show moderate deviations from additivity. To determine if the shielding effect of a fluoro substituent on a C_γ atom depends on the atoms bonded to the C_γ atom, we compare results for 1,3-difluoroallene with those for monofluoroallene. The changes in the C_α magnetic shielding tensor between these two systems are −13.7 (*xx*), −1.2 (*yy*), and 1.9 (*zz*) ppm. Thus, here also there is reasonable additivity for substituent effects on the *xx* and *yy* elements but not for effects on the *zz* element. This comparison suggests that it is mainly the lack of additivity of substituent effects on the *zz* component of the magnetic shielding tensor which is responsible for the different γ substituent effects obtained for the isotropic chemical shifts in the 1,1- and 1,3-difluoroallene substitution patterns.

We are currently continuing this study in an attempt to relate the nonadditive shielding effects of fluoro substituents to various features of molecular electronic structure.

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Nuclear Magnetic Relaxation Behavior of Lecithin Multilayers[1]

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Abstract: In an effort to elucidate the state of molecular motion in the lamellar liquid crystalline phase of lecithin, the nuclear magnetic relaxation properties of unsonicated lecithin multilayers have been investigated. A previously noted field dependence of the proton magnetic resonance line width is shown to be accounted for by the chemical shift differences among the various kinds of protons. Spin–lattice relaxation rates have been measured for these protons as a function of temperature and frequency, and these data have been interpreted in terms of models for the segmental motion of the choline head groups and the hydrocarbon chains. The influence of spin diffusion on the relaxation behavior of the various protons is also discussed.

The current interest in understanding the details of structure and function of biological membranes has prompted studies of model membrane systems. The lamellar liquid crystalline phase of lecithin and water increasingly is being used as a model for biological membranes.[3–7] Evidence that the liquid crystal phase is a close structural analog to real biomembranes comes from differential scanning calorimetry,[8,9] X-ray diffraction,[10–12] and spin label studies,[13,14] all of which have detected this phase in real biomembranes.

Coarse aqueous liquid crystal dispersions can be irradiated ultrasonically to produce bilayer vesicles which are useful models for studying membrane function, especially transport.[3,15] However, there is strong evidence that, at the molecular level, the vesicle bilayer is significantly disordered because of the high curvature of these small particles.[16,17] For this reason, coarse aqueous dispersions of lamellar liquid crystals, hereafter referred to as "multilayers," are better models of membrane structure, though they are not suitable for transport studies because of the absence of a well-defined inside and outside.

Nuclear magnetic resonance (nmr) is a method of established usefulness for studying molecular interactions. Furthermore, nmr is sensitive to the details of molecular motion. For these reasons, there is much interest in using nmr to investigate membrane systems. Lipid model membranes,[18–24] as well as erythrocyte[25] and

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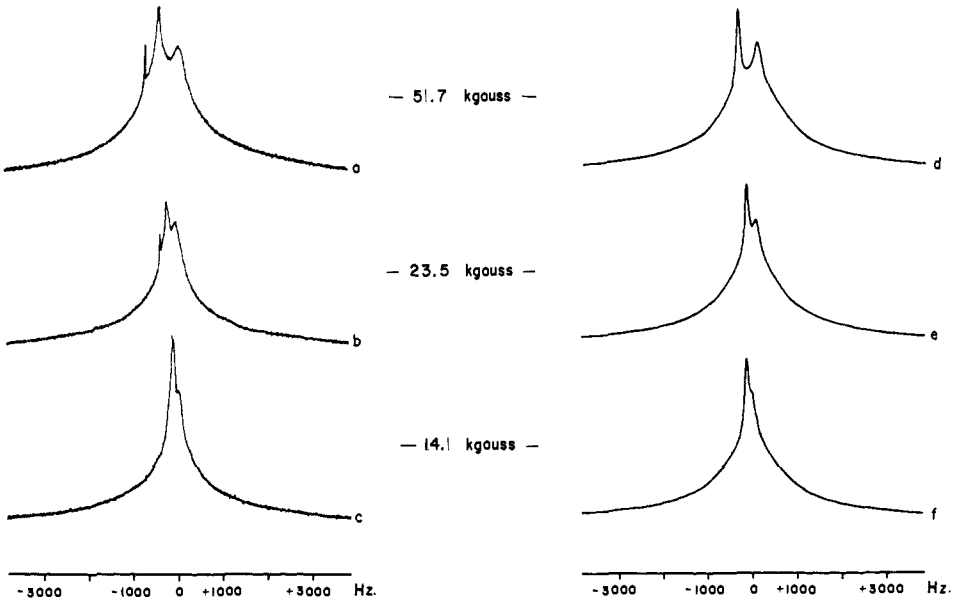

Figure 1. Magnetic field strength dependence of the line shape of the pmr spectra of dimyristoyl lecithin multilayers at 30° (a–c) and comparison of these experimentally observed spectra with computer simulated spectra (d–f). The simulated spectra are composed of the sum of the following Lorentzian lines: (i) a peak 3000 Hz wide containing 75% of the total signal intensity, centered at 0.0 ppm, corresponding to the methylene protons; (ii) a peak 100 Hz wide containing 5% of the total signal intensity, centered at −1.6 ppm, corresponding to the narrow choline methyl resonance; (iii) a peak 3000 Hz wide containing 10% of the total signal intensity, centered at −1.6 ppm, corresponding to the broad choline methyl resonance; (iv) a peak 200 Hz wide containing 5% of the total signal intensity, centered at +0.37 ppm, corresponding to the narrow terminal methyl resonance; and (v) a peak 3000 Hz wide containing 5% of the total signal intensity, centered at +0.37 ppm, corresponding to the broad terminal methyl resonance.

mitochondrial membranes,[26] have been studied with nmr.

Many previous nmr studies of model membranes have been done on ultrasonicated lipid bilayer vesicles, because such systems give rise to sharp nmr lines.[17,27] However, the nmr spectra of real biomembranes are dominated by broad resonances.[25,26] These spectral differences have been shown to be indicative of structural differences.[16] In this respect, the nmr spectra of biomembranes contain features which are just those found in the nmr spectra of lecithin multilayers. In order that nmr be a useful tool for studying biomembranes, complete and detailed interpretation must be made of the nmr properties of multilayers. In addition, the details of molecular motion in this simple model system will more clearly define the molecular motional state in real biomembranes. There is thus some urgency in understanding the molecular details responsible for the nmr properties of multilayers.

Work from this laboratory has shown that the principal features of the lecithin multilayer spectra are the broad methylene resonance and the presence of less intense and much narrower peaks from the choline and terminal methyl groups.[18] Recently, Seiter and Chan have developed a model of molecular motion to explain the line widths of the methylene and methyl resonances observed in the nmr spectra of lecithin multilayers.[16] In this model, the lecithin molecule undergoes restricted and anisotropic motion, resulting in certain solid-like features of the pmr spectrum. Still, two kinds of observations have remained unexplained. These are the field dependence of the pmr line width for lecithin multilayers[24,27,28] and the mechanism responsible for the spin–lattice relaxation time, T_1.[27,29]

In an earlier paper[18] we showed that the spin–spin relaxation time, T_2, for an entire sample of lecithin multilayers (frequently related to the line width) was independent of external magnetic field strength up to 14.1 kG. This observation was used to demonstrate that the magnetic dipolar interaction was the cause of the breadth of the pmr resonance. Recently, however, several workers have reported a dependence of the pmr line width on magnetic field strength at high field strengths, from 14.1 to 51.7 kG.[24,27] This field dependence implies the existence of a line broadening mechanism which is not dipolar in origin. In this paper we will try to resolve this problem of the field independence of T_2 and the field dependence of the line width.

An aspect of the nmr behavior of lecithin multilayers which is not well understood is the spin–lattice relaxation time, T_1. The unusual state of motion in the liquid crystal phase of these molecules presents theoretical and experimental difficulties in the interpretation of T_1. We have overcome some of these difficulties and will show how T_1 can be used to obtain details of molecular motion for lecithin in multilayers.

Experimental Section

Materials. Dimyristoyl lecithin, obtained from Nutritional Biochemicals, Inc., and dipalmitoyl lecithin, obtained from Calbiochem, gave a single spot by thin-layer chromatography and were used without further purification. Egg yolk lecithin was prepared by the method of Singleton, *et al.*[30] Deuterium oxide, 100.0%

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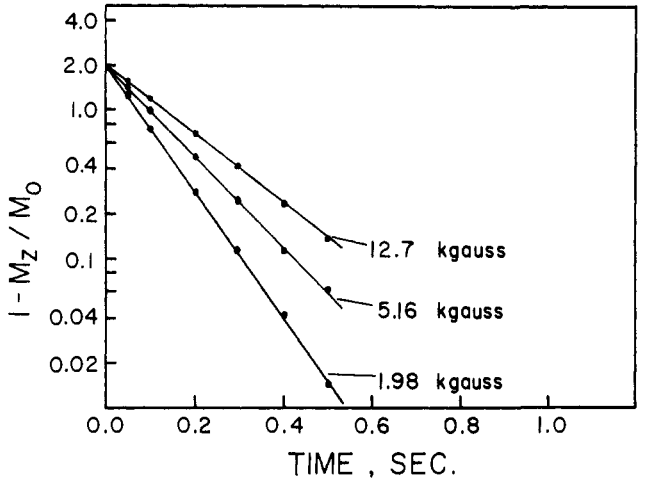

Figure 2. Recovery of the magnetization of all of the protons in an egg yolk lecithin multilayer sample after a 180° pulse at 30° for three different magnetic fields.

^2H, from Dia-Prep, was used in the preparation of dipalmitoyl and egg lecithin multilayers. Deuterium oxide, 99.7% ^2H from Columbia Organics, was used for samples of dimyristoyl lecithin. The samples were prepared by extensive agitation of lecithin and D_2O in an nmr tube, using a vortex mixer.

Instrumentation. The continuous wave spectra, as well as the Delayed Fourier Transform spectra, were obtained on Varian HA-100 and HR-220 spectrometers, equipped with Fourier transform accessories and interfaced to a Varian 620i computer. A data collection delay time of 475 μsec was used in the delayed Fourier transform measurements.[31] In all these experiments, the temperature was controlled with a Varian-4540 variable temperature controller.

The relaxation measurements for the bulk sample were made on a 1000-W pulse nmr spectrometer from Tomlinson Research Instruments, Inc., using a Varian 4013A 12 in. wide gap electromagnet. Probes of homemade construction were used for the measurements at different frequencies.[32] Temperature variation was achieved by equilibrating the 15-mm sample tube at the appropriate temperature outside the probe and then inserting it into the probe for the spectral observation.

The free induction decay and its Fourier transform at 14.1 kG were kindly provided by R. Vaughan and were obtained using pulse nmr equipment described elsewhere.[32]

Results

Spectra. The pmr spectra of dimyristoyl lecithin multilayers are shown in Figures 1a–c. The 51.7- and 23.5-kG spectra were obtained in the continuous wave mode, whereas the 14.1-kG spectrum is the Fourier transform of a free induction decay, with no data collection delay. The downfield spike arises from the residual protons in the D_2O. The sharp central line, about 100 Hz wide, is from the choline methyl protons. The sharp upfield line, about 200 Hz wide, has an origin in the terminal methyl protons on the lecithin side chains. The remaining protons, which are predominantly from methylene groups, appear as a spectral band several thousand hertz in width. There is clearly a field dependence of the line shape, although the precise magnitude of the dependence cannot be readily determined. This difficulty comes about because the line width depends upon a choice of the peak height, which is indeterminate because of the presence of sharp peaks superimposed on a broad peak. Also, the choice of the spectral base line is subject to considerable uncertainties.

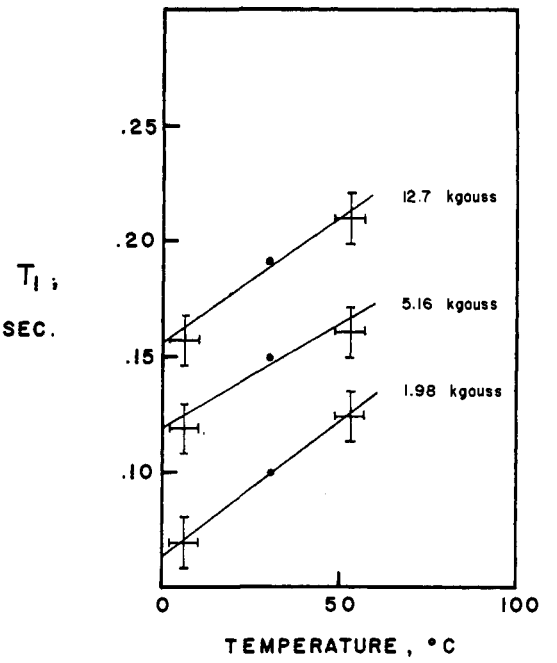

Figure 3. Temperature dependence of the proton spin-lattice relaxation time for a bulk sample of egg yolk lecithin multilayers for magnetic field strengths of 12.7, 5.16, and 1.98 kG.

The pmr absorption spectrum contains peaks from various methyl and methylene groups with significant chemical shift differences. In order to determine whether the chemical shift difference between the choline methyl, the terminal methyl, and the methylene groups could account for the observed magnetic field dependence of the line shape, we have computer simulated the spectra at 51.7, 23.5, and 14.1 kG, including the expected chemical shift differences between the peaks. These spectra are shown in Figures 1d–f.

T_2. The free induction decay was measured at 14.1, 12.7, and 5.16 kG, and T_2 was computed as the time for decay to $1/e$ of the initial value. These data are given in Table I. There is no field dependence evident in these data.

Table I. Magnetic Field Dependence of Line Widths and Transverse Relaxation Rates at 30°

Magnetic field, kG	Apparent line width	Obsd T_2, μsec	$1/\pi T_2$, Hz
51.7[a]	1700		
23.5[a]	1100		
14.1[a]	850	135	2360
12.7[b]		120	2650
5.16[b]		125	2550

[a] Dimyristoyl lecithin multilayers. [b] Egg yolk lecithin multilayers.

T_1. The magnetization recovery for the protons of an entire multilayer sample following a 180–90° pulse sequence is shown at three different magnetic field strengths in Figure 2. This magnetization recovery is exponential, indicating one value for T_1. The temperature dependence of the T_1 values at each field is given in Figure 3.

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DFT Spectra. The high resolution features of the spectra are themselves interesting and can usefully be studied by delayed Fourier transform nmr spectroscopy. This technique, which is described in detail elsewhere,[31] filters out the broad resonances, thereby providing a flat base line for the sharper resonances. The line width and intensity measurements for the choline and terminal methyl signals, determined using this method, are given in Table II for several different

Table II. Line Width and Intensity Results for DFT Experiments

Lecithin	Methyl group	% of protons obsd	Line width, Hz
Egg yolk[a]	Choline	30 ± 10	100 ± 20
	Terminal	60 ± 20	150 ± 50
Dipalmitoyl[b]	Choline	30 ± 10	110 ± 20
	Terminal	50 ± 20	180 ± 50
Dimyristoyl[a]	Choline	20 ± 10	110 ± 20
	Terminal	40 ± 20	180 ± 50

[a] At 30°. [b] At 50°.

varieties of lecithin. The intensity data are given as the percentage of the intensity expected on the basis of the molarity of the sample. It is clear that significantly less than 100% of the signal from the methyl groups appears with the line width indicated in the high resolution DFT spectrum.

The DFT method enables us to determine the T_1 values of the individual methyl resonances, since these resonances become isolated from the bulk of the signal and are thereby sufficiently distinct from each other. T_1 was determined from the magnetization recovery following a 180–90° pulse sequence and Fourier transforming the free induction decay after the 90° pulse. The magnetization recovery for the choline and terminal methyl resonances is shown in Figure 4. The recovery is exponential, again indicating the presence of only one value of T_1. The T_1 values at several temperatures at 51.7 and 23.5 kG are given in Table III.

Table III. Temperature and Magnetic Field Dependence of the Spin–Lattice Relaxation Times (sec) for the Methyl Protons of Dimyristoyl Lecithin Multilayers

	51.7 kG		23.5 kG	
Temp, °C	Choline	Terminal	Choline	Terminal
30	0.37	0.33	0.29	0.26
39	0.40	0.39	0.40	0.31
61	0.47	0.47	0.48	0.44
75	0.66	0.55	0.50	0.48

Discussion

Field-Dependent Line Shape. From Figure 1 it is clear that most of the field dependence of the line width can be accounted for by the chemical shift differences of the protons. Since the chemical shift is a necessary and perhaps sufficient line-broadening mechanism, an additional field-dependent line-broadening effect is not called for. It was possible to reproduce the shape of the experimentally observed spectra with broad components comprising the bulk of the signal, choline and terminal methyl signals which appear with less than 100% of their intensity in narrow signals, and

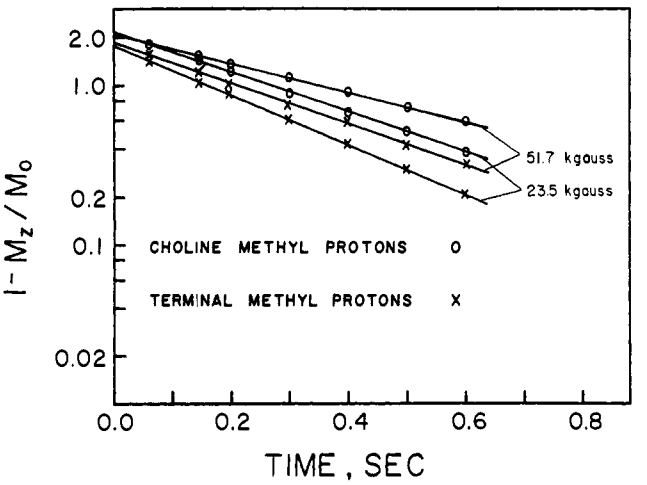

Figure 4. Recovery of the magnetization for choline methyl and terminal methyl protons of a dimyristoyl lecithin multilayer sample after a 180° pulse at 30° for magnetic fields of 51.7 and 23.5 kG.

choline and terminal methyl components of greater line width comprising the remainder of these signals. We point out that the principal feature of the computer simulated spectra, *i.e.*, the field-dependent line width, is not sensitive to details of the composition of the line. We find a similar field dependence with many different simulated compositions, as long as a chemical shift difference is included in the simulated spectra.

An important aspect of the line widths determined from the continuous wave spectra is that they are not at all in agreement with the values of $1/\pi T_2$, obtained from the free induction decay after a 90° pulse. This is especially apparent at 60 MHz where both the free induction decay and the Fourier transform of that free induction decay were available. This fact should not be surprising, since the relationship between the line width and T_2 computed from the $1/e$ value of the free induction decay is not known *a priori* for a complex line shape. When the line shape is given by a simple mathematical function, T_2 is related to the line width in a simple way. The two known examples of such simple relations are the Lorentzian line shape, for which

$$\Delta\nu = 1/\pi T_2$$

and the Gaussian line shape, for which

$$\Delta\nu = \left(\frac{\ln 2}{\pi}\right)^{1/2} \frac{1}{T_2}$$

where $\Delta\nu$ corresponds to the line width measured at half-maximum intensity. In the case of the lecithin molecule, not only are appreciable intensities of chemically shifted species present but also the individual resonance from each component cannot be presumed to be of a simple shape, since the molecule may be undergoing anisotropic and restricted motion.[16] Moreover, the presence of narrow lines in the observed continuous wave spectrum gives disproportionate weight to these lines when the width at half-height is determined. On the contrary, the initial height of the free induction decay after a 90° pulse contains each component weighted simply by its actual intensity, and, in time, each component of the free induction decay decays with its own value of T_2. If one component of the free induction decay is dominant in intensity, then

the overall T_2 is largely determined by this one component. This is the case for the lecithin studied here wherein the methylene protons make up over 70% of the signal.

In summary, the line width is not a meaningful measure of spin–spin relaxation for a complex spin system. The T_2 determined from the free induction decay is a reliable measure of the spin–spin relaxation, even for a complex system. In contrast to the apparent line width, T_2 is independent of magnetic field strength for lecithin multilayers.

The appearance in the pmr spectrum of less than 100% of the expected intensity for the methyl resonances has been interpreted to indicate solid-like features of the methyl spectrum.[16] The unseen intensity is *not* contained in other resonances, such as from methyl protons which are in a different motional state or at an unpropitious angle to the magnetic field. Rather, the signal which does show up with the indicated line width arises from a particular set of energy transitions and contains contributions from all protons from the particular chemical species of methyl group. We point this out because we later use T_1 values from the observed methyl resonances to establish a motional model for the entire system of methyl protons.

The Question of Spin Diffusion. An important experimental quantity to interpret is the value of T_1 for the entire sample. The initial part of the proton free induction decay for the entire multilayer sample contains proportionate contributions from all of the protons in the sample but may be considered to arise principally from the hydrocarbon chain methylene protons, since these account for about 70% of the protons in the lecithin molecule. These methylene protons would be expected *a priori* to be heterogeneous in their T_1 relaxation because of mobility differences among the various methylene groups along the hydrocarbon chains. However, we must first determine whether this measured T_1 value is a composition of T_1 values from the different methylene protons or if the measured T_1 is controlled by just a small fraction of the protons.

The former case, that individual protons have their own characteristic T_1, is the usual case in high resolution pmr. However, the latter possibility, that a specific few spins control the T_1 process for the entire spin system, occurs commonly in solids. Historically, the first example of this phenomenon was described by Bloembergen for crystals of alum, $KAl(SO_4)_2 \cdot 12H_2O$.[33] The immobility of the protons in this solid implies a longer T_1 than the value observed. Bloembergen showed that the spin–lattice relaxation was controlled by a very small percentage of a paramagnetic impurity. The mechanism for the relaxation originates in the weak coupling of the proton spins to the lattice, whereas the spins are strongly coupled to each other. The term "spin diffusion" was used to describe the diffusion of spin energy, *via* the magnetic dipolar coupling between the spins, from the entire spin system to the small number of proton spins near paramagnetic ions, which act as the heat sinks. The criterion for spin diffusion is that $T_2 \ll T_1$, together with the presence of some spins with relatively short T_1 values.

A second example of spin diffusion, somewhat more allied to our system of lecithin multilayers, is found in solid alkanes. Anderson and Slichter[34] showed that the reorienting methyl groups acted as the heat sinks for the entire proton spin system because of their rapid reorientation. It should be noted that the measured T_1 value did not correspond in magnitude to the T_1 calculated for an isolated methyl group. Instead, since the methyl protons relax the entire spin system, the measured T_1 is longer than the T_1 of the protons of an isolated methyl group by a factor of the ratio of the total number of protons in the spin system to the number of methyl protons.

We must determine whether or not spin diffusion is occurring in the proton spin system of lecithin multilayers. There may be spin exchange between the terminal methyl and methylene protons along the methylene chain and between the choline methyl and methylene protons. Resolving the first possibility will determine whether the T_1 measured for the terminal methyl group is the intrinsic T_1 of these protons or if the methyl group relaxation behavior is modified by coupling to methylene protons. The second possibility implicitly questions whether there is a motional difference among the methylene groups, with some fraction of the total relaxing the rest, or whether all of the chain methylene groups are in similar motional states for effecting T_1 relaxation. With regard to the last possibility, we note that the choline methyl T_1 behavior is not expected to be greatly modified by spin diffusion, since the nine choline methyl protons could be coupled to only four methylene protons, and the T_1 measured for the choline methyls would merely be increased by 35% even in the limit of infinitely fast spin diffusion.[34]

Abragam has shown how the spin-exchange rate (W) can be estimated for a solid from the second moment.[35] We shall extend this method of estimating the spin-exchange rate to the case of lecithin multilayers, wherein some motional narrowing of the line width occurs. We assume that the line shapes are Gaussian, so that we can replace the second moment by the square of the measured line width at half-height. In order to calculate the spin-exchange rate, it is necessary to determine the contribution to the line width from the particular set of spin-exchanging protons. The principal contribution to the observed line width is from the intrapair (geminal) protons, whereas we seek the lesser contribution from the more distant spin-exchanging protons of interest. Accordingly, if r_1 is the distance between the geminal protons (1.79 Å for methylene protons), and if r_2 is the distance between the spin-exchanging protons (2.48 Å for a methylene proton to the nearest proton on either the next methylene group or the methylene group two bonds away; 2.66 Å for the average distance from a reorienting methyl proton to the nearest methylene proton), then W can be estimated from $\Delta\omega$, the line width in radians per second determined from the free induction decay ($\approx$15,000 radians sec^{-1} for lecithin multilayers), as follows

$$W \approx \frac{\Delta\omega}{30}\left(\frac{r_1}{r_2}\right)^6$$

This expression predicts an exchange rate of $\approx 120\ sec^{-1}$ between nearest methylene groups and also between the

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methylene groups which are two bonds away, if the hydrocarbon chain is in the all-trans configuration.

The above calculation is applicable to spin exchange among magnetic nuclei with perfectly overlapping resonances. For chemically shifted spins, and for spins with grossly different line shapes, the spin-exchange rate is less than it would be when spectral overlap is perfect. Following Abragam,[35] this imperfect spectral overlap can be taken into consideration by multiplying W, calculated above, by the appropriate overlap integral. For example, the methyl resonance line shape for lecithin multilayers is composed of a broad resonance approximately 3000 Hz in width, comprising 50% of the signal intensity and arising from one class of transitions, and a superimposed narrow resonance about 200 Hz in width, comprising the other 50% of the signal and arising from a different class of transitions.[16,36] Spin exchange can occur when there is a simultaneous energy conserving transition among the methylene energy levels and among either class of methyl energy levels. However, because of differences in spectral overlap, there is a large difference in the value of W calculated for the two classes of methyl energy levels. Taking into account the number of different spin-exchange transitions which are possible between an interacting methylene pair of spins and a methyl three-spin system, together with the estimated overlap integrals, the overall spin-exchange rate between methylene and methyl protons can be estimated to be ≈ 130 sec^{-1}, with spin exchange involving those methyl energy levels giving rise to the broad methyl resonance occurring ten times more frequently than with those methyl levels associated with the narrow resonance.

We can now estimate the time for spin diffusion to occur over a certain distance along the methylene chain, as well as the time for spin diffusion to occur between the terminal methyl group and its neighboring methylene protons on the same hydrocarbon chain. The time for spin diffusion in the latter case is just the reciprocal of the spin-exchange rate, or ≈ 0.01 sec. For spin diffusion along the methylene chain, the successive spin exchanges along the various possible paths should be considered. Between methylene groups, spin exchange which occurs between protons on carbons separated by one methylene is much more efficient for effecting spin diffusion than the spin exchange between nearest neighbor interpair methylene protons, even though the spin-exchange rates W are comparable for both processes. This is so because in the latter case the spin energy gets propagated a distance a along the hydrocarbon chain axis, whereas in the former case this distance is $2a$. The time for spin energy to diffuse over a distance d along the hydrocarbon chain is thus given by[35]

$$t \cong \frac{d^2}{W[a^2 + (2a)^2]}$$

This result predicts a time of ~ 0.15 sec for spin diffusion over ten carbon–carbon bonds along the hydrocarbon chains of the lecithin molecules.

In the light of the above considerations, it is clear that the spin-exchange rate between terminal methyl and methylene protons is much faster than the rate of spin–lattice relaxation ($1/T_1$) of a methyl group. This means that the terminal methyl protons are strongly coupled to the methylene chain protons and must thereby share common heat sinks. Furthermore, because spin diffusion does occur over much of the methylene chain in a time shorter than the observed methylene T_1, the bulk of the methylene chain protons do not serve as their own heat sinks, in spin–lattice relaxation. It is likely that the less mobile methylene groups near the glycerol backbone, and thus most distant from the actual heat sinks, have line widths considerably greater then the average line width. Spin exchange among those protons would then be faster than the average rate for the chain methylenes, in which case the methylene chain would be sufficiently coupled that spin diffusion would occur over the entire chain in a time shorter than T_1.

We point out, however, that it is not possible for the entire methylene chain to be relaxed slowly *via* dissipation of the spin energy through the methyl heat sinks. This is because in general the measured T_1 of a strongly coupled spin system will be longer than the intrinsic T_1 of the relaxing spin by a factor of the ratio of the total number of spins coupled by spin diffusion to the number of relaxing spins.[36] Should relaxation of methylene protons be controlled by these methyl groups, the measured T_1 of ≈ 0.1 sec for the entire lecithin multilayer sample would imply an intrinsic T_1 of ≈ 0.01 sec for the methyl protons, a value which is about a factor of 3 shorter than the expected minimum possible methyl T_1 if the relaxation is purely of dipolar origin. Instead, the measured relaxation times indicate that the methyl group, together with perhaps 2–4 mobile methylene groups, serves as the heat sink for the entire methylene chain.

Interpretation of T_1 Data. The above considerations regarding spin diffusion in lecithin multilayers suggest that the methylene chain protons are strongly coupled among themselves and are coupled to the lattice *via* some fraction of their number. In addition, it appears that the spin–lattice relaxation behavior of the terminal methyl protons and the methylene protons of the hydrocarbon chains are interdependent. On the other hand, it could be said with some assurance that the choline methyl protons are not coupled to the protons on the hydrocarbon chains, although we expect that they are coupled to the two choline methylene groups. In the light of these considerations, interpretation of the data for the choline methyl groups can be made without qualification, since the T_1 measured for these protons is basically their intrinsic T_1. However, since the terminal methyl protons are coupled to a very large spin system, namely the methylene protons, the precise mechanism responsible for the terminal methyl T_1 is not clear. The T_1 observed for the bulk sample, however, can be considered as characteristic of the small number of the methylene protons which are acting as the heat sinks for the entire coupled spin system. The intrinsic value of T_1 for these methylene heat sinks would be considerably shorter than the observed bulk T_1 if these protons were not relaxing a large spin system.

We can now proceed to obtain a quantitative description of the motional state of the methyl and methylene groups of the lecithin molecule. We assume, for example, for a methylene pair, that the state of motion can be described in terms of a model in

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which the interproton magnetic dipolar vector reorients about the hydrocarbon chain axis with a correlation time $\tau_{||}$ and undergoes off-axis motion with a correlation time $\tau_{\perp}$, with the off-axis motion restricted to excursions of at most $\Delta\beta$ from the average interproton vector reorientation $\bar{\beta}$.[16] Similarly, the motion of a methyl rotor can be described by two motional correlation times, $\tau_{||}$ for the reorientation about the rotor axis and $\tau_{\perp}$ for the off-axis excursions, as well as a range for the excursions of the rotor axis, $\Delta\theta'$. Using this motional model, we seek appropriate combinations of the parameters $\tau_{||}$, $\tau_{\perp}$, $\Delta\beta$, or $\Delta\theta'$, which account for the following observations: (1) T_1 is a weak function of frequency, decreasing with decreasing frequency; (2) T_1 increases with increasing temperature at all frequencies observed; (3) the magnetization recovery is exponential at all frequencies observed.

In our analysis we have modified the well-known theory of Woessner[37] for relaxation of protons of methyl tops undergoing anisotropic reorientation to include the effects of motional restriction. The following expression has been obtained for an assembly of methyl rotors undergoing anisotropic and restricted motion[16]

$$\frac{1}{T_1} = \frac{9}{4}\frac{\gamma^4\hbar^4}{r^6}\left\{\frac{1}{4}\overline{(\sin\theta'\cos\theta')^2}\,\frac{2\tau_{\perp}}{1+\omega_0^2\tau_{\perp}^2} + \frac{1}{8}\overline{\sin^2\theta'(1+\cos^2\theta')}\,\frac{2\tau_c}{1+\omega_0^2\tau_c^2} + \frac{1}{4}\overline{(\sin^2\theta')^2}\,\frac{2\tau_{\perp}}{1+4\omega_0^2\tau_{\perp}^2} + \frac{1}{8}\overline{(1+6\cos^2\theta'+\cos^4\theta')}\,\frac{2\tau_c}{1+4\omega_0^2\tau_c^2}\right\}$$

In this expression, $1/\tau_c = 1/\tau_{\perp} + 1/\tau_{||}$, θ' is the orientation of the methyl rotor axis in the magnetic field, and the bars denote averaging over the appropriate range of $\Delta\theta'$. It should be noted that the intermolecular contribution to T_1, neglected in the above expression, has been shown to be of the order of 20% of the intramolecular relaxation rate for polymethylene chains.[38,39]

An analogous expression can be derived to calculate the T_1 of methylene protons which are reorienting freely about an axis, c. Here, θ' in the T_1 expression would denote the orientation of the c axis in the magnetic field, and the angular functions would be averaged over the appropriate range of β ($\Delta\beta$). Because $\bar{\beta} = 90°$ for methylene protons, this averaging over β is equivalent to a similar averaging of the angular functions over excursions in θ' of the c axis, and this latter procedure is actually used for convenience.

In order to clarify the above motional model, we now outline the method used to obtain the appropriate averages of the angular functions in the T_1 expression for the methyl protons. For a methyl rotor undergoing restricted motion, the rotor axis can wander over the angular range $\bar{\theta}' \pm \Delta\theta'$. This motion allows the rotor axis vector to trace out a cap on the surface of a unit sphere. For a given value of $\bar{\theta}'$, the angular averages in the T_1 expression may be obtained by averaging the angular functions over this range of angles. Statistical weighting of the angular functions must be included in the averaging procedure since the rotor axis has a greater probability of being at $\theta' = \bar{\theta}'$ than at any other angle, with $\theta' = \bar{\theta}' \pm \Delta\theta$ being the least probable angles. Using the weighting function

$$\sin\{(\pi/2)(\bar{\theta}' + \Delta\theta' - \theta')/2\Delta\theta'\}$$

T_1 can thus be evaluated at any angle $\bar{\theta}'$ given $\Delta\theta'$, $\tau_{||}$, and $\tau_{\perp}$. However, the samples contain methyl groups with all possible values of $\bar{\theta}'$. At any $\bar{\theta}'$ value, the number of methyl groups varies as $\sin\bar{\theta}'$. The T_1 which we determine experimentally is a composite of different T_1 values from protons with various average orientations of the axis vector relative to H_0. Since some of these orientations are more probable than others, the magnetization recovery of the various parts of the sample is weighted by $\sin\bar{\theta}'$, and the sum of the magnetization recoveries is fitted to a single value of T_1 for comparison with experiment.

As anticipated, our calculations predict that only certain ranges of the variables $\tau_{||}$, $\tau_{\perp}$, and $\Delta\theta'$ ($\Delta\beta$) can account for the complex temperature and frequency dependence observed for the T_1's. For the choline methyl protons, the observed T_1 and its temperature dependence (near room temperature) at 200 and at 100 MHz were accounted for only with the following parameters.

$$\tau_{||} = 1 \times 10^{-10} - 5 \times 10^{-11}\ \text{sec}$$
$$\tau_{\perp} \geq 4 \times 10^{-7}\ \text{sec}$$
$$\Delta\theta' \geq 60°$$

Similarly, the measured T_1 values at 54, 22, and 8.4 MHz, together with the observed temperature dependence (near room temperature), yielded

$$\tau_{||} = 1 \times 10^{-9} - 2 \times 10^{-10}\ \text{sec}$$
$$\tau_{\perp} \geq 1 \times 10^{-7}\ \text{sec}$$
$$\Delta\beta \geq 60°$$

for the motional parameters of the methylene protons which are relaxing the coupled spin system. In these results, the restriction on $\Delta\theta'$ and $\Delta\beta$ arises from the calculated nonexponential magnetization recovery for $\Delta\theta'$, $\Delta\beta < 90°$. In practice, the deviation from exponential behavior could be easily detected only for $\Delta\theta'$, $\Delta\beta \leq 60°$. Since the experimental magnetization recovery plots were always exponential, we conclude that for our samples $\Delta\theta' \geq 60°$.

The motional parameters for the terminal methyl group cannot be directly determined, because these methyl protons are coupled to the large methylene spin system, and are thereby affected in their relaxation behavior. Nonetheless, since the narrow methyl resonance is not strongly coupled to the methylene spin system, the intrinsic T_1 of the terminal methyl protons cannot be very different from the value of T_1 observed for the narrow methyl resonance. The motional parameters of our model may therefore be fitted to the observed T_1 data for the methyl resonance. The result of this treatment is that, for the terminal methyl protons on the hydrocarbon side chains, $\tau_{||} = 1 \times 10^{-10} - 5 \times 10^{-11}$ sec and $\tau_{\perp} \geq 1 \times 10^{-7}$ sec.

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Segmental Motion of the Hydrocarbon Chain. The preceding analysis has permitted us to ascertain those molecular motions of the lecithin molecule which serve to couple the spin energy into the lattice. An earlier line width analysis has enabled us to comment on the degree of motional restriction. The present T_1 studies confirm these line width results but in addition permit the description of the molecular motion in the lecithin molecule in terms of certain motional correlation times. Such motional correlation times have a precise meaning in magnetic resonance theory.

In a system as complex as a lecithin multilayer, interpretation of the correlation times $\tau_{||}$ and $\tau_{\perp}$ may present some difficulty. However, in one of our earlier papers[16] evidence was presented to suggest that only motions which preserve the overall orientation of the chain perpendicular to the multilayer surface are allowed. In fact, the off-axis motion, corresponding to $\tau_{\perp}$, has been ascribed to kink formation in lecithin multilayers[16,40] (or "crankshaft motion," as kinks have been called in polymers).[41] Kink formation results from + gauche rotation about one carbon–carbon single bond, simultaneous with − gauche rotation about another carbon–carbon bond which is separated from the first by one or more bonds. The intrapair, interproton vector on methylene groups underoing such rotation is taken through an angle of approximately 60° by this motion. Kink formation is expected to be most rapid near the free end of the methylene chain, since the initial activation energy is lowest there.[40] On the other hand, because of steric restrictions, the end of the methylene chain which is bonded to the glycerol backbone undergoes less rapid kink formation. On this basis, the off-axis motion of the methylene groups would be increasingly more rapid toward the methyl end of the chain.

The other type of methylene motion, described by $\tau_{||}$, is reorientation around the chain axis. The way in which such a reorientation comes about is of interest. The molecule as a whole cannot be reorienting at this rate of $\approx 10^9$ sec^{-1}, as indicated by the rate of $\approx 10^6$ sec^{-1} for comparable single chain molecules,[42] which have a much smaller moment of inertia than does the double chain lecithin and also whose cylindrical symmetry permits reorientation without great steric restraints, again different from lecithin. One type of motion which is occurring rapidly, and which could result in reorientation about the chain axis, is torsional oscillation about carbon–carbon single bonds. The motion about one bond is of low amplitude (<20°) and very fast, $\approx 10^{-14}$ sec^{-1}.[43] This frequency is much too high to be an effective source of T_1 relaxation. However, a long series of such torsional oscillators, weakly coupled, would give rise to larger amplitude motions of lower frequency, simply from statistical fluctuations in the phase of the many oscillations.

A quantitative treatment of the problem of a large number (*e.g.*, 16) of weakly coupled torsional oscillators which are loosely confined to a cylinder would be exceedingly difficult. We do not attempt even an approximate solution of such a problem. We only point out that such oscillations will give rise to a distribution of correlation times for motion around the methylene chain axis. These motions are bounded at the high frequency, low amplitude end by the single bond oscillation frequency of $\approx 10^{-14}$ sec. An example of a higher amplitude, lower frequency motion occurs when all oscillators are rotating in the same direction. The frequency of this occurrence is approximately $(1/2)^{16} \times 10^{14} \approx 1 \times 10^9$ (for a chain of 16 methylene groups). Our experimentally derived result that $\tau_{||} = 1 \times 10^{-9} - 2 \times 10^{-10}$ probably represents a weighted mean for a distribution of motions of the methylene group heat sinks.

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Summary

In the lamellar liquid crystalline phase of lecithin, there are proton spins with different chemical shifts and with different nmr line shapes. The spin–spin relaxation rate for the most abundant methylene proton spins can be determined from the free induction decay of the bulk sample. In contrast, the observed nmr line width of the bulk sample for such a complex spin system is not suitable for determining the spin–spin relaxation rate of the methylene protons because of the strong influence on the line width of chemically shifted spins and spins with narrow nmr signals, together with the difficulty of locating the true base line. The delayed Fourier transform technique was demonstrated to effectively filter out the broad methylene resonance, thereby providing a flat base line for the study of sharper methyl resonances.

This paper also demonstrates how the motional mechanisms responsible for the spin–lattice relaxation times of the lecithin methyl and methylene protons can be determined from a study of the temperature and the magnetic field dependence of the spin–lattice relaxation rate. For choline and terminal methyl protons, the motion which is most effective for T_1 relaxation was shown to be rapid reorientation around the methyl rotor axis, with a smaller influence from the slower, off-axis motion. The hydrocarbon chain methylene protons are most likely relaxed primarily *via* torsional oscillations of the methylene chain, with a smaller influence on the relaxation rate from kink formation. Spin diffusion was shown to have a pronounced influence on the T_1 of the hydrocarbon chain methylene protons. Furthermore, spin diffusion was demonstrated to give rise to mutual interdependence of the spin–lattice relaxation behavior of the terminal methyl and chain methylene protons. This study of spin–lattice relaxation behavior in lecithin multilayers complements and augments a recent lecithin line width study from this laboratory.[16] In our earlier work, the nmr line shape was shown to be a sensitive monitor of the details of slow molecular motions. This present work indicates that the spin–lattice relaxation is sensitive primarily to the time scale of the more rapid molecular motions.