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EFFECTS OF CHOLESTEROL ON THE ORIENTATIONAL ORDER OF UNSATURATED LIPIDS IN THE MEMBRANES OF *ACHOLEPLASMA LAIDLAWII*

A ^{2}H-NMR STUDY

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We have investigated by ^{2}H-NMR the effects of the incorporation of cholesterol on the orientational order of unsaturated lipid acyl chains in the membranes of *Acholeplasma laidlawii* B. This is the only ^{2}H-NMR study to date of the influence of cholesterol in a biological membrane using specifically labelled fatty acids. We observed the characteristic condensing effect of cholesterol on the lipid acyl chain order in the liquid crystalline phase. In terms of the percentage increase in the quadrupolar splittings, the presence of cholesterol has its greatest effect on the methyl end of the labelled oleoyl chains, with a maximum at the C-14 segment. In absolute terms, the perturbation is greatest in the carboxyl end of the chains. The temperature dependence of the ^{2}H spectra for the cholesterol-containing membranes is very similar to that for the cholesterol-free membranes. The broad phase transition of the membrane lipids, which is characteristic for the samples lacking cholesterol, is apparently little affected by the presence of up to 27 mol% cholesterol. In addition, the temperature of onset of the phase transition is not significantly depressed by the presence of cholesterol.

Introduction

Since cholesterol is a major component of many biomembranes, the understanding of its interaction with other membrane components is essential for a complete description of the hydrophobic environment in which various membrane processes occur [1,2]. One important aspect of this problem is the effect cholesterol has on the structure and dynamics of the lipid bilayer. Mixtures of cholesterol and phospholipids in model systems have been widely studied using a variety of physical techniques, including differential scanning calorimetry (DSC) and differential thermal analysis (DTA) [3–5], freeze-fracture electron microscopy [6–8], X-ray and neutron diffraction [9–11], fluorescence [12–14], Raman [15,16], electron spin resonance (ESR) [17,18] and nuclear magnetic resonance (NMR) [19–23] spectroscopy, and dielectric relaxation measurements [24,25].

In spite of the large number of investigations, a

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Abbreviations: DSC, differential scanning calorimetry; DTA, differential thermal analysis; ESR, electron spin resonance; NMR, nuclear magnetic resonance; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine.

clear picture of the cholesterol-lipid interaction has yet to be formulated. At present, it is still uncertain whether cholesterol forms specific complexes with a number of lipid molecules [9,26–28] or is randomly distributed throughout the lipid bilayer [29]. However, there are a number of well defined physical changes which occur upon incorporation of cholesterol into model membrane systems. The gel to liquid crystal phase transition is broadened and the heat absorbed at the transition decreases [4,9]. Monolayer studies [30,31] show that mixing of phosphatidylcholine with cholesterol above the phase transition results in a decrease in the mean cross-sectional area per molecule within the bilayer. This effect can be understood in terms of a decrease in the flexibility of the acyl chains of the lipid due to the influence of cholesterol on the *trans-gauche* isomerization of the methylene groups [30]. In lipid bilayers, NMR [19,20,29] and ESR [16,17] studies have shown that cholesterol interacts with the fatty acyl chains above the gel to liquid crystal phase transition to inhibit chain flexibility. Below the transition temperature NMR and ESR measurements of the acyl chain order parameters show that the presence of cholesterol inhibits the cooperative crystallization of the hydrocarbon chains. Deuterium NMR, in particular, has shown that in equimolar mixtures of cholesterol and DPPC [19,22] or DMPC [23,29] there is a continuous increase in the quadrupolar splittings with decreasing temperature, in contrast to the results for samples without cholesterol where there is a sharp discontinuity in the splittings at the phase transition temperature. By disordering the chain packing at low temperature and reducing the chain flexibility at high temperatures, cholesterol creates a so-called 'intermediate fluid condition' [32].

In a well defined model membrane system, such as DPPC-cholesterol, the observed physical behaviour will best be described in terms of a phase diagram [7]. While a complete phase diagram is not presently available, a number of research groups are working on this problem (Ref. 7, and Söderman, O. and Wennerström, H., personal communication, for example). However, the heterogeneity inherent in a biological membrane will probably preclude any discussion of the cholesterol-lipid interaction in terms of distinct phases. On the other hand, it is important that the influence of cholesterol on biological membranes be compared with the behaviour observed in the model systems consisting of phospholipid-cholesterol mixtures.

Acholeplasma laidlawii B, a prokaryote of the class *mollicutes,* provides a unique opportunity to study the influence of cholesterol on membrane structure and function [33]. The organism is structurally very simple, having no cell wall and no internal membrane systems. The plasma membrane can therefore be easily isolated in large quantities without contamination by other membranes. While *A. laidlawii* does not require cholesterol for growth, if cholesterol is present in the growth medium it will be incorporated into the cell membrane. Furthermore, *A. laidlawii* readily incorporates exogenous deuterium-labelled fatty acids into its membrane lipids. It has been demonstrated that ^{2}H-NMR of specifically-deuterated lipids provides a non-perturbing probe of the molecular structure and dynamics within membrane [34,35]. Detailed studies on the *A. laidlawii* system [36,37] have shown that there is a remarkable similarity between the ^{2}H-NMR results for model and biological membranes. In particular, the order parameter profiles observed for *A. laidlawii* membranes containing selectively-deuterated palmitic and oleic acid closely resemble those obtained in phospholipid dispersions [38,39]. DTA [40], X-ray diffraction [41], and ^{2}H-NMR [42] have shown that the membrane lipids of *A. laidlawii* undergo a relatively well defined gel to liquid crystal phase transition similar to those observed in model systems. *A. laidlawii* grown in the presence of a high concentration of cholesterol incorporates as much as 10% by weight of the membrane lipid. With the addition of cholesterol there is a substantial decrease in the area under the endothermic peak as observed by DSC [43]. In a recent study the spectra of perdeuterated palmitoyl chains incorporated biosynthetically into the *A. laidlawii* membrane were observed with and without cholesterol [44]. The addition of cholesterol removed the phase transition. While the order-parameter profile in the liquid crystalline phase region maintains its general shape, the cholesterol-containing membranes have a higher average order. In the original gel phase region, the addition of

cholesterol causes rather dramatic changes in the ^2H spectra, suggestive of complex motional and/or phase behaviour [44].

The purpose of the present study is to characterize further the influence of cholesterol on the structure and dynamics of the lipids in a biological membrane, namely the plasma membrane of *A. laidlawii* B. In particular, the influence of cholesterol on acyl chains containing a *cis*-unsaturated bond is investigated using ^2H-NMR. In a previous study [37] the orientational order of oleic acid-enriched membrane lipids in cholesterol-free *A. laidlawii* was characterized and thus a direct comparison can be made between ^2H-NMR results for membranes with and without cholesterol.

Materials and methods

Specifically-dideuterated oleic acids ([5-$^2H_2$]-, [9,10-$^2H_2$]-, [14-$^2H_2$]oleic acid) and [18-$^2H_3$]oleic acid [45,46] were incorporated biosynthetically into the membranes of *A. laidlawii* as described previously [47]. The growth medium was enriched with the appropriate specifically-deuterated oleic acid (20 mg/l) and with cholesterol (approx. 20 mg/l). The acyl chain distribution and the exact cholesterol content, as determined by gas chromatography, are given in Table I. The NMR samples consisted of approx. 200 mg of freeze-dried membrane, hydrated with deuterium-depleted water (Aldrich Chemical Co., Milwaukee, WI) in a weight ratio of about 1:1.2. The samples were mixed by centrifugation through a constriction, then sealed in glass ampoules.

The ^2H-NMR spectra were obtained at 41.3 MHz using a home-built spectrometer [48] and the modified quadrupolar echo pulse sequence [37]. The $\pi/2$ pulses were 5–6 μs in length and the spacing between the two $\pi/2$ pulses was 50–60 μs. The frequency of the spectrometer was set to the center of the symmetric quadrupolar powder pattern. All of the spectra were obtained using quadrature detection, but in order to improve the signal-to-noise ratio by a factor of $\sqrt{2}$, the spectra for the (5-$^2H_2$) and (14-$^2H_2$) labels were folded so that the two halves were superimposed.

The quadrupolar echo technique effectively eliminates spectral distortion due to the finite recovery time of the radiofrequency (rf) receiver. For the rf field strengths used in the present experiments (as monitored by the 90° pulse lengths), the influence of the quadrupolar interaction during the rf pulses leads to another distortion, characterized by reduced intensity in the wings of some of the broader spectra. No corrections were made for this effect, as its only significant consequence here is the underestimation of the second moments for the lower temperatures in Fig. 5, which does not affect the qualitative analysis given.

Results

Liquid crystalline phase spectra

The NMR spectra of the various deuterium-labelled *A. laidlawii* membrane samples grown with and without cholesterol are shown in Fig. 1. The spectra were obtained at a temperature of 25°C, where all of the membrane lipids are in the liquid crystalline state. The most obvious change in the spectra upon the incorporation of cholesterol is the increased width of the quadrupolar powder patterns. The separation of the peaks in the spectra, $\Delta\nu_Q$, is shown as a function of temperature in the liquid crystalline phase in Fig. 2; the data for the samples without cholesterol were presented earlier in Ref. 37. Rather than using the actual peak maxima to obtain the $\Delta\nu_Q$ values, the separation of the points which lie about 85% of the way up the outside of the peaks (as measured from the baseline) was determined. Simulation studies (unpublished) have shown that the latter criterion for measuring $\delta\nu_Q$ yields a more accurate value when significant line-broadening is present in the spectra. The addition of cholesterol results in an increase in $\Delta\nu_Q$ at each position studied on the oleoyl chains, and at each temperature. The quadrupole splittings, and hence the degrees of molecular ordering, decrease with increasing temperature in a roughly linear fashion. Due to differing cholesterol levels in the various samples (Table I), a direct comparison of the effect of the cholesterol at the different labelled positions cannot be made. It is possible, however, to normalize the data to a uniform cholesterol level. In Table II the $\Delta\nu_Q$ values at 25°C for samples with and without cholesterol are listed. The percentage increase resulting from the addition of cholesterol is

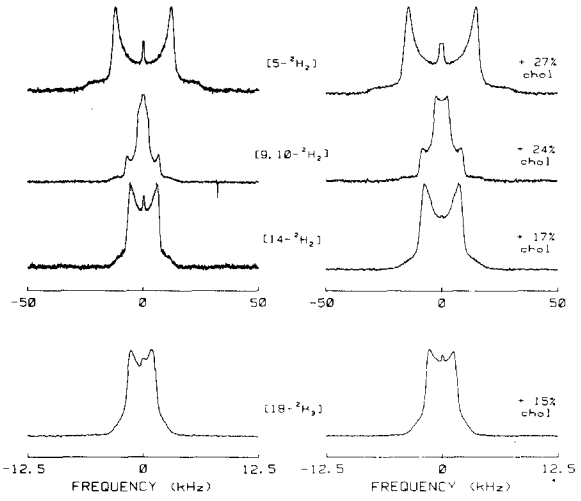

Fig. 1. Comparison of ^2H spectra for *A. laidlawii* membranes with and without cholesterol, at 25°C. The oleoyl chains are deuterated at the positions indicated. The spectra on the left are for the samples lacking cholesterol; those on the right are for membranes containing 15–27 mol% cholesterol. Typical experimental parameters are a pulse spacing of 50 μs, a pulse length of 6 μs, a 0.2 s recycle time (1.5 s for the methyl sample), and 64000 scans.

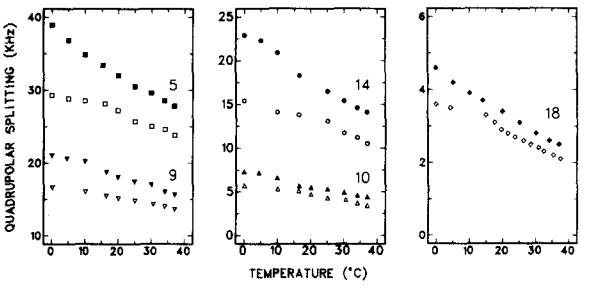

Fig. 2. Temperature dependence of the quadrupolar splittings for the specifically deuterated oleoyl chains in the *A. laidlawii* membranes. The solid symbols represent the data for the membranes containing cholesterol, the open symbols for the corresponding samples lacking cholesterol. The estimated error is indicated by the size of the symbols.

given in column four. To take into account the differing amounts of cholesterol, the percentage increases in $\Delta\nu_Q$ were divided by the cholesterol concentration to obtain the percentage increase/mol% cholesterol, which is listed in the last column of Table II. The justification for this

TABLE I

FATTY ACID AND CHOLESTEROL COMPOSITION OF THE TOTAL MEMBRANE LIPIDS OF *ACHOLEPLASMA LAIDLAWII* B GROWN ON SPECIFICALLY-DEUTERATED OLEIC ACID AND CHOLESTEROL AT 37°C

Carbon atom labelled	Mol% cholesterol	Mol% fatty acid					
		12:0	14:0	16:0	18:0	18:1	Other
5	27	1.9	5.5	21.2	1.9	64.6	4.9
9,10	24	–	4.0	18.6	2.5	73.3	1.6
14	17	1.8	5.1	27.2	3.1	60.8	2.0
18	15	0.9	4.0	19.0	–	71.3	4.8

TABLE II

CHANGE IN THE QUADRUPOLAR SPLITTING DUE TO THE ADDITION OF CHOLESTEROL

Carbon atom labelled	$\Delta\nu_Q$ (kHz)		Increase (kHz)	% Increase	% Increase per mol% cholesterol
	With	Without			
5	30.8	26.0	4.8	19	0.7
9	17.5	14.7	2.8	19	0.8
10	5.3	4.4	0.9	21	0.9
14	16.5	12.4	4.1	33	1.9
18	3.1	2.7	0.4	15	1.0

final step lies in the observations on model systems [20,22,29] that a plot of quadrupole splitting, $\Delta\nu_Q$, as a function of cholesterol content is approximately linear, at least up to 30 mol% cholesterol. A comparison of the entries in the last column of Table II shows that cholesterol causes the greatest relative perturbation in the C-14 position and the least in the plateau region at position C-5. On the other hand, in terms of absolute changes, the greatest effect is seen at the C-5 position.

As well as increasing the quadrupolar splittings, the addition of cholesterol causes $\Delta\nu_Q$ to have a greater temperature dependence, as shown in Fig. 2. The temperature coefficients of the quadrupolar splittings are given in Table III. These coefficients represent the slopes of the lines which best fit the data above 10°C in Fig. 2. At each carbon position measured, cholesterol causes an increase in the temperature coefficient.

The phase transition region

In view of the interesting results that have been observed at the gel to liquid crystal phase transition upon the addition of cholesterol, an investigation of the low temperature behaviour of the oleate -labelled *A. laidlawii* membranes containing cholesterol was carried out. These results are to be compared with those for samples lacking cholesterol [37]. The spectra shown in Fig. 3 are for the C-14 position, and those shown in Fig. 4 are for the C-18 position. Above 5°C the spectra

TABLE III

THE INFLUENCE OF CHOLESTEROL ON THE TEMPERATURE DEPENDENCE OF THE QUADRUPOLAR SPLITTING

Carbon atom labelled	Temperature coefficient of $\Delta\nu_Q$ (kHz/K) (×100)	
	with cholesterol	without cholesterol
5	26.0	15.4
9	15.1	8.8
10	8.2	6.9
14	20.7	12.7
18	5.4	4.3

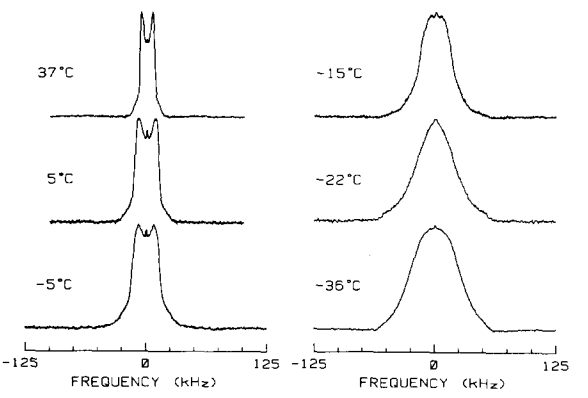

Fig. 3. Temperature dependence of the spectra for the (14-$^2H_2$) -labelled oleoyl chains in *A. laidlawii* membranes containing 17 mol% cholesterol. Spectra were acquired with 64000 scans, a 0.17 s recycle time, a 50 μg pulse spacing, and a 5 μs pulse length.

are characteristic of the liquid crystalline phase. Below this temperature there is a marked increase in the intensity of the wings of the spectra, signalling the transition to the gel phase. The intensity in the wings of the spectrum continues to increase as the temperature decreases. The spectra shown in Figs. 3 and 4 are similar to those observed for the [12-$^2H_2$]oleate-labelled sample without cholesterol, presented in Fig. 6 of Ref. 37.

The second moment of the ^{2}H-NMR spectra,

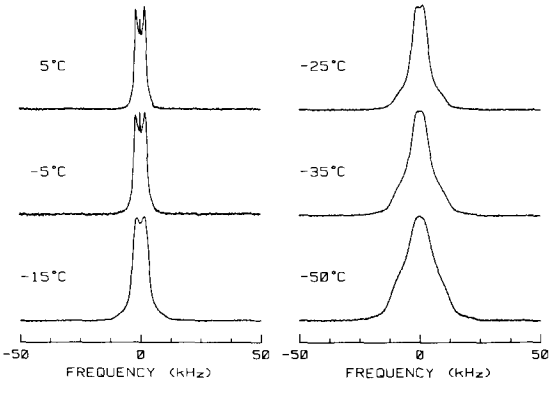

Fig. 4. Temperature dependence of the spectra for the methyl-labelled oleoyl chains in membranes of *A. laidlawii* containing 15 mol% cholesterol. The spectra were acquired with 8000 scans, a 1.4 s recycle time, a 55 μs pulse spacing, and 5 μs pulse lengths.

defined by the equation

$$M_2 = \int_0^{w_m} w^2 F(w)\, dw \Big/ \int_0^{w_m} F(w)\, dw \qquad (1)$$

is a useful parameter when trying to quantify the width of the quadrupole spectrum, especially when the peaks in the spectrum are not well defined. In Eqn. 1, $F(w)$ is the spectral lineshape function, with $w = 0$ corresponding to the center of the symmetric powder pattern and w_m is the cutoff frequency beyond which there is only noise. Fig. 5 shows the variation in M_2 with temperature for samples with and without cholesterol. The moments for the (14-$^2H_2$)- and (18-$^2H_3$)-labelled samples with cholesterol are presented along with the data from Ref. 37 for the (12-$^2H_2$)-labelled sample lacking cholesterol. As a consequence of rapid rotation about the last carbon-carbon bond, the quadrupole splitting for the methyl group is reduced by a factor of 3, and thus the second moment by a factor of 9, from that for a comparable methylene group [34,39]. Therefore, for the purposes of comparison, the data for the methyl group label were multiplied by a factor of 9 for presentation in Fig. 5. It is apparent from Fig. 5 that the incorporation of cholesterol into the *A. laidlawii* membranes, at least to the level of 17 mol%, causes no gross changes in the width or position of the phase transition.

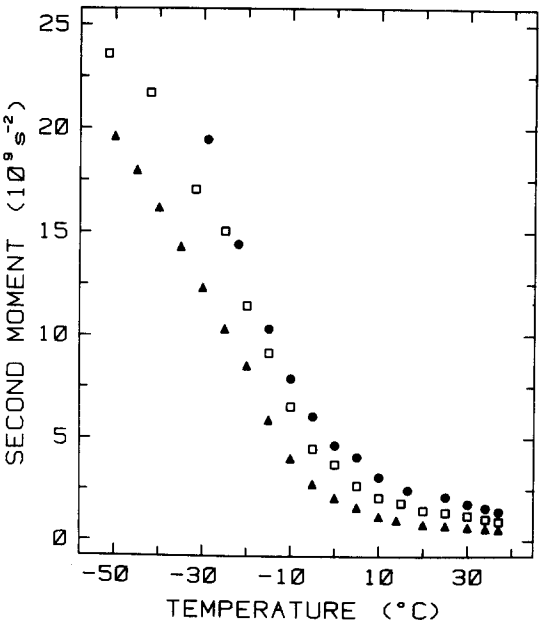

Fig. 5. Temperature dependence of the second moments for the ^2H-labelled oleoyl chains in the *A. laidlawii* membranes. (●), [14-$^2H_2$]+17 mol% cholesterol. (▲), [18-$^2H_3$]+15 mol% cholesterol. (□), [12-$^2H_2$] with no cholesterol. The data for the methyl position were multiplied by a factor of 9 for comparison.

Discussion

Molecular motion in the liquid crystal phase and the influence of cholesterol

Any molecular motion will reduce the measured quadrupolar splitting $\Delta\nu_Q$ from its rigid lattice value of 126 kHz. The measured $\Delta\nu_Q$ can be expressed in terms of the C-^2H bond order parameter [34],

$$\Delta\nu_Q = \frac{4}{4}\left(\frac{e^2qQ}{h}\right) S_{CD} \qquad (2)$$

In the liquid crystalline state, the molecular packing is sufficiently loose to allow rotational and translational motion of the molecule as a whole. Rapid rotation of the lipid molecules about the bilayer normal [34] reduces S_{CD} from unity to the value $(3\cos^2\beta - 1)/2$, where β is the angle between the C-^2H bond and the bilayer normal. Tilting of the molecule with respect to the bilayer normal, and internal motions of the acyl chains resulting from *trans-gauche* isomerization, cause β to be time-dependent, with the result that

$$S_{CD} = \langle (3\cos^2\beta(t) - 1)/2 \rangle \qquad (3)$$

where the angular brackets represent the appropriate time average. The *trans-gauche* isomerization gives rise to the fluid nature of the acyl chains characteristic of the liquid crystalline phase. Statistical mechanical calculations performed by Marčeljá [50], Schindler and Seelig [51], and more recently by Gruen [52] and Dill and Flory [53] have lead to a better understanding of the internal motions of the acyl chains.

Due to the packing of the lipid molecules within the bilayer, there are severe constraints on the possible molecular conformations. The beginning

of the chain is confined by its attachment to the glycerol backbone of the lipid molecule. The plateau in the order parameter profile [34,35], a common feature of saturated acyl chains, is a direct consequence of the limitation of molecular motions by steric interaction between neighbouring chains. The steric hindrance results in the predominance of defect conformations which have the chains nearly parallel to the bilayer normal, such as the kink ($g^{\pm}tg^{\mp}$) and jog ($g^{\pm}ttg^{\mp}$).

The studies carried out on chains having a *cis* double bond [37,39] have emphasized the fact that the observed quadrupolar splittings or order parameters depend on both the amplitudes of the various molecular motions and the average orientation of the C-^{2}H bond. In the oleoyl chain the sharp decrease in the order parameter at the C-9,10 double bond is primarily a geometric effect.

Recently it has been pointed out [23,54] that rigid molecule tilting motions may also make a significant contribution to the observed order parameter. There are a number of mechanisms which could lead to a tilting of the molecule with respect to the bilayer normal. Each molecule acting independently could tilt as a rigid rod with respect to its neighbours. It would be expected that this motion would be of small amplitude and high frequency because of the steric hindrance involved with single particle motions. A mechanism requiring less energy is a cooperative tilting of many molecules, giving rise to an undulation mode in the bilayer [55]. The tilting motion of the molecules can be enhanced if there is an undulation wave pattern set up in the bilayer and the molecules are able to diffuse in the plane of the bilayer [56].

The separation of the measured order parameter, obtained from Eqn. 3, into a part resulting from internal modes of motion and a part accounting for motion of the molecule as a whole depends on a number of assumptions [57–59]. The reorientational motion of the molecule as a rigid unit must be independent of the configuration of the molecule. As well, there must be free rotational motion about the long axis of the molecule. The validity of these assumptions has yet to be adequately investigated by experiment, even in model membrane systems [60].

Proceeding in the manner previously suggested by Petersen and Chan [54], we rewrite Eqn. 3 as

$$S_{CD} = \langle \frac{3\cos^2\alpha - 1}{2} \rangle \langle \frac{3\cos^2\gamma - 1}{2} \rangle$$

$$= S_\alpha S_\gamma \qquad (4)$$

where α is the instantaneous angle between the bilayer normal and the direction of the long axis of the molecule, and γ is the angle between the C-^{2}H bond and the long axis of the molecule. The order parameter S_α, which applies to motion of the molecule as a whole, is constant over all the segments in the chain. S_γ is affected by *trans-gauche* isomerizations, and will therefore be a function of the position along the acyl chain.

One important question is whether the addition of cholesterol to the membrane influences S_α, S_γ, or both S_α and S_γ simultaneously. If S_α alone is changed with the addition of cholesterol then the percentage increase in S_{CD} should be constant over each position in the chain, since

$$\frac{\Delta S^i_{CD}}{S^i_{CD}} = \frac{\Delta S_\alpha S^i_\gamma}{S_\alpha S^i_\gamma} = \frac{\Delta S_\alpha}{S_\alpha} \qquad (5)$$

The data listed in Table II show that the percentage increase in S_{CD} varies by about a factor of two over the various positions in the chain. The percentage increase seems to be largest near the center of the bilayer rather than in the plateau region. It must be concluded therefore that the observed changes in order parameter as a result of cholesterol addition do not come solely from changes in S_α, the order parameter describing the motion of the molecule as a whole.

Earlier results of Oldfield et al. [23] using selectively deuterated cholesterol in DMPC-water systems were interpreted as evidence for rigid body motions in the bilayer. The order parameter for deuterium in the rigid ring structure of the cholesterol molecule, in the limit of small cholesterol concentrations, was less than the value of 0.5 expected for the case where there was only rotation about the long axis of the molecule. At high temperature (37°C above the phase transition) higher concentrations of cholesterol lead to an increase in S_{CD}, suggesting that cholesterol does in fact have an influence on the amplitude of

a rigid body motion. This evidence applies only to rigid body motions of cholesterol itself, and has no direct implication for the fatty acyl chains.

In order that Eqn. 2 be valid, the correlation times describing the various possible molecular motions must be short compared to $1/2\pi(\Delta\nu_Q)_{rigid}$. In the critical region where $\tau \sim 1/2\pi\Delta\nu_Q$ the usual powder pattern is not observed, and the splitting between the peaks is not a reliable experimental parameter [63]. Lipid-water systems which are gently mixed usually form multilamellar structures with a diameter of approx. 1.0 μm [61]. Assuming a diffusion constant (D) of about $5 \cdot 10^{-8}$ cm$^2 \cdot$ s^{-1} [62], the correlation time τ_c for diffusion over the curved surfaces.

$$\tau_c = r^2/4D \qquad (6)$$

has the value $1.25 \cdot 10^{-2}$ s. Previous ^{31}P results indicate that while such slow motions will have little effect on the quadrupole splittings of ^{2}H spectra, they do influence the observed lineshapes. In the liquid crystalline phase the lineshapes obtained for the *A. laidlawii* membranes with and without cholesterol (see Fig. 1) cannot be simulated by Lorentzian-broadened powder patterns. There are, however, reasonably well defined peaks in most of the spectra with splittings much less than the rigid lattice value of 126 kHz, indicating that there must be rotation about the long axis of the molecule and some *trans-gauche* isomerization taking place on a time scale fast compared to $1/2\pi(\Delta\nu_Q)_{rigid} = 1.3 \cdot 10^{-6}$ s. It should be noted that the spectra with the smallest quadrupole splittings, namely the C-10 and C-18 positions, show the largest deviation from the ideal quadrupole powder pattern. The lineshapes for the C-10 and C-18 segments suggest the presence of molecular motion with a correlation time of the order of 10^{-4} s. At the present time attempts are being made to simulate the ^{2}H spectra using various models, in order to identify this motional mode.

The main effect of cholesterol addition on the ^{2}H-NMR spectra is to cause an overall increase in the width of the power pattern, with little or no change in the lineshape. This suggests that the correlation time for the slow motion responsible for the lineshape is not significantly affected by the addition of cholesterol. It is also possible to conclude that the rotation of the molecule about the long axis remains relatively rapid, such that $\tau_R \ll 1/2\pi(\Delta\nu_Q)_{rigid}$. If this rotation were to become slow on the NMR time scale there would be a very dramatic increase, at least a factor of two, in the width of the spectra.

The phase transition

Due to the breadth of the gel to liquid crystal phase transition of the *A. laidlawii* membranes, as indicated by both ^{2}H-NMR and DSC techniques, it is very difficult to measure small shifts in the temperature range of the transition due to cholesterol addition. However, our results show that there is certainly no significant change in this range. This observation is in contrast to the results of Davis et al. [44] in their study of the *A. laidlawii* membranes enriched with perdeuterated palmitic acid. Whereas we observed no significant decrease in the temperature at which gel phase lipid started to appear, and no qualitative differences in the appearance of the ^{2}H spectra through the phase transition region between samples with and without cholesterol, Davis et al. [44] found that the onset of purely liquid crystalline phase spectra was depressed by about 20°C, and that the spectra of the sample containing cholesterol gave no indication of a clearly defined phase transition. The reason for the different results obtained in the two studies could be the dissimilar levels of cholesterol incorporation; in our samples the highest concentration of cholesterol was 27 mol%, as opposed to the 39 mol% present in the sample studied by Davis et al. [44]. However, we cannot presently rule out the possibility that the observed differences are due to different interactions of the cholesterol with membranes enriched with oleic and palmitic acids. For example, the ratio between the major lipids monoglucosyldiacylglycerol and diglucosyldiacylglycerol is altered depending on the ratio of unsaturated to saturated fatty acids in the membranes of *A. laidlawii* A [64]. This compositional difference could conceivably affect the lipid-cholesterol interaction in the membranes by altering the packing constraints of the molecules in the bilayers [64–66]. In order to facilitate the determination of the factors responsible for the different results observed in our own study and that of Davis et al. [44], spectra should be obtained

from samples of oleate- and palmitate-labelled *A. laidlawii* membranes with the same content of both fatty acids and the same concentration of cholesterol. Such studies are currently in progress.

References

1 Demel, R.A. and De Kruijff, B. (1976) Biochim. Biophys. Acta 457, 109–132
2 Quinn, P.J. and Chapman, D. (1980) CRC Crit. Rev. Biochem. 8, 1–117
3 Ladbrooke, B.D. and Chapman, D. (1969) Chem. Phys. Lipids 3, 304–367
4 Mabrey, S., Mateo, P.L. and Sturtevant, J.M. (1978) Biochemistry 17, 2464–2468
5 Lundberg, B., Svens, E. and Ekman, S. (1978) Chem. Phys. Lipids 22, 285–292
6 Verkleij, A.J., Ververgaert, P.H.J.T., De Kruyff, B. and Van Deenen, L.L.M. (1974) Biochim. Biophys. Acta 373, 495–501
7 Lentz, B.R., Barrow, D.A. and Hoechli, M. (1980) Biochemistry 19, 1943–1954
8 Kleeman, W. and McConnell, H.M. (1976) Biochim. Biophys. Acta 419, 206–222
9 Ladbrooke, B.D., Williams, R.M. and Chapman, D. (1968) Biochim. Biophys. Acta 150, 333–340
10 Franks, N.P. (1976) J. Mol. Biol. 100, 345–358
11 Worcester, D.L. and Franks, N.P. (1976) J. Mol. Biol. 100, 359–378
12 Veatch, W.R. and Stryer, L. (1977) J. Mol. Biol. 117, 1109–1113
13 Owicki, J.C. and McConnell, H.M. (1980) Biophys. J. 30, 383–398
14 Copeland, B.R. and McConnell, H.M. (1980) Biochim. Biophys. Acta 599, 95–109
15 Lippert, J.L. and Peticolas, W.L. (1971) Proc. Natl. Acad. Sci. U.S.A. 68, 1572–1576
16 Bush, S.F., Levin, H. and Levin, I.W. (1980) Chem. Phys. Lipids 27, 101–111
17 Hubbell, W.L. and McConnell, H.M. (1971) J. Am. Chem. Soc. 93, 314–326
18 Taylor, M.G. and Smith, I.C.P. (1980) Biochim. Biophys. Acta 599, 140–149
19 Gally, H.U., Seelig, A. and Seelig, J. (1976) Hoppe-Seyler's Z. Physiol. Chem. 357, 1447–1450
20 Stockton, G.W. and Smith, I.C.P. (1976)Chem. Phys. Lipids 17, 251–263
21 Opella, S.J., Yesinowski, J.P. and Waugh, J.S. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 3812–3815
22 Haberkorn, R.A., Griffin, R.G., Meadows, M.D. and Oldfield, E. (1977) J. Am. Chem. Soc. 99, 7353–7355
23 Oldfield, E., Meadows, M., Rice, D. and Jacobs, R. (1978) Biochemistry 17, 2727–2740
24 Shepherd, J.C.W. and Büldt, G. (1979) Biochim. Biophys. Acta 558, 41–47
25 Henze, R. (1980) Chem. Phys. Lipids 27, 165–175
26 Engelman, D.M. and Rothman, J.E. (1972) J. Biol. Chem. 247, 3694–3697
27 Phillips, M.C. and Finer, E.G. (1974) Biochim. Biophys. Acta 356, 199–206
28 Martin, R.B. and Yeagle, P.L. (1978) Lipids 13, 594–597
29 Jacobs, R. and Oldfield, E. (1979) Biochemistry 18, 3280–3285
30 Chapman, D., Owens, N.F., Phillips, M.C. and Walker, D.A. (1969) Biochim. Biophys. Acta 255, 458–465
31 Demel, R.A., Bruckdorfer, K.R. and Van Deenen, L.L.M. (1972) Biochim. Biophys. Acta 255, 311–320
32 Chapman, D. (1968) Biological Membranes (Chapman, D., ed.), Academic Press, New York
33 Razin, S. and Rottem, S. (1978) Trends Biochem. Sci. 3, 51–55
34 Seelig, J. (1977) Q. Rev. Biophys. 10, 353–415
35 Mantsch, H.H., Saitô, H. and Smith, I.C.P. (1977) Prog. N.M.R. Spectr. 11, 211–272
36 Stockton, G.W., Johnson, K.G., Butler, K.W., Tulloch, A.P., Boulanger, Y., Smith, I.C.P., Davis, J.H. and Bloom, M. (1977) Nature 269, 267–268
37 Rance, M., Jeffrey, K.R., Tulloch, A.P., Butler, K.W. and Smith, I.C.P. (1980) Biochim. Biophys. Acta 600, 245–262
38 Seelig, A. and Seelig, J. (1974) Biochemistry 13, 4839–4846
39 Seelig, J. and Waespe-Šarčevič, N. (1978) Biochemistry 17, 3310–3315
40 McElhaney, R.N. (1974) J. Mol. Biol. 84, 145–157
41 Engelman, D. (1971) J. Mol. Biol. 48, 153–165
42 Smith, I.C.P., Butler, K.W., Tulloch, A.P., Davis, J.H. and Bloom, M. (1979) FEBS Lett. 100, 57–61
43 McElhaney, R.N. (1974) Pan Am. Assoc. Biochem. Soc. Rev. 3, 753–760
44 Davis, J.H., Bloom, M., Butler, K.W. and Smith, I.C.P. (1980) Biochim. Biophys. Acta 597, 477–491
45 Tulloch, A.P. (1979) Chem. Phys. Lipids 23, 69–76
46 Tulloch, A.P. (1979) Chem. Phys. Lipids 25, 225–235
47 Stockton, G.W., Johnson, K.G., Butler, K.W., Polnaszek, C.F., Cyr, R. and Smith, I.C.P. (1975) Biochim. Biophys. Acta 401, 535–539
48 Jeffrey, K.R. (1980) J. Magn. Resonance 37, 465–468
49 Davis, J.H. (1979) Biophys. J. 27, 339–358
50 Marčelja, S. (1974) Biochim. Biophys. Acta 367, 165–176
51 Schnidler, H. and Seelig, J. (1975) Biochemistry 14, 2283–2287
52 Gruen, D.W.R. (1980) Biochim. Biophys. Acta 595, 161–183
53 Dill, K.A. and Flory, P.J. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 3115–3119
54 Petersen, N.O. and Chan, S.I. (1977) Biochemistry 16, 2657–2667
55 De Genne, P.G. (1974) Physics of Liquid Crystals, Oxford University Press, Clarendon, Oxford
56 Pincus, P. (1969) Solid State Commun. 7, 415–417
57 Burnell, E.E. and De Lange, C.A. (1980) J. Magn. Resonance 39, 461–480
58 Emsley, J.W. and Luckhurst, G.R. (1980) Mol. Phys. 41, 19–29
59 Burnell, E.E., De Lange, C.A. and Mouritsen, O.G. (1982) J. Magn. Resonance, in the press
60 Vaz, M.J., Vaz, N.A., Doane, J.W. and Westerman, P.W. (1979) Biophys. J. 28, 327–338

61 Papahadjopoulos, D. and Miller, N. (1967) Biochim. Biophys. Acta 135, 624–638
62 Kuo, A. and Wade, C.G. (1969) Biochemistry 18, 2300–2308
63 Stockton, G.W., Polnaszek, C.F., Tulloch, A.P., Hasan, F. and Smith, I.C.P. (1976) Biochemistry 15, 954–966
64 Wieslander, Å., Christiansson, A., Rilfors, L. and Lindblom, G. (1980) Biochemistry 19, 3650–3655
65 Israelachvili, J.N. and Mitchell, D.J. (1975) Biochim. Biophys. Acta 389, 13–19
66 Carnie, S., Israelachvili, J.N. and Pailthorpe, B.A. (1979) Biochim. Biophys. Acta 554, 340–357