ANISOTROPIC ^{2}H–NUCLEAR MAGNETIC RESONANCE SPIN-LATTICE RELAXATION IN CEREBROSIDE-AND PHOSPHOLIPID-CHOLESTEROL BILAYER MEMBRANES

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ABSTRACT The axially symmetric powder pattern ^{2}H–nuclear magnetic resonance (NMR) lineshapes observed in the liquid crystalline phase of pure lipid or lipid/cholesterol bilayers are essentially invariant to temperature, or, equivalently, to variations in the correlation times characterizing C—^{2}H bond reorientations. In either of these melted phases, where correlation times for C—^{2}H bond motions are shorter than 10^{-7} s, information on the molecular dynamics of the saturated hydrocarbon chain would be difficult to obtain using lineshape analyses alone, and one must resort to other methods, such as the measurement of ^{2}H spin-lattice relaxation rates, in order to obtain dynamic information. In pure lipid bilayers, the full power of the spin-lattice relaxation technique has yet to be realized, since an important piece of information, namely the orientation dependence of the ^{2}H spin-lattice relaxation rates is usually lost due to orientational averaging of T_1 by rapid lateral diffusion. Under more favorable circumstances, such as those encountered in the lipid/cholesterol mixtures of this study, the effects of orientational averaging by lateral diffusion are nullified, due to either a marked reduction (by at least an order of magnitude) in the diffusion rate, or a marked increase in the radii of curvature of the liposomes. In either case, the angular dependence of ^{2}H spin-lattice relaxation is accessible to experimental study, and can be used to test models of molecular dynamics in these systems. Simulations of the partially recovered lineshapes indicate that the observed T_1 anisotropies are consistent with large amplitude molecular reorientation of the C—^{2}H bond among a finite number of sites. Furthermore, from the observed orientation dependence of the ^{2}H spin-lattice relaxation rates, we conclude that order director fluctuations cannot provide the dominant relaxation pathway for acyl chain deuterons.

INTRODUCTION

Cholesterol (CHOL) is an integral component of most mammalian cell membranes, yet its biophysical function in these membranes is poorly understood. The amount of membrane cholesterol varies greatly between mammalian tissues with relatively high concentrations found in red blood cells, liver cells, and myelin membranes. The apparent tissue-specific variability of cholesterol deposition is further underscored in myelin membrane in which the sterol is suggested to be asymmetrically distributed between membrane monolayers (Caspar and Kirschner, 1971). This asymmetry may in turn be related to the glycosphingolipid asymmetry (Linington and Rumsby, 1978, 1980) in myelin membrane, as suggested on physical-chemical grounds in a recent study (Ruocco and Shipley, 1984). The concentration and positional variability of cholesterol in cell membranes, as well as the preeminent role of cholesterol in pathological conditions continue to raise important issues about the function of cholesterol in biomembranes.

Many of the effects of cholesterol in both model and biological membranes are now well characterized. An understanding of how cholesterol influences the physical properties of membranes requires a description of the interaction between cholesterol and lipid at the molecular level. For this reason, the phase diagram of the phosphatidylcholine (PC)/CHOL system and its interpretation has remained a subject of intense study by a variety of techniques. The picture which emerges from a synthesis of the observed effects of cholesterol and the models which have been proposed to explain them can be summarized as follows: In melted liquid crystalline bilayers, cholesterol has a profound "condensing" effect on the hydrocarbon chains which is manifest as an increase in the *d* spacing observed by x-ray diffraction and in bilayer thickness (Lecuyer and Dervichian, 1969; McIntosh, 1978). In contrast, below the chain-melting transition temperature,

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the continuous broadening of wide-angle x-ray diffraction lines observed with increasing cholesterol content in DMPC/CHOL dispersions implied that the molecular arrangement became increasingly disordered, and the separation between neighboring acyl chains increased (Hui and He, 1983). This disruption of the lattice by cholesterol evidently does permit more rapid axial diffusion of the lipid molecules, as shown by the sharp axially symmetric ^{2}H–nuclear magnetic resonance (NMR) spectra observed for dipalmitoylphosphatidylethanolamine (DPPE) in DPPE/CHOL dispersions, for example, but the lack of any significant reduction in the breadth of these spectra also indicated that cholesterol does not appear to substantially alter the *trans* population in the acyl chains, which remain reasonably well ordered (Blume and Griffin, 1982). The increased ordering effect of cholesterol above the chain-melting transition temperature is also manifest in solid state ^{2}H–NMR spectra. In particular, studies of specifically ^{2}H-labeled dipalmitoylphosphatidylcholine (DPPC)/ and DMPC/CHOL dispersions have shown that quadrupole splittings from the fatty acyl chains increase with cholesterol concentration (Gally et al., 1976; Haberkorn et al., 1977; Oldfield et al., 1978; Jacobs and Oldfield, 1979). As well as modifying the phospholipid order in the liquid crystalline phase, cholesterol modifies the phase behavior of the system. In addition, solid state ^{2}H– and ^{13}C–NMR spectra of DPPE/CHOL mixtures (Blume and Griffin, 1982) have been used to elucidate the changes in molecular order and dynamics which accompany changes in the phase behavior observed in differential scanning calorimetry (DSC) experiments.

Until very recently, most of our knowledge of the lipid/cholesterol interaction revealed by solid state ^{2}H–NMR has been obtained either through a comparison of ^{2}H quadrupole splittings ($\Delta\nu_Q$) above T_c (Gally et al., 1976; Haberkorn et al., 1977; Oldfield et al., 1978; Jacobs and Oldfield, 1979; Siminovitch et al., 1987), or by simulating gel phase ^{2}H–NMR lineshapes (Blume and Griffin, 1982).

In the liquid crystalline phase, where correlation times for C—^{2}H bond motions are shorter than 10^{-7}, the NMR lineshape is essentially invariant to temperature. Thus only the magnitude and the temperature-dependence of the quadrupole splitting, $\Delta\nu_Q$, remain as experimental parameters which any successful model must predict. In contrast, ^{2}H–NMR lineshapes of gel phase lipids are extremely sensitive to temperature both in the absence (Huang et al., 1980; Blume et al., 1982) and in the presence (Blume and Griffin, 1982) of perturbing molecules such as cholesterol. It is this fact which has allowed such a wealth of information on the rates and type of molecular motion to be extracted from the simulation of gel phase lineshapes. Therefore, in the liquid crystalline phase, additional information obtainable from spin-lattice relaxation experiments is essential in fully characterizing the interactions and dynamic properties of lipid/cholesterol mixtures. Such studies have only just begun, using either specifically ^{2}H-labeled cholesterol (Taylor et al., 1981, 1982; Dufourc, 1983) or lipid (Ghosh and Seelig, 1982).

Here, we have investigated the effects of spin-lattice relaxation on the NMR lineshapes of lipid/cholesterol mixtures, since there are theoretical reasons for expecting that molecular reorientation should give rise to orientation-dependent relaxation (Torchia and Szabo, 1982; Wittebort et al., 1987). With the recognition that spin-lattice relaxation in solids is often anisotropic, the lineshapes observed in inversion recovery experiments may be used to test motional models. Orientation-dependent spin-lattice relaxation is potentially an important source of information on molecular dynamics, and therefore ^{2}H spectra of pure lipid bilayers have been recorded in an attempt to observe these effects. However, a previous attempt to observe anisotropic spin-lattice relaxation in pure lipid bilayers was unsuccessful (Brown and Davis, 1981). A probable explanation for this effect is that rapid lateral diffusion of lipid molecules over the curved two-dimensional surface of multilamellar liposomes is sufficiently fast to prevent observation of any orientation-dependence in T_1 (Brown and Davis, 1981). However, due to fortuitous changes in the lateral diffusion rate or the radii of curvature of the liposomes, the effects of orientational averaging of T_1 by lateral diffusion are nullified in the lipid/cholesterol mixtures of this study. These changes in the physical properties of the lipid/cholesterol dispersions are sufficient to render observable anisotropic relaxation effects in spin-lattice relaxation experiments. Simulations of the lineshapes indicate that the observed anisotropies are consistent with large amplitude molecular reorientation of the C—^{2}H bond among a finite number of sites. Furthermore, the orientation-dependence of spin-lattice relaxation in these lipid/cholesterol systems is not that expected if slow, collective director fluctuations provide the dominant relaxation mechanism, as proposed for pure lipid bilayers (Brown, 1982; Brown et al., 1983; Williams et al., 1985).

MATERIALS AND METHODS

Sample Preparation

[7,7-$^2H_2$]N-palmitoylgalactosylsphingosine (NPGS) was synthesized using the method of Radin (1972) and [4,4-$^2H_2$]DPPE was synthesized as described previously (Blume et al., 1982). The synthesis of [7,7-$^2H_2$]DPPC was carried out by Avanti Polar Lipids, Inc., Birmingham, AL using [7,7-$^2H_2$]palmitic acid prepared according to methods described in Das Gupta et al. (1982). Samples consisted of ~70 mg of labeled lipid together with 40–50 mol % cholesterol. Lipid and cholesterol were mixed in chloroform/methanol or in dichloromethane/methanol, and the solvent was evaporated under a stream of nitrogen. The lipid mixtures were dried under high vacuum for 24 h to remove residual solvent, dispersed in ^{2}H-depleted water (~70 wt% H_2O, Aldrich Chemical Co., Inc., Milwaukee, WI), quickly frozen in liquid nitrogen and sealed under vacuum in 7-mm outside diameter glass tubes.

NMR Spectroscopy

^{2}H–NMR spectra were obtained at a frequency of 45.3 MHz using a home-built solid-state pulse spectrometer and a super-conducting sole-

noid (6.8 T). To study the effect of spin-lattice relaxation, ^{2}H–NMR lineshapes were obtained as a function of a variable interval τ using a three-pulse inversion recovery sequence ($180_x - \tau - 90_x - t - 90_y$) for T_1 experiments, as follows. In order to observe the recovery of magnetization after the application of a single π pulse, the magnetization was sampled at various intervals τ after the inverting pulse using the quadrupole echo (Davis et al., 1976; Solomon, 1958):

$$\pi - \tau - (\pi/2)_0 - t - (\pi/2)_{\pm 90} - t - \text{ACQ}. \qquad (1)$$

Between 2,000 and 30,000 echo signals were accumulated, depending on the absolute magnitude of longitudinal magnetization τ ms after the inverting pulse. For example, in the region of the null ($\tau \approx (1n2)T_1$), an appropriate increase in the amount of signal averaging was required to enhance the signal-to-noise ratio. The Fourier transforms of the echo were then used to monitor any orientation-dependence in the recovery of magnetization. The recycle delay ($\leq$0.4 s) was chosen to be at least five times the longest T_1 of the mixture under investigation. The pulse separation t between the two sampling pulses of the quadrupole echo was typically 50 μs. The dwell time was 2 μs, corresponding to a spectral width of $\pm$250 kHz. The ^{2}H $\pi/2$ pulse width was 2.0–2.2 μs. Phase cycling and quadrature detection were used for all NMR experiments (Griffin, 1981).

Selective inversion recovery experiments were carried out using a Dante pulse sequence (Bodenhausen et al., 1976; Morris and Freeman, 1978) consisting of a train of 16 pulses of 0.4 μs duration (corresponding to a $\pi/2$ pulse of 3.2 μs), and spaced 15 μs apart. At some time τ after the Dante pulse train, the extent of magnetization recovery was monitored by the quadrupole echo sequence. The complete pulse sequence can then be written as

$$[(\alpha)_0 - t_2]^n - \tau - \left(\frac{\pi}{2}\right)_0 - t_1 - \left(\frac{\pi}{2}\right)_{\pm 90} - t_1 - \text{ACQ}, \quad (2)$$

where $n\alpha \simeq \pi$, t_2 = 15 μs, and t_1 = 50 μs.

RESULTS

To study the effects of spin-lattice relaxation on the NMR lineshape, we have performed a set of inversion recovery experiments on several lipid/cholesterol bilayer systems. Representative results of some of these experiments are shown in Figs. 1 and 2. Each one of the spectra displayed is the result of one inversion recovery experiment in which the magnetization has been sampled by the quadrupole echo sequence (see [Eq. 1] above) at a fixed time τ after a single inverting pulse. The Fourier transforms of the corresponding quadrupole echos for a set of delays τ then immediately reveal any angular variation in T_1 across the powder pattern spectra.

The partially relaxed spectra of Figs. 1 and 2 clearly demonstrate that there is a significant orientation-dependence of the ^{2}H spin-lattice (T_1) relaxation times in both the NPGS/ and DPPE/cholesterol mixtures. A comparison of Figs. 1 and 2 reveals that the T_1 anisotropy evident in these partially relaxed spectra is very similar in both mixtures, although for any particular orientation, the relaxation rate is clearly much faster in the DPPE/cholesterol bilayers. Liquid crystalline [7,7-$^2H_2$]DPPC bilayers containing 50 mol % cholesterol also exhibit very similar anisotropic T_1 relaxation (results not shown). The basic features of the T_1 anisotropy in the partially relaxed spectra of an equimolar mixture of 1,2-bis(perdeuterio)

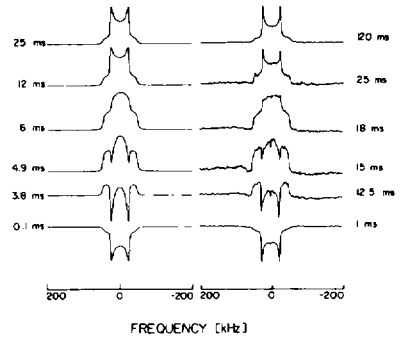

FIGURE 1 (*Right*) Partially recovered (T_1) spectra of 2[4,4-$^2H_2$]DPPE/50 mol% cholesterol at 31°C as a function of τ in the inversion recovery sequence. (*Left*) Simulations of the partially recovered lineshapes at the indicated τ delays. Spectral simulations were performed using a nine-site model, which includes both *gauche-trans* isomerization and long axis rotational diffusion, as described in the text. The two *gauche* sites are equally populated ($P_{g+} = P_{g-} = 0.125$), and the population of the *trans* site ($P_t = 0.75$) is chosen so that the observed splitting is reproduced by the simulation. The rotational diffusion rate is 2.2×10^9 s^{-1}, determined from the jump rate k ($k = 5 \times 10^8$ s^{-1}) between N sites ($N = 3$) according to the expression $D = (4\pi^2/N^2)k$ (Torchia and Szabo, 1982), while the *gauche-trans* isomerization rate is 5×10^7 s^{-1}.

palmitoyl-*sn*-glycero-3-phosphocholine (DPPC-d_{62}) and cholesterol shown in Fig. 3 are very similar to those of the specifically labeled lipid/cholesterol mixtures shown in Figs. 1 and 2, which suggest that both the orientation dependence and the magnitude of T_1 are very similar for most positions on the palmitoyl chain (excluding the methyl group). In any of the lipid/cholesterol mixtures we have investigated, the angular-dependence of the relaxation rates can be characterized by the ordering:

$$\frac{1}{T_1(0°)} > \frac{1}{T_1(54.7°)} > \frac{1}{T_1(90°)},$$

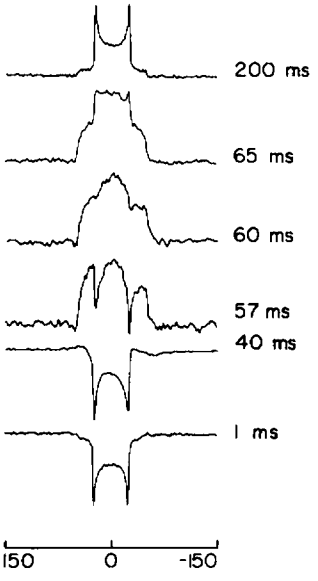

FIGURE 2 Partially recovered (T_1) spectra of [7,7-$^2H_2$]NPGS/44 mol% cholesterol at 75°C as a function of τ in the inversion recovery sequence.

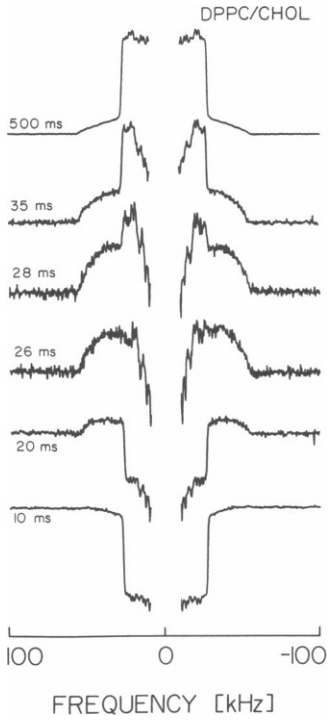

FIGURE 3 Partially recovered spectra of an equimolar DPPC-d_{62}/CHOL mixture at 33°C as a function of τ in the inversion recovery sequence. For the sake of clarity in presentation, the intensity from the longer relaxing methyl groups are not shown in these spectra.

where 0°, 54.7°, and 90° correspond to the parallel edges, center, and perpendicular edges of the powder spectrum, respectively. The absence of any orientation-dependence of T_1 in the partially relaxed spectra of pure liquid crystalline NPGS bilayers at 76.5°C[1] (results not shown) corroborates the observations of Brown and Davis (1981) who noted the experimental invariance of T_1 with bilayer orientation in specifically deuterated DPPC bilayers. The failure to detect any significant orientation-dependence of T_1 in pure lipid bilayers contrasts sharply with the T_1 anisotropy evident in the partially relaxed lineshapes of lipid/cholesterol mixtures shown in Figs. 1, 2, and 3.

Brown and Davis (1981) have shown that, after a long, weak pulse designed to selectively invert only the center of the powder pattern, there is a rapid transfer of magnetization from domains oriented near the magic angle (i.e., the inverted component) to domains having other orientations. This is a direct consequence of rapid lateral diffusion in the liquid crystalline DPPC bilayers. In the gel phase, they report that this transfer of magnetization does not occur, a result which may simply be a consequence of slower lateral diffusion (see Discussion). In order to determine whether or not magnetization transfer occurs in the liquid-gelatin phase (Blume and Griffin, 1982) of cerebroside/cholesterol bilayers, we have performed the same selective inversion recovery experiment originally performed by Brown and Davis (1981) on specifically deuterated DPPC bilayers, with the minor modification that a Dante pulse sequence instead of a long, weak pulse was used to accomplish the selective inversion (Fig. 4). For delay times $\tau \ll 5$ ms, the difference spectra $[F(\omega, \tau \rightarrow \infty) - F(\omega, \tau)]$ of Fig. 4 obtained by subtraction of the selectively inverted spectrum $F(\omega, \tau)$ from the equilibrium spectrum $F(\omega, \tau \rightarrow \infty)$ reveals that part of the spectrum which was selectively inverted. For longer values of τ, these difference spectra may indicate transfer of magnetization from domains oriented near the magic angle to domains having other orientations. The difference spectra of the NPGS/CHOL mixture in Fig. 4 clearly demonstrate that even 20 ms after selective inversion, there has been no significant transfer of magnetization to other regions of the powder pattern. In particular, these difference spectra show that there is no rapid increase in the intensity of the 90° peaks as there is, for example, in DPPC bilayers deuterated either at the 4-position (Brown and Davis, 1981) or the 7-position (Fig. 4, *top*).

In the lipid/cholesterol mixtures we have studied, what is true for the lipid component should also be true for the cholesterol component, so that the absence of orientational averaging should also allow the observation of anisotropic spin-lattice relaxation of ^{2}H nuclei on specifically labeled cholesterol. The partially recovered lineshapes of an equimolar NPGS/3α-^{2}H-CHOL mixture shown in Fig. 5 demonstrate that this is indeed the case.

DISCUSSION

In the melted liquid crystalline phase of lipid/cholesterol mixtures, the ^{2}H–NMR lineshape of specifically deuterated lipid is an axially symmetric powder pattern. Although these powder patterns are amenable to a lineshape analysis, very little of the underlying molecular dynamics would be revealed since, in contrast to the gel phase where molecular fluctuations of the C—^{2}H bond are relatively slow, the rates of *gauche-trans* isomerization and rotational diffusion are both in fast limit ($>10^7$ s^{-1}). For

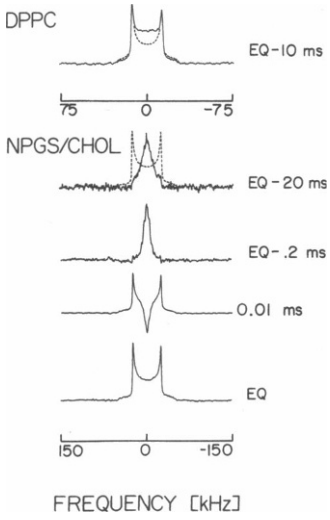

FIGURE 4 Selective inversion experiments on [7,7-$^2H_2$]DPPC bilayers at 45°C (*top*), and [7,7-$^2H_2$]NPGS/50 mol% cholesterol bilayers at 76°C (*bottom*). The equilibrium spectrum $F(\omega, \tau \rightarrow \infty)$ of the NPGS/CHOL mixture is labeled by EQ, while the selectively inverted spectrum $F(\omega, \tau)$ is labeled by the τ delay τ = 0.01 ms. In this notation, difference spectra $[F(\omega, \tau \rightarrow \infty) - F(\omega, \tau)]$ for DPPC bilayers (*top*) or NPGS/CHOL bilayers (*bottom*) are then labeled as EQ – τ ms.

[1]Hydrated *N*-palmitoylgalactosylsphingosine bilayers have a high T_c (82°C) (Ruocco et al., 1981). The cerebroside bilayers can, however, be supercooled and maintained in a stable, melted liquid crystalline bilayer phase at 82°C $\geq T \geq$ 73°C (Ruocco, 1983).

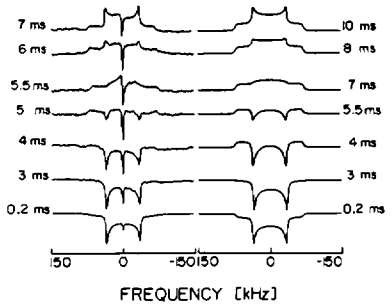

FIGURE 5 (*Left*) Partially recovered (T_1) spectra of an equimolar NPGS/3α-^{2}H-CHOL mixture at 69°C as a function of τ in the inversion recovery sequence. (*Right*) Simulations of the partially recovered lineshapes at the indicated τ delays. Spectral simulations were performed using a three-site jump model in which C—^{2}H bond of the 3-carbon in cholesterol is oriented at 73.5° with respect to the axis of motional averaging. The exchange rate was 7.8×10^8 s^{-1}.

this reason the simulated lineshapes are relatively insensitive to the rates of molecular motion. Accordingly, any model of the microscopic mechanisms of molecular reorientation which lead to these spectra must be content with predicting the $\Delta\nu_Q$ alone, leaving unanswered all questions concerning the time scales of molecular reorientation involved. Clearly, this is an unsatisfactory situation for testing any proposed model.

In principle, NMR relaxation can yield valuable information about the mechanism and rates of molecular motions which is not available from the powder pattern lineshapes of liquid crystalline lipid (Kimmich et al., 1983). Significant progress in this area has already been made by interpreting the frequency- and order parameter-dependence of ^{2}H spin-lattice relaxation rates in DPPC bilayers (Brown, 1982; Brown et al., 1983; Williams et al., 1985). Spin-lattice (T_1) experiments are especially useful if the predicted T_1 anisotropies are model-dependent, since the anisotropic relaxation behavior observed in these experiments can be used to discriminate between models. Provided these anisotropic relaxation effects are not obscured or obliterated by rapid lateral diffusion (Brown and Davis, 1981), the measurement of ^{2}H spin-lattice relaxation rates and their orientation dependence can be a useful strategy for probing molecular dynamics in lipid bilayer phases (Pope et al., 1982; Siminovitch et al., 1985; Jarrell et al., 1987).

The presence of high concentrations of cholesterol in the lipid/cholesterol mixtures of this study has allowed the observation of orientation-dependent spin-lattice relaxation in the partially relaxed powder pattern spectra of melted bilayers. Brown and Davis (1981) have shown that in the liquid crystalline phase of pure DPPC, molecular diffusion is rapid enough to allow each molecule to sample all orientations during times of the order of T_1, thus preventing the observation of the orientation-dependence of spin-lattice relaxation. An immediate corollary is that whenever anisotropic relaxation behavior is observed, such as in the gel phase of pure lipid bilayers (Siminovitch et al., 1985) or in the melted phase of lipid/cholesterol mixtures (Figs. 1–3), either the lateral diffusion rate must be slow enough to prevent any significant orientational averaging of T_1, or there must be a significant increase in the radii of curvature of the multilamellar liposomes. That low temperatures can significantly reduce the diffusion rate is well-documented in the case of DPPC bilayers (Rubenstein et al., 1979; Tamm and McConnell, 1985). If the lateral diffusion rate is slow enough, or if the lamellae of the lipid/cholesterol dispersions have only a slight curvature, then in a selective inversion experiment, there should be no transfer of magnetization across the spectrum after inversion of the center of the powder pattern. In the gel phase of pure DPPC bilayers (Brown and Davis, 1981) and in the liquid crystalline phase of NPGS/cholesterol bilayers (Fig. 4), no such transfer of magnetization occurs. The most reasonable interpretation, therefore, of the results of the selective inversion experiment presented in Fig. 4 is that in the NPGS/cholesterol system and, by analogy in the other lipid/cholesterol mixtures we have investigated, mechanisms for orientational averaging of spin-lattice relaxation rates are not effective.

The partially recovered lineshapes of an equimolar NPGS/3α-^{2}H-CHOL mixture shown in Fig. 5 demonstrate that in these lipid/cholesterol systems, anisotropic T_1 relaxation behavior is observed for both lipid and cholesterol. Simulations of these lineshapes employ a model in which the C—^{2}H bond vector at position 3 is oriented at 73.5° with respect to the axis of motional averaging, and executes a three-site hop about this axis at a rate (k) of 7.8×10^8 s^{-1}, corresponding to a correlation time ($\tau_c = (3k)^{-1}$) of 4.2×10^{-10} s rad^{-1}. These simulation parameters agree very well with previously determined values of 79° between the 3-position C—^{2}H bond and the averaging axis (Taylor et al., 1981), and 3.5×10^{-9} s rad^{-1} for the correlation time of cholesterol molecular motion (Taylor et al., 1982), both quantities measured in equimolar egg lecithin/cholesterol mixtures. Note that the correlation time for cholesterol in egg lecithin was estimated (Taylor et al., 1982) from the T_1 minimum observed at 30°C ($\omega_0\tau_c \approx 1$). At higher temperatures corresponding to those of this NPGS/CHOL study, the variation of T_1 with temperature in the egg lecithin/CHOL system (Taylor et al., 1982) indicates that cholesterol motion enters the motional narrowing regime, leading to much shorter correlation times as found for the NPGS/CHOL system at 69°C. Further studies of specifically labeled cholesterol in lipid/cholesterol mixtures are now in progress to characterize the molecular dynamics of cholesterol in these systems. In the absence of orientational averaging, the orientation-dependence of spin-lattice relaxation becomes accessible to experimental study, as the spectra of Figs. 1–3 so clearly demonstrate. More importantly, we are now in a position to assess the validity of models of molecular motion and dynamics in the liquid crystalline phase for the

liquid gelatin phase of lipid/cholesterol mixtures, and to probe in more detail the nature of the interaction between phospholipid and cholesterol in the lipid bilayer.

It has been shown (Brown, 1982; Brown et al., 1983; Williams et al., 1985; Brown et al., 1986) that a relaxation law of the form

$$\frac{1}{T_1} = A\tau_f + B S^2_{C-^2H} \omega_0^{-1/2} \quad (3)$$

can provide a unified picture of molecular dynamics in the liquid crystalline phase of pure DPPC bilayers. In Eq. 3, A and B are constants, ω_0 denotes the nuclear Larmor frequency and S_{C-^2H} is the segmental order parameter determined from the ^2H $\Delta\nu_Q$ according to the relationship

$$\Delta\nu_Q = \frac{3}{4}\frac{e^2qQ}{h} S_{C-^2H}. \quad (4)$$

The first A term of Eq. 3 includes contributions from rapid, local motions, due to *gauche-trans* isomerizations and long-axis rotational diffusion of the lipid chains, characterized by the effective correlation time τ_f, whereas the second B term describes slower, collective fluctuations in the local ordering. If the relaxation rate is dominated by collective order fluctuations, then Eq. 3 predicts that the relaxation rate should be proportional to $S^2_{C-^2H}$, and should disperse as $\omega_0^{-1/2}$. The dependence of T_1^{-1} on ordering, as determined from ^2H–NMR $\Delta\nu_Q$, and the dependence of T_1^{-1} on frequency, as determined from ^{13}C and ^2H–NMR spin-lattice relaxation studies, are both consistent with the order parameter and frequency-dependence predicted by the B term of Eq. 3. Moreover, the collective model for slow motions (Brown, 1982), embodied in the B term of Eq. 3, also predicts a characteristic orientation-dependence of T_1^{-1} if the slow, collective fluctuations are the dominant relaxation mechanism. Specifically, the relaxation rate is given by (Brown, 1982):

$$\frac{1}{T_1} \cdot \alpha \left\{\frac{3}{2}\left[d_{11}(\beta)^2 + d_{1-1}(\beta)^2\right] + 3\sqrt{2}\left[d_{21}(\beta)^2 + d_{2-1}(\beta)\right]\right\}, \quad (5)$$

where $d_{MN}(\beta)$ are the reduced Wigner rotation matrix elements, and β is the angle between the director and the magnetic field. If these motions dominate the observed acyl chain relaxation rates in lipid/cholesterol mixtures, the characteristic anisotropy in spin-lattice relaxation predicted by Eq. 5 should be manifest in the liquid crystalline phase of these systems. However, the orientation-dependence observed experimentally (Figs. 1–3) and calculated on the basis of the nine-site model (Fig. 1) is not that predicted by the slow, collective model (Brown, 1982).

The nine-site model includes both *gauche-trans* isomerization and long axis rotational diffusion. *Gauche-trans* isomerization is treated as a three-site chemical exchange process in which the C—^2H bond vectors are oriented along three of the principal directions of a tetrahedron, one corresponding to the *trans* site and the other two corresponding to *gauche* sites. The two *gauche* sites are equally populated, and the population of the *trans* site is chosen so that the observed splitting is reproduced by the simulation. Long axis rotational diffusion is modeled as a three-site hop about the director, and thus there are a total of nine sites in the model. As with previous lineshape simulations of gel phase spectra (Blume et al., 1982), 3-fold (120°) rotational jumps about the director would seem to be a more appropriate model for rotational diffusion in the liquid-gelatin phase of these lipid/cholesterol mixtures than a continuous diffusion model. The formalism required to simulate lineshapes from this model, and to calculate the anisotropy of the relaxation, is described in Torchia and Szabo (1982) and Wittebort et al. (1987). A comparison of the nine-site model simulations of Fig. 1 with the partially relaxed lineshapes of Fig. 6, which were calculated on the basis of the orientation-dependence predicted by Eq. 5, clearly illustrates the differences between the two models in the anisotropic spin-lattice relaxation behavior which they predict. For example, from the simulations of Fig. 1, it is clear that in the nine-site model

$$\frac{1}{T_1(54.7°)} > \frac{1}{T_1(90°)},$$

whereas Fig. 6 shows that

$$\frac{1}{T_1(54.7°)} < \frac{1}{T_1(90°)}.$$

Certainly, the different orientation-dependence predicted by the model of Brown (1982) is not surprising, since it arises from a consideration of slow, collective director fluctuations, a process which has been explicitly neglected

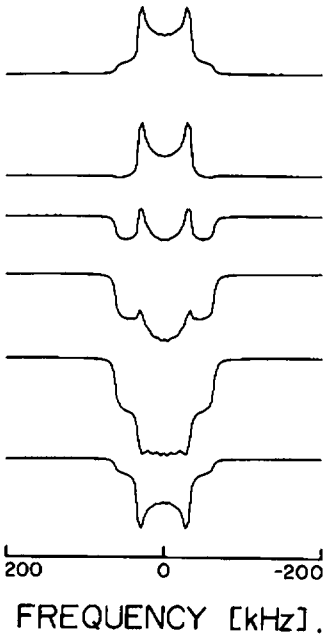

FIGURE 6 Simulations of partially recovered spectra assuming the orientation dependence of the relaxation rate is governed by Eq. 5 (director fluctuations provide the dominant relaxation pathway).

in the nine-site model. Nevertheless, the discrepancy between the anisotropic relaxation behavior predicted by the nine-site model (and which we observe experimentally in lipid/cholesterol bilayers) and that predicted by the slow, collective model (Brown, 1982) may have important and far-reaching implications, and deserves further experimental investigation. Since the T_1 anisotropy predicted by the slow collective model is not observed in the partially relaxed powder spectra of unoriented lipid/cholesterol bilayers, nor is it observed in the spectra of oriented bilayers of DMPC or DPPC (Jarrell et al., 1987), we conclude that order director fluctuations cannot provide the dominant relaxation mechanism for saturated acyl chain deuterons at these Larmor frequencies (the MHz regime). This conclusion is supported by a recent study of the ^{1}H spin T_1 relaxation dispersion of DMPC bilayers by Rommel et al. (1988), who concluded that collective order director fluctuations contribute to the relaxation process only at extremely low frequencies in the kHz regime, whereas the conventional high frequency range (MHz) is dominated by reorientation of individual molecules. Thus it would appear that isolated rather than collective motions, specified in terms of single rather than a continuous distribution of correlation times, are the distinguishing feature of models which best describe molecular dynamics in lipid bilayers.

In conclusion, the work we have described above is an example of the information which may be extracted from an analysis of the partially relaxed powder pattern lineshapes observed in T_1 experiments (Torchia and Szabo, 1982; Wittebort et al., 1987). In the framework of the nine-site model, cholesterol substantially increases the *trans* isomer population. Increased *trans* conformations in hydrocarbon chains are consistent with an increased lamellar repeat, d, or bilayer thickness, d_1, of melted NPGS (Ruocco and Shipley, 1984) and phosphatidylcholine bilayers (McIntosh, 1978), respectively, in the presence of cholesterol. Such changes in these x-ray diffraction parameters are a manifestation of the well-known condensation effect of cholesterol (Lecuyer and Dervichian, 1969). Agreement between the simulations and the partially recovered experimental lineshapes shown in Fig. 1 suggest that the angular dependence of the ^{2}H relaxation rates is dominated by axial diffusion, and not by *gauche-trans* isomerization. This T_1 anisotropy is dependent on the number of sites N chosen to model rotational diffusion about an axis of $3N$-fold symmetry. For example, we have shown that the T_1 anisotropies that are observed for 9-, 18-, or 27-site models, which include *gauche-trans* isomerization between three sites and which employ either three, six, or nine sites, respectively, for rotational jumps about the long axis changes (jump rates k were scaled to leave the correlation time $\tau_c = (Nk)^{-1}$ invariant). The nine-site model probably represents an oversimplification of molecular dynamics in the hydrocarbon chain, and there are undoubtedly other molecular motions which contribute to the spectral density functions. This may explain the discrepancy between the experimental times and the calculated times in Fig. 1. The explicit neglect of off-axis motions of the lipid long axis (including wobble, for example) in our analysis does not rule out a role for such motions in more sophisticated models of ^{2}H relaxation in these systems. Rather, our approach has been to seek the simplest explanation for the observed T_1 anisotropies, without the introduction of an unwieldy number of fit parameters. Given that there are at least two modes of molecular reorientation in these systems which could contribute to the spin-lattice relaxation, namely *trans-gauche* isomerization and long-axis diffusion, the relative rates of these processes, and the proximity of their correlation times to the T_1 minimum ($\omega_0\tau_c \approx 1$), will ultimately determine the T_1 anisotropy observed. Note that the rotational jump constant ($k = 5 \times 10^8\ s^{-1}$) is an order of magnitude faster than the *gauche-trans* isomerization rate ($k = 5 \times 10^7\ s^{-1}$). In this respect (i.e., the relative rates of axial diffusion and local motions), the liquid-gelatin phase of the DPPE/CHOL mixture bears a close resemblance to the gel state of pure DPPE bilayers, whose lineshape simulations also employed a model whose rotational jump constants were an order of magnitude faster than the *gauche-trans* isomerization rate (Blume et al., 1982).

Our original motivation in developing the nine-site model was to provide a simple physical explanation of the observed hydrocarbon chain ^{2}H quadrupolar splittings of the liquid crystalline phase of lipids, without recourse to an order parameter analysis. In lipid bilayers, the S_{C-^2H} order parameter provides a description of the angular fluctuations of the C—^{2}H bond with respect to an axis of motional averaging (the director):

$$S_{C-^2H} = \left\langle \frac{1}{2}(3\cos^2\beta - 1) \right\rangle, \qquad (6)$$

where the orientational average $\langle\ \rangle$ denotes an ensemble average over a time scale long compared with $1/\Delta\nu_Q$, and where β is the instantaneous angle between the C—^{2}H bond and the director. If the amplitudes and time scales of the molecular motions of the C—^{2}H bond can be parametrized in a model, then the orientational average of Eq. 6 can be explicitly evaluated (Spiess, 1980; Rosenke et al., 1980) and compared directly with an experiment, an approach we have adopted here. A problem inherent in the use of order parameters is that it is never possible to disentangle orientation effects (i.e., a change in the average orientation of the C—^{2}H bond) from motional averaging effects (i.e., a change in the amplitude of the C—^{2}H bond vector excursions about the axis of motional averaging). Without independent corroborative evidence from an adjacent labeled segment, this problem necessarily leads to a certain ambiguity in the interpretation of order parameters, particularly in the case of phospholipid headgroups

(Akutsu and Seelig, 1981; Siminovitch et al., 1984). While it is not our intention to supplant order parameters which, in the absence of specific models of molecular motion, often provide a useful description of the average orientational order in anisotropic systems, the approach we have taken here can provide considerable additional insight into the dynamics and character of the motional averaging process.

In interpreting the axially symmetric ^{2}H–NMR lineshapes observed in the liquid crystalline phase of lipids, the conventional practice has been to invoke motions which consist of an effectively continuous distribution of possible C—^{2}H bond orientations. For this reason, it is not surprising that an anisotropic rotational diffusion model, involving small jumps among a large number of possible orientations, has enjoyed considerable favor in selecting models of molecular reorientation in the liquid crystalline phase (e.g., Brown, 1982). However, it is important to recognize that axially symmetric lineshapes can also arise, in the limit of fast motion, from a discrete process which consists of large amplitude jumps among a small number of sites (Griffin, 1981; Rice et al., 1981). In the liquid crystalline phase of lipid/cholesterol bilayers, molecular reorientation of the C—^{2}H bond among nine such sites provides a comprehensive interpretation of the magnitude of the S_{C-^2H} order parameter and the anisotropic spin-lattice relaxation behavior. Whether or not this model will provide an adequate description of molecular ordering and dynamics in the liquid crystalline phase of pure lipid systems remains to be seen. Further experiments are underway in our laboratory designed to address this question.

A copy of the Fortran code used for the lineshape simulations of this study is available from the authors on request.

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