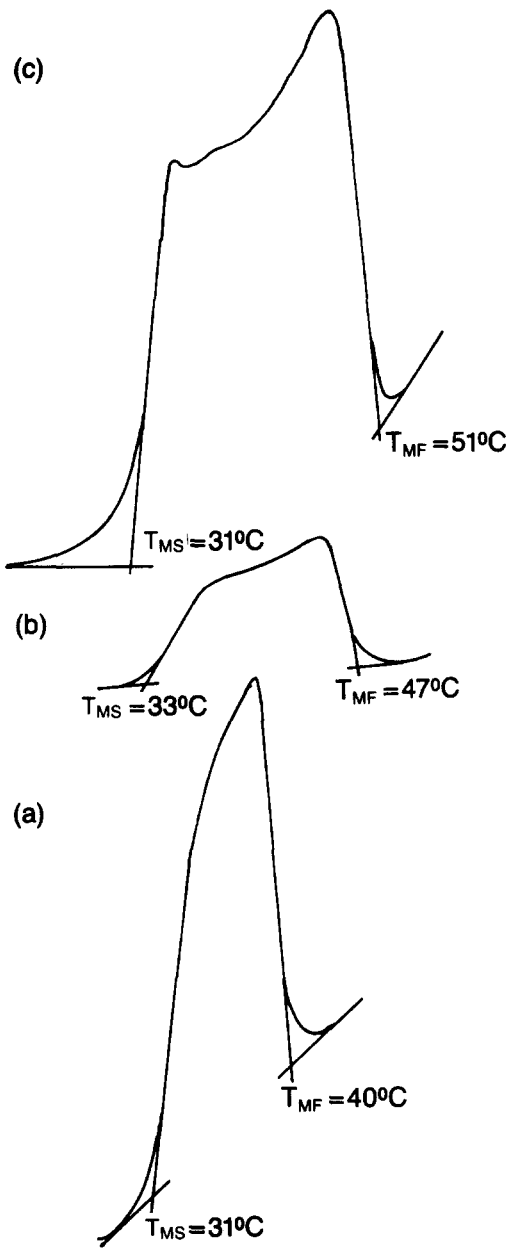

Fig. 6. DSC thermograms of the gel to liquid-crystal transition in DMPC bilayers containing 33 mol% *n*-alkanols: (a) DMPC/25 H_2O/0.5 dodecanol; (b) DMPC/25 H_2O/0.5 tetradecanol; (c) DMPC/25 H_2O/0.5 hexadecanol. In the case of *n*-butanol and *n*-octanol being the solute, T_{MF} is 20.5° C and 18° C, respectively, under identical conditions.

solute concentration. The implication of these results is that the ordering of membrane solutes at very low concentrations may be measured at T_{MF}

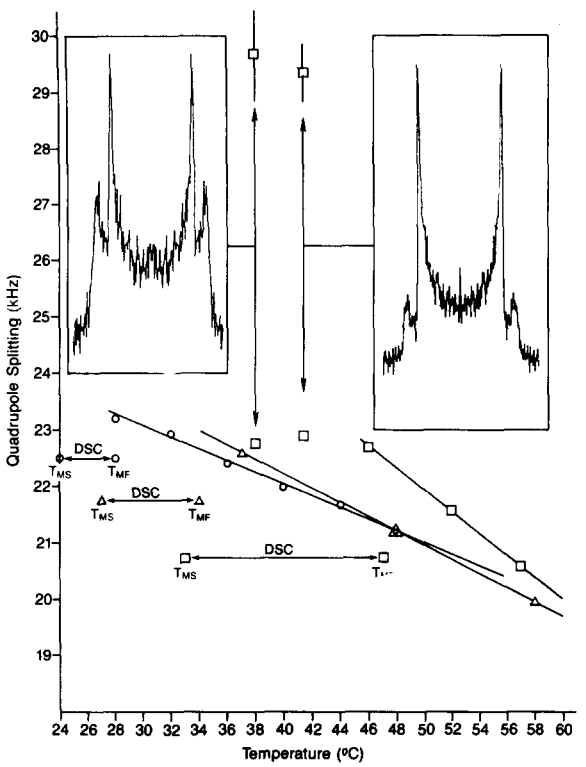

Fig. 7. Temperature dependence of ^{2}H-NMR splitting for the 1-segment of tetradecanol showing the influence of solute concentration for bilayers of molar composition 1.0 DMPC/25 H_2O/X *n*-tetradecanol-d_2 with $X = 0.1$ (○); $X = 0.2$ (△) and $X = 0.5$ (□). Limits of the thermal transition measured by DSC are indicated for each concentration. The two splittings observed at each concentration for temperatures within the limits of the thermal transition are also indicated. Representative ^{2}H-NMR spectra obtained at the highest solute concentration are shown in insets.

using higher solute concentrations. Such a conclusion is supported by results using *n*-dodecanol (data not shown). Also, for the same bilayer concentrations of *n*-dodecanol and *n*-tetradecanol the dependences of the quadrupole splitting on temperature are the same.

To determine the influence of *n*-alkanols on bilayer structure the same ternary systems have been studied with the ^{2}H-label on DMPC, rather than on the *n*-alkanol. Particular emphasis has been placed on acquiring data for ^{2}H-labelled sites at the interfacial region between the hydrophilic choline headgroup and the hydrophobic acyl chains. This region which is probably the most rigid portion of the DMPC molecule in bilayer systems, offers the most promise for a quantitative analysis of solute-induced conformational changes in the lipid [50].

Representative ^{2}H-NMR spectra, in the L_α phase, of bilayers formed from selectively deuterated DMPC in the presence of 33 mol% *n*-butanol are shown in Fig. 8. Spectra in Fig. 8 (a), (b) and (c) were obtained using lipid deuterated at sites on the upper regions of the acyl chains, on the glycerol backbone, and in the choline headgroup, respectively. The convention used in numbering sites in these regions is indicated in Fig. 1. Assignment of the quadrupole splittings in the ^{2}H-NMR spectra of DMPC, ^{2}H-labelled in the 2′ positions of the *sn*-1 and -2 chains (Fig. 8a) was based on reported values for

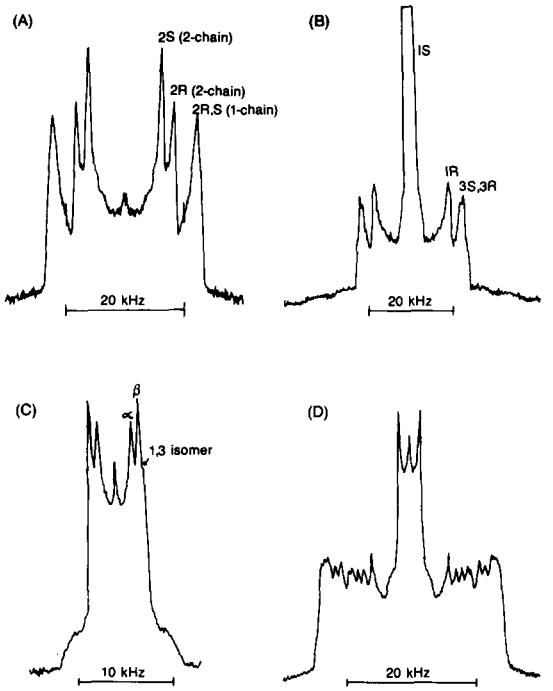

Fig. 8. ^{2}H-NMR spectra in the L_α phase of an aqueous multilamellar dispersion of specifically ^{2}H-labelled DMPC containing 33 mol% *n*-butanol as solute. (A) DMPC, ^{2}H-labelled on 2′-positions of *sn*-1,2 chains at 47 °C; (B) DMPC, ^{2}H-labelled at 1*R*, 1*S*, 3*R*, 3*S* of glycerol backbone at 35 °C; (C) DMPC, ^{2}H-labelled at α- and β-methylenes of choline at 32 °C. Peak labelled 1,3-isomer arises from choline α- and β-methylene groups in the 1,3-diacyl isomer of DMPC. (D) Acyl chain perdeuterated (d_{54}) DMPC at 24 °C.

these splittings [41,51]. In assigning splittings in the spectrum of DMPC, deuterated at the 1*R*, 1*S*, 3*R* and 3*S* sites in the glycerol backbone (Fig. 8(b)), it was assumed that these splittings were very similar to values reported for the same positions in DPPC and other phospholipids [35,53,54]. Stereospecific monodeuteration of several of these phospholipids showed that the two splittings from the 1-glycerol segment and the two splittings from the 3-glycerol segment reflect motional inequivalence of the individual deuterons [53]. α- and β-choline data (Fig. 8(c)) were obtained on tetradeuterated material and assignments are based on previously published data [52,55]. Some results were obtained with DMPC, ^{2}H-labelled at the 1*R*, 1*S*, 3*R* and 3*S* sites on the glycerol as well as the α- and β-methylene groups of the choline headgroup. A representative spectrum in the L_α phase of bilayers formed from DMPC labelled in these eight sites, in the presence of *n*-dodecanol, is shown in Fig. 9. The influence of four *n*-alkanols on ordering at the 3*S* site of the glycerol backbone is shown in Fig. 10(a) where the quadrupole splitting is plotted as a function of reduced temperature both in pure DMPC bilayers and DMPC bilayers containing 33 mol% *n*-alkanol. In Fig. 10(b) quadrupole splittings at the 3*R* and 3*S* sites are plotted as a function of *n*-alkanol chain length for a temperature of $T_{MF} + 10$ C°. Equivalent data for ^{2}H labels on the 1*R*, 1*S* sites of the glycerol backbone, the 2′ positions of the *sn*-1,2 chains, and the α- and β-methylene groups on the choline headgroup were made (data not shown). The different effects on quadrupole splittings produced by these four *n*-alkanols at the twelve ^{2}H-labelled sites in the interfacial region of DMPC may be grouped into four categories. Firstly, the largest effect occurs at the 3*R*,*S* sites of the glycerol backbone, where a plot of chain length versus quadrupole splitting shows a minimum at *n*-butanol (i.e., a maximum disordering by *n*-butanol). This observation probably reflects the greater fraction of time the hydroxyl group of the *n*-butanol spends hydrogen bonded to the phosphate group of the lipid, compared with the more hydrophobic longer-chain *n*-alkanols, which are more deeply embedded in the bilayer. The second type of effect, of smaller magnitude, is found for the 1*R* glycerol and β-methylene groups where

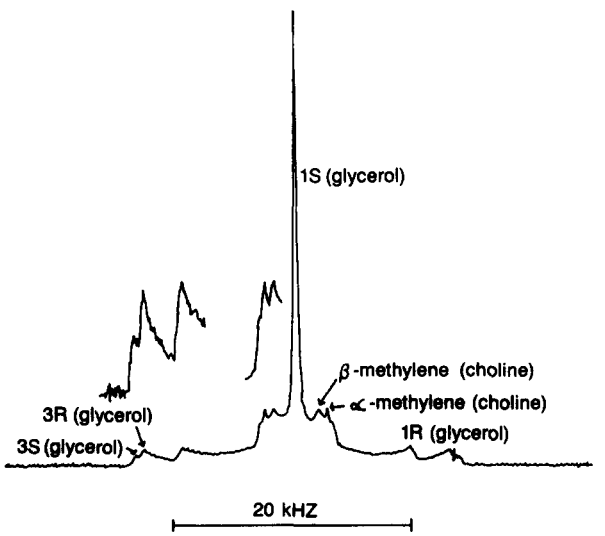

Fig. 9. ^{2}H-NMR spectrum at 42° C of bilayers of DMPC containing 33 mol% *n*-dodecanol. The lipid is ^{2}H-labelled at the 1*R*, 1*S*, 3*R*, 3*S* sites of the glycerol backbone and the α- and β-methylenes of the choline headgroup.

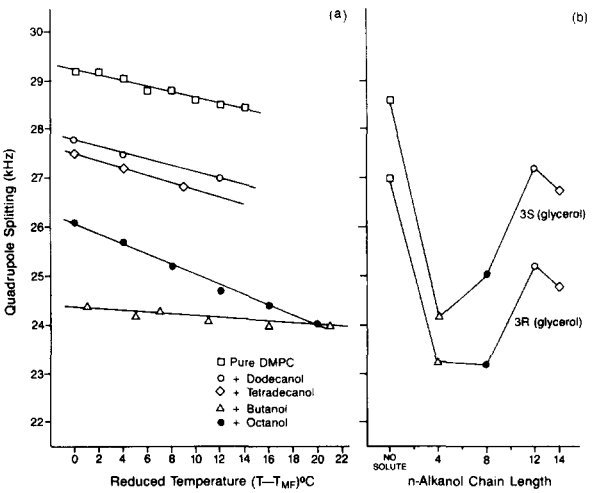

Fig. 10. (a). Temperature dependence of the ^{2}H-NMR quadrupole splittings for the 3*S* site of the glycerol backbone in an aqueous multilamellar dispersion of selectively ^{2}H-labelled DMPC containing 33 mol% *n*-alkanol. Reduced temperatures ($T - T_{MF}$) are the differences between sample temperature, and the finishing temperature of the main thermal transition as determined from the DSC thermogram. (b) Dependence of ^{2}H-NMR splittings at the 3*R* and 3*S* sites of the glycerol backbone, as a function of alkanol chain length for 33 mol% *n*-alkanol in DMPC bilayers at 10° C above T_{MF}.

increasing chain length produces increasing disorder. Thirdly, at the 2*R*,*S* sites on the *sn*-1 acyl chain and the 2*R* site of the *sn*-2 chain a maximal ordering is observed for *n*-octanol. Finally, there are four sites (1*S* (glycerol), 2′*S* (*sn*-2 chain) and α-CH_2 (choline)) where orientational ordering is insensitive to the presence of *n*-alkanol in the bilayer. The interpretation, in molecular terms, of many of the above smaller effects on ordering, is complicated by subtle factors such as the change in surface area per lipid molecule produced by intercalation of the *n*-alkanol in the membrane, and by the ability of the various *n*-alkanols to influence whole-body reorientations, depending on the mass (chain length) of the alkanol.

The effect of *n*-alkanols on ordering in the glycerol backbone, choline headgroup and upper regions of the acyl chains of DMPC has been compared, under identical conditions with the effect of cholesterol at the same sites (Table I). The patterns of change produced in lipid ordering by *n*-alkanols are quite different from those produced by cholesterol. For example, at the 1*R*, 3*R*, 3*S* glycerol sites *n*-alkanol decreases ordering, whereas cholesterol has little effect. The 1*S* site is insensitive to the presence of *n*-alkanol but in the case of cholesterol an increase in ordering is observed. At the upper sites of the acyl chains, cholesterol increases order to a greater extent than the *n*-alkanols whose effect is dependent on chain length. The influence of the *n*-alkanols at the choline β-methylene position is disordering, while that of cholesterol is negligible. It is apparent that these differences reflecting subtle differences between *n*-alkanols and cholesterol in the way in which each solute influences whole-body reorientations of the lipid molecules as well as the time-averaged conformation of the lipid interfacial region.

The influence of *n*-alkanols on orientational ordering at sites over the whole acyl chain length, was determined from spectra of samples prepared using chain perdeuterated DMPC. Fig. 8(d) shows the spectrum obtained when *n*-butanol is the solute. As we have shown earlier [13], changes in the

TABLE I

EFFECTS OF SEVERAL ALCOHOLS ON ^{2}H-NMR QUADRUPOLE SPLITTINGS ($\Delta\nu$) OF SPECIFICALLY ^{2}H-LABELLED DMPC [a]

Position of ^{2}H label	$\Delta\nu$ (kHz) no solute [b]	Effect of solute (kHz) [c]				
		butanol	octanol	dodecanol	tetradecanol	cholesterol [d]
3*S* (glycerol) [e]	28.3	−4.4	−3.5	−1.5	−2.0	−0.2
3*R* (glycerol) [e]	26.3	−3.7	−3.9	−1.5	−2.2	0.3
1*R* (glycerol) [e]	25.4	−1.9	−1.5	−1.9	−2.9	0.0
1*S* (glycerol) [e]	0.0	0.0	0.0	0.0	0.0	1.3
2′*R*,*S* (*sn*-1-chain) [f]	26.0	0.6	1.5	0.0	−0.1	6.9
2′*R* (*sn*-2-chain) [f]	17.5	0.7	0.9	−0.4 [h]	−0.4 [h]	7.1
2′*S* (*sn*-2-chain) [f]	12.4	0.3	0.1	1.0 [h]	1.0 [h]	4.0
α-CH_2 (choline) [g]	6.0	−0.5	−0.7	−0.7	−0.7	−0.2
β-CH_2 (choline) [g]	5.2	−1.3	−0.9	−1.7	−2.3	0.3

[a] For 33 mol% solutions of alcohol in lipid at 10 C° above T_{MF} unless otherwise indicated. Error estimated to be ±0.3 kHz.

[b] Multilamellar dispersion of pure DMPC in excess H_2O, for which chain melting temperature is 22–24°C. Error estimated to be ±0.2 kHz.

[c] Positive value indicates an increase in the quadrupole splitting.

[d] 22 mol% solution in bilayer.

[e] Measured with either DMPC-d_4 specifically ^{2}H-labelled at the 1,1,3,3 sites of the glycerol moiety, or with DMPC-d_8 specifically ^{2}H-labelled at both the choline methylene groups and the 1,1,3,3 sites of the glycerol moiety.

[f] Measured with DMPC-d_4 specifically ^{2}H-labelled at the 2′-positions of the *sn*-1 and *sn*-2 acyl chains.

[g] Measured with either DMPC-d_4 specifically ^{2}H-labelled at the choline methylene groups, or with DMPC-d_8 specifically labelled at the choline methylene groups and the 1,1,3,3 sites of the glycerol moeity.

[h] Error estimated to be ±0.5.

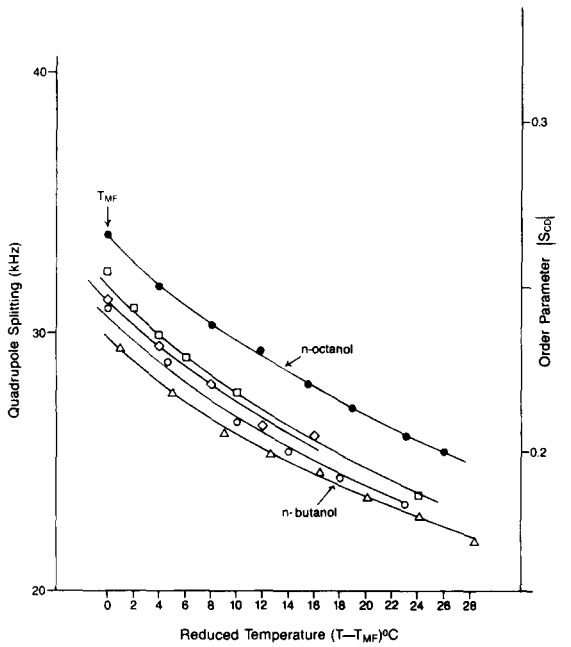

Fig. 11. Temperature dependence of the width of ^{2}H-NMR spectra for bilayers of perdeuterated DMPC containing 33 mol% *n*-alkanol. □, no alkanol; △, *n*-butanol; ●, *n*-octanol; ○, *n*-dodecanol ◇, *n*-tetradecanol.

spectra of perdeuterated DMPC on addition of solutes can be substantial, and in general prevent a complete assignment of the splittings in those cases. This would require spectra from samples containing selectively deuterated lipids in the presence of solute. We have not done this but it is clear that the central resolved splitting reflects the order of the terminal C^2H_3 groups of the lipid chains, while the outer edges of the main part of the spectrum yield the order parameter for the most ordered segments of the acyl chains. The width of this order parameter plateau reflects ordering from segments near the glycerol backbone region of the bilayer (although the 2-position of the *sn*-2 chain exhibits anomalous behavior) and is most marked just above the gel to liquid-crystal transition. The temperature dependence of splittings corresponding to this plateau is shown for several samples with and without solutes in Fig. 11. At comparable reduced temperature *n*-octanol has a pronounced ordering effect, *n*-butanol a significant disordering effect, while *n*-dodecanol and *n*-tetradecanol have little effect on acyl chain ordering. By comparison all four solutes have little effect on the splitting of the terminal methyl end of the chains, when corrected for changes in T_{MF}.

Discussion

Previous studies [21,25] have shown that *n*-alkanols C_3–C_{18} dissolve in lipid bilayers to varying degrees in the L_α phase, without producing gross changes in bilayer structure, such as non-lamellar aggregates. Data on the location of the solutes within the bilayer and the extent to which they intercalate between the lipid chains is, however, much more limited [11–14]. All the ^{2}H-NMR data obtained in this study, of which the spectra in Fig. 2 are representative, show that *n*-alkanols from C_4 to C_{14}, at concentrations up to 33 mol% in the L_α phase, dissolve completely in DMPC bilayers. Furthermore, the uniaxial nature of the spectra (Fig. 2) indicate that the motions of the solute, responsible for time-averaging the quadrupole interaction are rapid on the NMR time-scale ($\tau < 10^{-5}$ s).

Whether the solute molecules are intercalated between the alkyl chains of the lipid molecules or form an ordered sandwich between monolayers at the bilayer center, has not been determined by measuring the direction of the principal axis of the time-averaged electric field-gradient tensor with oriented bilayers. However, the former option is favoured on the basis of the order parameter profiles for *n*-octanol, *n*-dodecanol and *n*-tetradecanol shown in Fig. 4. For these *n*-alkanols, the magnitudes of the quadrupole splittings from the most ordered segments are comparable to those of the lipid chain (Fig. 11), while the presence of a significant order parameter plateau reflects that exhibited by the lipid chains [40,42]. This confirms that, as expected, the terminal hydroxyl is anchored near the aqueous interface, probably at times projecting significantly into the aqueous phase beyond the most ordered 'glycerol backbone' region of the bilayer, giving rise to a reduced order parameter for this segment (Fig. 4). Similar effects have been observed for *n*-decanol in soap bilayers [46,47] and in DPPC bilayers [14]. Anchoring of the hydroxyl group in the vicinity of the aqueous interface of the bilayer is also re-

flected in the increased order parameter for the methyl group of *n*-octanol and *n*-dodecanol, (S = 0.039 and 0.034, respectively) with respect to that for the acyl chain methyls of the lipid molecules at a comparable temperature ($\Delta\nu = 3.5$ kHz or S = 0.027 at $T_{MF} + 10$ C°) [56]. This results from the shorter length of the *n*-octanol and *n*-dodecanol chains so that the alcohol methyl groups are pulled into more ordered regions of the lipid bilayer [57].

A comparison of the order profiles for the *n*-alkanols in Fig. 4 is informative about their time-averaged dispositions in DMPC bilayers. The measured order parameter for the 1-methylene segment shows a maximum for a chain length of twelve. This result indicates that 1-dodecanol is maximally ordered in DMPC bilayers, suggesting that this chain is of optimal length for intercalation between the acyl chains of this phospholipid. If the *n*-alkanol is longer as in *n*-tetradecanol, it is too large to be ideally accommodated and this is reflected by increased disorder in the solute molecule. *n*-Tetradecanol is the first *n*-alkanol in the series where the order parameter for its terminal methyl group ($S = 0.023$ at $T_{MF} + 10$ C°) is less than that of the terminal methyl groups of the acyl chains in DMPC ($S = 0.027$ at $T_{MF} + 10$ C°) [56]. Consideration of the relative lengths of the all-*trans* configuration of the hydrocarbon chains in *n*-dodecanol and DMPC using space-filling models suggests that the hydroxyl group of *n*-dodecanol is ideally positioned to be hydrogen bonded to the ester carbonyl groups of the *sn*-1 and -2 acyl chains in DMPC. The larger quadrupole splitting of the terminal methyl group in *n*-dodecanol compared with the terminal methyl groups of DMPC, however, show that this methyl group is, at times, located further up in the bilayer, allowing the hydroxyl group to hydrogen bond with the phosphodiester group in DMPC as well as water molecules closely associated with the polar headgroup region. Such an arrangement would account for the drop in the order parameter towards the hydroxyl end of the molecule. If the *n*-alkanol is shorter than *n*-dodecanol, the lower molecular weight and fewer opportunities for van der Waals interactions with the acyl chains, would allow these movements of the *n*-alkanol out into the headgroup region to assume more significance. Thus, the order at the 1-methylene segment decreases with decreasing *n*-alkanol chain length. In the case of *n*-butanol, there is no plateau region in the order profile so that the fraction of time spent by the alkyl chain intercalated between the lipid acyl chains must be quite small. Since the most ordered segment in *n*-butanol is the 1-methylene group, it appears that the hydroxyl group is anchored to hydrogen bond acceptor groups in the polar headgroup region. This binding must be sufficiently strong to make the rate of exchange of *n*-butanol with the bulk water environment, slow on the NMR time scale (Fig. 2(a)).

The presence in Fig. 5 of an ordering maximum at *n*-dodecanol is also evident at T_{MF}, as well as at $T_{MF} + 10$ C°. Since the data for *n*-tetradecanol in Fig. 7, and equivalent results for *n*-dodecanol, show that ordering at the 1-methylene segment at T_{MF} is independent of solute concentration, the above comments regarding the dispositions of *n*-dodecanol and *n*-tetradecanol in the bilayer are still applicable for much lower solute concentrations. Thus, at the lower levels of solute concentration which produce anesthesia, ordering of *n*-alkanols in DMPC bilayer still should show a maximum at *n*-dodecanol. That this ordering maximum in *n*-alkanols occurs at the same chain length as the 'cut-off' in anesthetic potency in the same series may however be coincidental. DMPC bilayers bear little resemblance to the biological membranes in tadpoles, in which anesthetic potencies for *n*-alkanols were measured. We are currently determining whether the ordering maximum in a homologous series is a function of the acyl chain length of the lipid, by measuring the ordering of the *n*-alkanols in DPPC bilayers.

The difference observed between the ordering of *n*-dodecanol and *n*-tetradecanol in DMPC bilayers is not reflected in their respective effects on the ordering of DMPC. At 33 mol% *n*-alkanol concentration and at comparable temperatures above T_{MF}, there is virtually no difference between the effects of *n*-dodecanol and *n*-tetradecanol on ordering at most sites in the choline headgroup, glycerol backbone and acyl chains of DMPC (Figs. 10, 11 and unpublished data). That both *n*-alkanols perturb the time-averaged conformation of the interfacial region of DMPC is evident by comparing their effect with the data for DMPC in the absence of *n*-alkanol. Significant

changes are seen at the β-methylene segment of the choline, and the $1R,3S$ sites of the glycerol backbone, but almost no changes in the acyl chain ordering are apparent (Table I). These results are consistent with the *n*-alkanols being intercalated between the acyl chains of DMPC, thus increasing the average area per lipid molecule in the bilayer and resulting in a change in time-averaged conformation of the interfacial region of the lipid. The ordering properties of the acyl chains would not be expected to change significantly, since there is little difference for a given acyl chain in DMPC, between having a long alkyl chain of an *n*-alkanol or another lipid acyl chain as a nearest neighbor in the bilayer.

The influence of the long chain *n*-alkanols on DMPC ordering, however, is quite different from that observed for *n*-octanol and *n*-butanol. Fig. 10 shows that *n*-butanol has a large effect on ordering at the $3R,S$ sites of the glycerol backbone. This change is caused partially by an increased area per lipid molecule in the bilayer, but the predominant contribution probably arises from new hydrogen bond interactions between the *n*-alkanol and the phosphodiester group in DMPC, causing a change in the time-averaged conformation of the interfacial region. This binding also appears to be important in the case of *n*-octanol (Fig. 10). The ordering of the α-methylene segment of the choline group which is the second methylene group directly bonded to the phosphodiester linkage is not altered significantly by such hydrogen bonding (Table I). The decreased ordering observed at the $1R$ site and the absence of any effect at the 1S site in the case of all four *n*-alkanols, suggest a change in time-averaged conformation of this segment with respect to the most odered molecular axis of the lipid. Similar effects at two sites on the same methylene group are also observed at the $2'R,S$-positions of the *sn*-2 acyl chains where the $2'S$ site, unlike the $2'R$ site is relatively insensitive to the presence of *n*-alkanol in the bilayer. The effect of both *n*-octanol and *n*-butanol on acyl chain ordering in DMPC has been discussed previously [13]. It was argued that the anchorage of *n*-butanol at the aqueous interface gives rise to an increase in bilayer area which the lipid chains accommodate by disordering below those segments directly affected by the presence of solute. It is more difficult to explain the ordering effect of *n*-octanol (Fig. 11), although for *n*-alkanols of intermediate chain length it is possible that an additional factor comes into play. Disordering of those parts of the lipid chains which entered beyond the terminal methyl of the *n*-alkanol clearly involves an energy penalty, since the additional energy associated with formation of a gauche bond is 500 ± 100 cal/mol [58]. This is only partially offset by the increased entropy contribution to free energy. Alternatively, the extent of the disordering can be reduced and the Van der Waals interactions with neighboring chains can be minimized if the lipid chains adjacent to the *n*-alkanol straighten, giving rise to the observed increase in order. This in turn will reduce the degree to which the ends of the lipid chains must disorder to fill space in the bilayer center, since the alkanol itself experiences a more ordered environment and consequently has a smaller projected area at the bilayer surface than it would have in the absence of such effects. Both these factors result in a reduction in free energy at the expense of some entropy contribution. For the short chain *n*-alkanols (*n*-butanol) the latter is clearly dominant, so that the lipid chains disorder over their entire length. Once the alcohol chain length approaches that of the lipid chains, the propensity for increased order of the lipid chains in the 'plateau' region is again reduced, since a better match can be achieved between the order and packing of both types of chain.

The effect of these four *n*-alkanols on DMPC ordering at comparable temperatures and concentrations are quite unlike those produced by the alcohol, cholesterol. For this solute the hydrophobic portion of the molecule comprises a larger component than in the case of the *n*-alkanols so we would predict this solute to be more deeply embedded in the bilayer. The minimal effects of cholesterol on the order parameter at the $1R,3R,3S$ sites on the glycerol backbone and the α- and β-methylene segments of the choline (Table I) suggests that there is little interaction between its hydroxyl group and the phosphodiester group of the lipid, as well as little change in the average area per lipid molecule in the bilayer. In conclusion, it appears that the data in Table I can be interpreted qualitatively in terms of the relative

depth of penetration of the various alcohol molecules into the lipid bilayer.

Acknowledgements

This work was supported in part by the Australian Research Grants Scheme, the National Institutes of Health (GM 27127 to P.W.W.), the National Science Foundation (DMR 85/03219), and by a Research Challenge Award Grant of the Ohio Board of Regents. We are grateful to Sutin Horvath for synthesizing specifically ^{2}H-labelled DMPC and *n*-alkanols.

References

1 Janoff, A.S. and Miller, K.W. (1982) in Biological Membranes, (Chapman, D., ed.), Vol. 4, pp. 417–476, Academic Press, London.
2 Franks, N.P. and Lieb, W.R. (1982) Nature (London) 300, 487–493.
3 Roth, S.H. (1979) Annu. Rev. Pharmacol. Toxicol. 19, 159–178.
4 Kaufman, R.D. (1977) Anaesthesiology 46, 49–62.
5 Seeman, P. (1972) Pharmacol. Rev. 24, 583–655.
6 Franks, N.P. and Lieb, W.R. (1985) Nature 316, 349–351.
7 Franks, N.P. and Lieb, W.R. (1986) Proc. Natl. Acad. Sci. USA 83, 5116–5120.
8 Bangham, A.D., Standish, M.M. and Miller, N. (1965) Nature 208, 1295–1297.
9 Johnson, S.M. and Bangham, A.D. (1969) Biochim. Biophys. Acta 193, 92–104.
10 Johnson, S.M., Miller, K.W. and Bangham, A.D. (1973) Biochim. Biophys. Acta 307, 42–57.
11 Pope, J.M., Walker, L.W. and Dubro, D. (1984) Chem. Phys. Lipids 35, 259–277.
12 Thewalt, J.L., Wassall, S.R., Gorissen, H. and Cushley, R.J. (1985) Biochim. Biophys. Acta 817, 355–365.
13 Pope, J.M. and Dubro, D.W. (1986) Biochim. Biophys. Acta 858, 243–253.
14 Thewalt, J.L., Tulloch, A.P. and Cushley, R.J. (1986) Chem. Phys. Lipids 39, 93–107.
15 Pang, K.-Y.Y., Braswell, L.M., Chang, L., Sommer, T.J. and Miller, K.W. (1980) Mol. Pharmacol. 18, 84–90.
16 Pringle, M.J. and Miller, K.W. (1979) Biochemistry 18, 3314–3320.
17 Richards, C.D., Keightley, C.A., Hesketh, T.R. and Metcalfe, J.C. (1980) in Molecular Mechanisms of Anesthesia (Progress in Anesthesiology, Vol. 2) (Fink, B.R., ed.), pp. 337–351, Raven Press, New York.
18 Lawrence, D.K. and Gill, E.W. (1975) Mol. Pharmacol. 11, 595–602.
19 Lyon, R.C., McComb, J.A., Schreurs, J. and Goldstein, D.B. (1981) J. Pharmacol. Exp. Ther. 218, 669–675.
20 Jain, M.K., Wu, N.Y.-M. and Wray, L.V. (1975) Nature 255, 494–496.
21 Eliasz, A.W., Chapman, D. and Ewing, D.F. (1976) Biochim. Biophys. Acta 448, 220–230.
22 Hui, F.K. and Barton, P.G. (1973) Biochim. Biophys. Acta 296, 510–517.
23 Jain, M.K. and Wu, N.M. (1977) J. Membr. Biol. 34, 157–201.
24 Kamaya, H., Matubayasi, N. and Ueda, I. (1984) J. Phys. Chem. 88, 797–800.
25 Lee, A.G. (1976) Biochemistry 15, 2448–2454.
26 Kamaya, H., Ma, S. and Lin, S.H. (1986) J. Membr. Biol. 90, 157–161.
27 Rowe, E.S. (1983) Biochemistry 22, 3299–3305.
28 Zavoico, G.B. and Kutchai, H. (1980) Biochim. Biophys. Acta 600, 263–269.
29 Hill, M.W. (1974) Biochim. Biophys. Acta 356, 117–124.
30 Miller, K.W. and Pang, K.-Y.Y. (1976) Nature 263, 253–255.
31 Aasen, A.J., Lauer, W.M. and Holan, R.T. (1970) Lipids 5, 869–877.
32 Dinh-Nguyen, N. and Stenhagen, E.A. (1967) Chem. Abstr. 67, 63814x.
33 Perly, B., Dufourc, E.J. and Jarrell, H.C. (1984) J. Lab. Comp. Radiopharm. 21, 1–13.
34 Hermetter, A. and Paltauf, F. (1981) Chem. Phys. Lipids 28, 111–115.
35 Wohlgemuth, R., Waespe-Sarcevic, N. and Seelig, J. (1980) Biochemistry 19, 3315–3321.
36 Eibl, E. (1980) Chem. Phys. Lipids 26, 239–247.
37 Taylor, M.G. and Smith, I.C.P. (1981) Chem. Phys. Lipids 28, 119–138.
38 Davis, J.H., Jeffrey, K.R., Bloom, M., Valic, M.I. and Higgs, T.P. (1976) Chem. Phys. Lett. 42, 390–394.
39 Rance, M., Jeffrey, K.R., Tulloch, A.P., Butler, K.W. and Smith, I.C.P. (1980) Biochim. Biophys. Acta 800, 245–262.
40 Seelig, J. (1977) Q. Rev. Biophys. 10, 353–418.
41 Seelig, J. and Seelig, A. (1980) Q. Rev. Biophys. 13, 19–61.
42 Davis, J.H. (1983) Biochim. Biophys. Acta 737, 117–171.
43 Griffin, R.G. (1981) Methods Enzymol. 72, 108–174.
44 Burnett, L.J. and Muller, B.H. (1971) J. Chem. Phys. 55, 5829–5831.
45 Katz, Y. and Diamond, J.M. (1974) J. Membr. Biol. 17, 101–120.
46 Klason, T. and Henriksson, V. (1982) in Solution Behavior of Surfactants (Mittal, K.L. and Fondler, E.J., eds.), p. 417, Plenum Press, New York.
47 Seelig, J. and Neiderberger, W. (1974) Ber. Bunsenges. Phys. Chem. 78, 947–949.
48 MacDonald, A.G. (1978) Biochim. Biophys. Acta 507, 26–37.
49 McIntosh, T.J., Simon, S.A. and McDonald, R.C. (1980) Biochim. Biophys. Acta 597, 445–463.
50 Strenk, L.M., Westerman, P.W. and Doane, J.W. (1985) Biophys. J. 48, 765–773.
51 Engel, A.K. and Cowburn, D. (1981) FEBS Lett. 126, 169–171.

52 Gally, H.U., Niederberger, W. and Seelig, J. (1975) Biochemistry 14, 3647–3652.
53 Gally, H.U., Pluschke, G., Overath, P. and Seelig, J. (1981) Biochemistry 20, 1826–1831.
54 Browning, J.L. and Seelig, J. (1980) Biochemistry 19, 1262–1270.
55 Seelig, J., Gally, H. and Wohlgemuth, R. (1977) Biochim. Biophys. Acta 467, 109–119.
56 Oldfield, E., Meadows, M., Rice, D. and Jacobs, R. (1978) Biochemistry 17, 2727–2739.
57 Pope, J.M., Dubro, D., Doane, J.W.and Westerman, P.W. (1986) J. Am. Chem. Soc. 108, 5426–5433.
58 Gruen, D.W.R. (1982) Chem. Phys. Lipids 30, 105–120.