Measurement of Fast Lateral Diffusion of Lipids in Vesicles and in Biological Membranes by ^1H Nuclear Magnetic Resonance†

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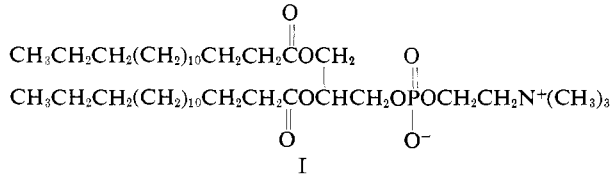
ABSTRACT: The proton spin–lattice relaxation times (T_1) for lecithin in deuteriomethanol are dominated by intramolecular motion. In contrast, the proton T_1 values of lecithin diluted in vesicles of the same lipid with perdeuterated chains show that intermolecular relaxation is important in the fully protonated bilayer. There are a number of possible molecular motions which could dominate this intermolecular relaxation. We have considered the relative rotational motion of close neighbors, oscillatory motions perpendicular to the surface of the vesicle, and lateral diffusion of the lipid molecules in the plane of the membrane. We conclude that lateral diffusion is the most plausible candidate for the intermolecular motion dominating the transverse relaxation (T_2), and on this assumption we calculate a self-diffusion coefficient for egg lecithin at 20° of *ca.* 0.9×10^{-8} cm²/sec. This can be compared with values recently obtained by analysis of electron spin resonance (esr) data for spin-labeled lipids of *ca.* 1.8×10^{-8} cm²/sec for diffusion of spin-labeled lecithin in lecithin multilayers at 25° (Devaux, P., and McConnell, H. M. (1972), *J. Amer. Chem. Soc. 94*, 4475) and *ca.* 1.0×10^{-8} cm²/sec for the diffusion of spin-labeled androstane molecules in a phosphatidylcholine monolayer (Trauble, H., and Sackmann E. (1972), *J. Amer. Chem. Soc. 94, 4499*). While it is not possible at present to prove rigorously that lateral diffusion is the only significant intermolecular mechanism for proton T_2 relaxation of the fatty acid chains, the values for the diffusion coefficient which are calculated on this assumption will certainly provide a lower limit for the rate of lateral diffusion. We suggest that it is also possible to obtain a lower limit for the self-diffusion coefficient simply by measuring ^1H nuclear magnetic resonance (nmr) line widths for the fatty acid chain. The lower limit calculated for the self-diffusion coefficient for the fluid lipid component of sarcoplasmic reticulum (Davis, D. G., and Inesi, G. (1971), *Biochem. Biophys. Acta 241*, 1; Robinson, J. D., *et al.* (1972), *Biochemistry 11*, 2903) would be 8×10^{-9} cm²/sec at 30° which can be compared with a value for spin-labeled lecithin fused with sarcoplasmic reticulum of 7.5×10^{-8} cm²/sec at 50° measured by Scandella, C. J., *et al.* ((1972), *Proc. Nat. Acad. Sci. U. S. 69*, 2056). Similarly, a major lipid component of rabbit sciatic nerve appears to have a self-diffusion coefficient of $\geq 5 \times 10^{-9}$ cm²/sec^{-1} at 31°. By contrast, it seems probable from both proton nmr data and several independent physical techniques that the lateral diffusion of the lipids in the erythrocyte membrane may be at least an order of magnitude slower than in these fluid membrane structures.

We have shown elsewhere how ^{13}C nuclear magnetic resonance (nmr) relaxation measurements can be used to map the gradients of motion in lipid molecules unperturbed by the presence of labeling groups (Metcalfe *et al.*, 1971; Levine *et al.*, 1972a,b). In particular, for bilayers of phosphatidylcholines I in lipid vesicles in water we have shown increasing



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motion from the glycerol backbone of the molecule, both toward the terminal methyl groups of the fatty acid chains and toward the N^+Me_3 choline head groups. We have also shown a correlation between the effect of changes in chain length on the motion of the fatty acid chains as detected by ^{13}C nmr relaxation measurements (Metcalfe *et al.*, 1972a,b) and the effect on permeability to neutral solute molecules as described, for example, by de Gier *et al.* (1968) and Van Deenen (1971). The ^{13}C relaxation times can be interpreted in terms of intramolecular motion within the lipid molecules because it has been shown that ^{13}C relaxation is dominated by directly bonded protons (Levine *et al.*, 1972b).

Although the proton relaxation times of the same lecithin molecules are also sensitive to molecular motions within the bilayer (Birdsall *et al.*, 1971), we now show that the proton relaxation times cannot be accounted for solely in terms of intramolecular chain motion, since they do not indicate the same motional gradient as the ^{13}C relaxation times. By measuring the relaxation times of protonated lipid chains which are highly diluted in bilayers of the same lipid with perdeuterated chains, we have shown that ^1H nmr relaxation is sensitive to intermolecular effects.

In this paper we consider the possible motions which might determine the intermolecular relaxation in the bilayer and the extension of this analysis to several biological membranes which give well-defined proton spectra.

Materials and Methods

Experimental Methods. Dipalmitoyllecithin was obtained from Koch-Light. Dioleyllecithin and perdeuteriodipalmitoyllecithin were synthesized from oleic acid (Sigma) and perdeuteriopalmitic acid (>97% deuterated, Merck, Sharp & Dohme, Canada), respectively, by the method of Robles and Van den Berg (1969).

Lipid samples were sonicated at 50° in deoxygenated D_2O

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buffer (45 mM NaCl–30 mM sodium acetate–5 mM sodium phosphate, pH 7.4) or in neat D_2O in glass vials under nitrogen until the sample was translucent and the residual light scattering was minimized.

Nuclear magnetic resonance (nmr) spectra were obtained on a Varian XL100-15 spectrometer, locked onto deuterium in the solvent (D_2O) and operating at 100 MHz for protons. Spin–lattice (T_1) relaxation measurements were made using the Fourier transform technique described elsewhere (Lee *et al.*, 1972).

Theoretical Treatment. MECHANISM OF ^1H AND ^{13}C RELAXATION. ^{13}C spin–lattice relaxation times, T_1, in long-chain hydrocarbons are dominated by dipole–dipole interactions (N. J. M. Birdsall, A. G. Lee, and J. C. Metcalfe, unpublished observations) and only in rare cases are dipole–dipole interactions not dominant in ^1H relaxation.

The dipole–dipole interaction between two nuclear spins depends upon the position vector of one spin with respect to the other. The relative positions of nuclei in liquid or liquid-like molecules alter with time because of the translational and rotational motions of the molecules. Variation of the dipole–dipole interactions with time results in the relaxation of the nuclear magnetization.

Since nuclei in the same molecule effectively have a constant distance of separation, the time dependence of the intramolecular dipole–dipole interactions in liquid molecules is due only to the rotational motion of the molecules. The time dependence of the relative position of two nuclei in different molecules, however, depends on both the rotational and translational motions of the molecules (unless both nuclei are at the centers of their respective molecules, when rotational motion becomes unimportant). Nuclear magnetic relaxation caused by intermolecular dipole–dipole interactions in general therefore depends on both the rotational and translational motions of molecules.

INTRAMOLECULAR RELAXATION. The ^1H relaxation times T_1 and T_2 due to intramolecular dipole–dipole interactions between two protons in a CH_2 group undergoing isotropic rotation are given by (Abragam, 1961)

$$\frac{1}{T^{\text{intra}}} = \frac{3}{10}\frac{\gamma_H{}^4\hbar^2}{b^6}\left[\frac{\tau_c}{1+\omega_H{}^2\tau_c{}^2}+\frac{4\tau_c}{1+4\omega_H{}^2\tau_c{}^2}\right] \quad (1)$$

$$\frac{1}{T_2{}^{\text{intra}}} = \frac{3}{20}\frac{\gamma_H{}^4\hbar^2}{b^6}\left[3\tau_c+\frac{5\tau_c}{1+\omega_H{}^2\tau_c{}^2}+\frac{2\tau_c}{1+4\omega_H{}^2\tau_c{}^2}\right] \quad (2)$$

Here γ_H is the magnetogyric ratio for the proton, ω_H is the Larmor frequency of the proton in the applied magnetic field, b is the proton–proton distance in a CH_2 group, and τ_c is the correlation time for the motion of the CH_2 group.

The ^{13}C relaxation time T_1 due to ^{13}C–^1H intramolecular relaxation in a CH group is similarly given by

$$\frac{1}{T_1{}^{CH}} = \frac{\gamma_c{}^2\gamma_H{}^2\hbar^2}{10r^6}\left\{\frac{\tau_c}{1+(\omega_H-\omega_c)^2\tau_c{}^2}+\frac{3\tau_c}{1+\omega_c{}^2\tau_c{}^2}+\frac{6\tau_c}{1+(\omega_H+\omega_c)^2\tau_c{}^2}\right\} \quad (3)$$

where γ_H and γ_c are the magnetogyric ratios for ^1H and ^{13}C, respectively, ω_H and ω_C are the Larmor frequencies for ^1H and ^{13}C and r is the proton–carbon distance. These equations show that if intramolecular dipolar relaxation is the dominant mechanism, then when $\omega\tau_c \ll 1$, T_1 should be greater for a ^1H nucleus in a CH_2 group than for the ^{13}C nucleus. For example, for isotropic motion of a CH_2 group with $\tau_c = 1.1 \times 10^{-11}$ sec, $T_1{}^{\text{intra}}$ values of 4 and 2.5 sec are obtained for ^1H and ^{13}C, respectively.

^1H INTERMOLECULAR DIPOLAR RELAXATION BY DIFFUSION. Intermolecular dipolar relaxation has been treated by Torrey (1953; Resing and Torrey, 1963). The steps in the calculation of T_1 and T_2 are similar to those that led to eq 1 and 2, except that instead of considering molecular rotation it is now necessary to consider molecular diffusion. Torrey adopted a model for the diffusive motion in a liquid with uniform spin density, which corresponds to a situation in which a nucleus can exist in one of two states: (a) bound in a potential well and (b) a thermally excited state in which the nucleus can move rapidly about in a random diffusive type of motion before it is again trapped in a potential well.

Kruger (1969) has shown that a correlation time, τ_c, can be written for this type of motion as

$$\tau_c = \left(\frac{1}{5}d^2+\frac{1}{12}\langle r^2\rangle\right)\frac{1}{D} \quad (4)$$

where d is the distance of closest approach of two nuclei, $\langle r^2\rangle$ is the mean-square jump distance and D is the diffusion coefficient.

T_1 and T_2 for intermolecular proton dipolar relaxation can then be written as (Kruger, 1969; Resing and Torrey, 1963)

$$\frac{1}{T_1} = k\{\phi(\alpha,\chi)+4\phi(\alpha,2\chi)\}\chi = kG_1(\alpha,\chi) \quad (5)$$

$$\frac{1}{T_2} = k\left\{\frac{3}{2}+\frac{5}{2}\phi(\alpha,\chi)+\phi(\alpha,2\chi)\right\}\chi = kG_2(\alpha,\chi) \quad (6)$$

where $\chi = \omega_H\tau_c$, $\alpha = \langle r^2\rangle/12d^2$, and

$$\phi(\alpha,\chi) = \frac{3}{\chi^2}\left(\frac{1}{5}+\alpha\right)\left\{v\left(1-\frac{1}{u^2+v^2}\right)+\left[v\left(1+\frac{1}{u^2+v^2}\right)+2\right]e^{-2v}\cos 2u+u\left(1-\frac{1}{u^2+v^2}\right)e^{-2v}\sin 2u\right\} \quad (7)$$

with

$$u = \frac{1}{2}\left[\frac{q(1-q)}{\alpha}\right]^{1/2} \quad (8)$$

and

$$v = \frac{1}{2}\left[\frac{q(1+q)}{\alpha}\right]^{1/2} \quad (9)$$

where

$$q = \left[1+\left(1+\frac{1}{5\alpha}\right)^2\frac{1}{\chi^2}\right]^{-1/2} \quad (10)$$

For protons

$$k = \frac{2\pi\gamma_H{}^4\hbar^2N}{5\ d^3\omega_H} \quad (11)$$

where N is the number density of protons.

TABLE I: ^1H and ^{13}C Relaxation Times for Phosphatidylcholines in CD_3OD and in Vesicles in D_2O.[a]

Nucleus	T_1 (sec)[b] $N(CH_3)_3^+$	$(CH_2)_n$[c]	CH_3
	Egg lecithin in CD_3OD		
^1H	1.24 ± 0.02	1.84 ± 0.02	4.93 ± 0.30[d]
^{13}C	0.94 ± 0.19	1.75 ± 0.07	5.8 ± 1.5
	Dipalmitoyllecithin as vesicles in D_2O		
^1H	0.57 ± 0.02	0.53 ± 0.04	0.84 ± 0.08
^{13}C	0.70 ± 0.03	0.53 ± 0.01	3.34 ± 0.25

[a] Data from Lee *et al.* (1972) and Levine *et al.* (1972a). [b] ^1H data at 54° and 100 MHz, ^{13}C data at 52° and 25.1 MHz. [c] This value represents a weighted average obtained from the decay of the composite resonances observed in the ^1H and ^{13}C spectra. [d] This value may be artificially low because of the presence of some residual oxygen in the sample.

TABLE II: ^1H Data for Dipalmitoyllecithin/Di(perdeuteriopalmitoyl)lecithin Vesicles in 1:10 Molar Ratio at 52°.

	$(CH_2)_n$[a]	CH_3
T_1 (sec)	0.8 ± 0.2	2.0 ± 0.5
$\Delta\nu_{1/2}$ (Hz)[b]	14	13[c]
$\Delta\nu_{1/2}$ (Hz)[b] for pure dipalmitoyllecithin bilayers	25	16[c]

[a] This value represents a weighted average for the composite resonance observed. [b] Uncorrected for magnetic field inhomogeneities. [c] The CH_3 resonance in CD_3OD as solvent appears as a distorted triplet of total line width *ca.* 11 Hz, due to ^1H–^1H coupling.

Plots of $G_1(\alpha,\chi)$ and $G_2(\alpha,\chi)$ against χ for various values of α are shown in Figure 1. Our calculations of $G_2(\alpha,\chi)$ agree with those of Kruger (1969) for a more limited range. For $\chi > 10$, $G_2(\alpha,\chi)$ is virtually independent of α, and for $\chi < 10$, the dependence on α is only slight. T_1 is independent of α only for $\chi \ll 1$. In the region where $\omega_H\tau_c \ll 1$ (the region of extreme narrowing), T_1 and T_2 will be almost equal. In the region where $\omega_H\tau_c \gg 1$, T_2 will be very much less than T_1.

The insensitivity of T_2 to α is in agreement with the intuitive arguments put forward by Abragam (1961). In the region $\omega_H\tau_c \ll 1$ the effective local fields near ω_H which will determine T_1 arise primarily from remote nuclei, since the neighboring nuclei contribute mainly very high-frequency terms, so that details of the motion producing intermolecular relaxation are relatively unimportant. In the region where $\omega_H\tau_c \gg 1$, however, it is the near neighbors which make the main relaxation contribution to T_1, with the remote nuclei providing mainly static terms, so that details of the motion will now have a considerable effect on T_1. For T_2 however in the region $\omega_H\tau_c \gg 1$ the static terms will be the most important for relaxation, and thus the remote nuclei will be of importance for relaxation whatever the value of $\omega_H\tau_c$, so that T_2 will always be relatively insensitive to details of the motion, including α. An important feature of these plots of relevance to the following discussion is the rapid increase in T_2 as τ_c increases, so that when $\omega_H{}^2\tau_c{}^2 \gg 1$ *only the slowest motion present in the system will be of importance in* T_2 *relaxation.*

Similar equations can be derived for the relaxation caused by dipolar interactions between two unlike spins. In this case (Kruger, 1969) for nucleus I

$$\frac{1}{T_1^I} = k'\left\{\frac{3}{2}\phi(\alpha,\chi_I) + \frac{7}{2}\phi(\alpha,\chi_S)\right\}\chi_I \qquad (12)$$

$$\frac{1}{T_2^I} = k'\left\{1 + \frac{3}{4}\phi(\alpha,\chi_I) + \frac{13}{4}\phi(\alpha,\chi_S)\right\}\chi_I \qquad (13)$$

where $\chi_I = \omega_I\tau_c$, $\chi_S = \omega_S\tau_c$, and

$$k' = \frac{16\pi\gamma_I{}^2\gamma_S{}^2\hbar^2 N_S}{45\, d^3\omega_I}S(S+1) \qquad (14)$$

where N_S is the number density of spins of spin quantum number S.

Results and Discussion

The Relative Importance of Intramolecular and Intermolecular Relaxation in ^1H Nmr of Lipids. Equations 1 and 3 predict that for $\omega\tau_c \ll 1$, the T_1 relaxation time for a ^1H nucleus in a CH_2 (or CH_3) group will be greater than that of a ^{13}C nucleus in the same group, if the relaxation is dominated by dipolar interactions within that group. Table I contains a comparison of some ^1H and ^{13}C T_1 values for phosphatidylcholines dissolved in CD_3OD and as vesicles in D_2O. Whereas values of the relaxation times in CD_3OD as solvent are at least equal for all three resonances within experimental error, for lipid vesicles in water the ^1H T_1 value for the terminal methyl group in particular is very considerably less than the ^{13}C value. In CD_3OD, phosphatidylcholine exists as aggregates of, on average, three molecules or less (Price and Lewis, 1929; Elworthy and Macintosh 1961). Coupled with the fact that because of the small magnetogyric ratio of deuterium (one-seventh of that for the proton) CD_3OD can make only a small contribution to the relaxation, this means that intermolecular relaxation is relatively unimportant in this system. In tightly packed lipid bilayers, however, intermolecular interactions between adjacent fatty acid chains are to be expected. Such interactions could produce efficient intermolecular relaxation and reduce T_1 values for ^1H nuclei which have inefficient intramolecular relaxation. The ^{13}C T_1 values will be relatively unaffected since intermolecular relaxation is generally unimportant for ^{13}C (Kuhlmann *et al.*, 1970). The short ^1H T_1 values observed for the terminal methyl and N^+Me_3 protons are consistent with an important contribution from intermolecular relaxation. For the $(CH_2)_n$ protons of the fatty acid chains, the intramolecular contribution to T_1 is expected to be more important.

The effect of intermolecular relaxation for the terminal methyl protons can be studied experimentally by measuring the ^1H relaxation times for a normal protonated lipid inserted into a bilayer of deuterated lipids. The results of such an experiment are given in Table II; the relaxation times T_1 for the fatty acid protons increase on incorporation into a deuterated bilayer, while the line widths decrease, in qualitative agreement with significant intermolecular relaxation. It is possible to treat the T_1 data for the terminal methyl group in more detail. The packing of the fatty acid chains in the bilayer

is generally assumed to be hexagonal. Any one fatty acid chain will then be surrounded by six other chains of which one will belong to the same lipid molecule as the surrounded chain. Because of the strong inverse distance dependence of relaxation (see eq 1, 5, and 11) we only need consider relaxation due to the six nearest-neighbor fatty acid chains at any instant in time. The intramolecular contribution (T_1^{intra}) to the ^1H T_1 value of the terminal methyl group can be estimated from the ^{13}C value (3.34 ± 0.25 sec) using eq 1 and 3 to be between 3.7 and 4.3 sec. If it is assumed that all six surrounding fatty acid chains produce an equal intermolecular contribution to ^1H relaxation, then from the observed T_1 value $T_{1,H}^{obsd}$ in the normal protonated bilayer the contribution $T_{1\,chain}^{inter}$ of one protonated chain to the intermolecular relaxation can be estimated from

$$\frac{1}{T_{1\,chain}^{inter}} = \frac{1}{6}\left(\frac{1}{T_{1,H}^{obsd}} - \frac{1}{T_1^{intra}}\right)$$

to be between 3.8 and 4.6 sec.

For a 1:10 ratio of protonated lipid to deuterated lipid, then at any instant in time approximately 55% of the protonated fatty acid chains will have only one protonated chain among its nearest neighbors, 35% will have two, and 10% will have three. We then have: for 55% of the protonated chains, $1/T_1' = 1/T_1^{intra} + 1/T_{1\,chain}^{inter}$; for 35% of the protonated chains, $1/T'' = 1/T_1^{intra} + 2/T_{1\,chain}^{inter}$ and for 10% of the protonated chains, $1/T_1''' = 1/T_1^{intra} + 3/T_{1\,chain}^{inter}$.

The observed proton relaxation is then characterized by a relaxation time

$$\frac{1}{T_1} = \frac{1}{100}\left(\frac{55}{T_1'} + \frac{35}{T_1''} + \frac{10}{T_1'''}\right)$$

The relaxation time T_1 will then be between 1.6 and 1.8 sec.

If it is now assumed that protonated fatty acid chains within the same lipid molecule produce relatively ineffective intermolecular ^1H relaxation (which would be the case if relaxation were largely caused by the relative motions of adjacent lipid molecules), then a T_1 relaxation time of between 2.4 and 2.8 sec is calculated. The agreement between the T_1 value observed in the deuterated bilayer and that estimated from intramolecular and intermolecular terms shows the importance of intermolecular interactions in determining ^1H T_1 values for the terminal CH_3 group. In the normal fully protonated bilayer, we have for the CH_3 group

$$\frac{1}{T_1^{obsd}} = \frac{1}{T_1^{intra}} + \frac{1}{T_1^{inter}}$$

so that with T_1^{intra} of *ca.* 4 sec, T_1^{obsd} is dominated by intermolecular effects

Unfortunately it is not yet possible to analyze the T_1 data for the $(CH_2)_n$ protons in detail, since the ^{13}C data for each (CH_2) group in the chain is not available, and these data are required to estimate T_1^{intra} for the protons of each (CH_2) group.

The observed decrease in line width corresponds to an increase in T_2^* defined as $T_2^* = 1/\pi\Delta\nu_{1/2}$, where $\Delta\nu_{1/2}$ is the line width. There is considerable evidence (Sheard, 1969) that the line width of the signal due to the $(CH_2)_n$ protons contains contributions from effects other than the dipolar relaxation time T_2, and the line width of the CH_3 protons will certainly contain some contribution from proton–proton coupling (Birdsall *et al.*, 1971). If the line width were caused solely by effects of T_2 relaxation, then in the 1:10 deuterated bilayer, the proton line width due to the $(CH_2)_n$ protons would be some 4 Hz; if the line width contains contributions independent of whether the surrounding fatty acids are protonated or deuterated, then the observed line width will be greater then 4 Hz. The observed line sharpening is certainly in agreement with an important intermolecular contribution to T_2.

Horwitz *et al.* (1972) have recently published some T_1 and T_2 data for vesicles of egg lecithin at 220 MHz. The T_1 data are in broad agreement with our previously published data (Birdsall *et al.*, 1971; Lee *et al.*, 1972), but they have been able to measure T_2 values by a spin-echo technique. Their T_2 value for the terminal methyl protons at 20° is 0.036 sec. The T_2 decay of the $(CH_2)_n$ proton signal was nonexponential, corresponding to a distribution of T_2 values, estimated as 20% at 0.056 sec and 80% at <0.02 sec (Horwitz *et al.*, 1972).

We have already concluded that for the terminal methyl protons at least, T_1 relaxation is dominated by an intermolecular process. If the intramolecular contribution to ^1H T_2 is caused by the same motion that produces the intramolecular ^{13}C T_1 relaxation, then since we know from the ^{13}C data that for this motion $\omega^2\tau_c^2 \ll 1$, it follows that $T_1^{intra} = T_2^{intra}$, and thus that the intramolecular contribution to ^1H T_2 will have a value greater than the ^{13}C T_1 value—for the $(CH_2)_n$ protons, this means a value greater than *ca.* 0.6 sec. It is conceivable however that the short T_2 value observed by Horwitz *et al.* (1972) is caused by some slow intramolecular motion which will contribute to T_2 but which is too slow to make a significant contribution to T_1 ($\omega_H\tau_c \gg 1$). However, calculations suggest that although such a slow motion could be of importance for the first few –(CH_2)– protons of the chain, it cannot be of importance for the last few; ^{13}C T_1 data (Levine *et al.*, 1972a,b) show such rapid bond oscillations about the final C—C bonds in the chain, with the motion becoming sufficiently isotropic, that the details of any slow intramolecular motion are effectively lost (Levine *et al.*, 1972b; Y. K. Levine, N. J. M. Birdsall, A. G. Lee, and J. C. Metcalfe, unpublished observations). We therefore conclude that at the terminal methyl end of the chain, ^1H T_2 is dominated by an intermolecular mechanism.

The Relative Importance of Rotational and Translational Motions in Intermolecular Relaxation. Both magnetic resonance (Metcalfe *et al.*, 1971; Hubbell and McConnell, 1971) and X-ray diffraction techniques (Levine and Wilkins, 1971) have shown that the hydrocarbon chains in lipid bilayers above the liquid-crystalline transition temperature are highly fluid, with the terminal methyl groups undergoing the most rapid internal rotational motions (Levine *et al.*, 1972b). The X-ray diffraction data also suggest that the terminal methyl groups occupy a relatively diffuse region at the center of the bilayer. Intermolecular proton–proton relaxation of the terminal methyl groups can then be reasonably treated by a "liquid-like" model.

Intermolecular relaxation of polyatomic molecules in a liquid depends both on the translational and on the rotational motions of the molecules. Following Hubbard (1963) we will treat separately the rotational and translational motions. Hubbard (1963) has shown that in a liquid when $\omega_H^2\tau_c^2 \ll 1$ the intermolecular relaxation is dominated by translational motion, and the effects of rotation are negligible. For aqueous dispersions of lipid vesicles there will, however, be a number of slow rotational motions present ($\omega_H^2\tau_c^2 \gg 1$). Thus lipid vesicles of diameter 250 Å tumble in solution with a correla-

tion time which can be estimated from the Stokes equation as *ca.* 10^{-6} sec. If this motion were effective in causing T_2 relaxation, then it can be seen from eq 2 that it would produce a very efficient relaxation and thus very broad resonances. Further, the line width would depend directly on the viscosity of the medium. However, we find that addition of glycerol to an aqueous sonicated dispersion of dioleyllecithin causes no change in line width for the $(CH_2)_n$ and CH_3 protons. Kornberg and McConnell (1971) have made a similar observation. We therefore conclude that vesicle rotation is not important in determining T_2 relaxation for these protons. This can be rationalized in a similar way as for the independence of T_2 to slow intramolecular motions. Thus the rotational motion of the terminal methyl end of the fatty acid chain (due to rapid C–C bond oscillations in that chain) is so fast and sufficiently isotropic that the effect of slow vesicle rotation in modulating dipole–dipole interactions is swamped. If a similar argument can be applied for the terminal methyl group end of the chain to any rotational motion which is much slower than that caused by internal bond rotations, then intermolecular relaxation will be determined by translational motions.

In a number of other studies, it has also been assumed that translational motions determine ^1H intermolecular relaxation. The temperature and frequency dependence of the relaxation times in glycerol are consistent with relaxation dominated by translational diffusion (Fiorito and Meister, 1972). ^1H relaxation in the plastic phase of pivalic acid and other organic molecules has also been interpreted in terms of relaxation by translational diffusion, and the self-diffusion coefficients obtained are consistent with data from tracer studies (Jackson and Strange, 1971). Finally, Watkins and Johnson (1971) have noted that ^1H relaxation in nematic liquid crystals is consistent with relaxation by translational diffusion. We now show that by making this assumption in the lipid bilayer system, it is possible to calculate rates of translational diffusion which agree with all available data on these rates.

Analysis of ^1H Relaxation Times in Terms of Translational Diffusion. Relaxation caused by translational diffusion can be treated by eq 5 and 6. For egg lecithin bilayers the area per molecule at 24° has been measured as 59.3 Å and the thickness of the hydrocarbon region of the bilayer as 35.8 Å (Reiss-Husson, 1967; Small, 1967). This then gives a proton density $N \simeq 6.0 \times 10^{22}$ spins/cm^3 which can be compared to that of $N \simeq 6.6 \times 10^{22}$ spins/cm^3 in glycerol (Burnett and Harmon, 1972).

A value of d, the distance of closest approach of two nuclei, of 5 Å has been used in studies of relaxation in glycerol (Bloembergen *et al.*, 1948; Burnett and Harmon, 1972) although a recent study suggests that $d \simeq 2.5$ Å (Fiorito and Meister, 1972). The value of k in eq 5 and 6 with $d = 5$ Å is 0.55 at 100 MHz and 0.25 at 220 MHz. A value of $d = 5$ Å is used for our calculations: using the smaller value of d (2.5 Å), the diffusion coefficients obtained will be larger by a factor of 2. The uncertainties in the theoretical treatment cannot be estimated at present, since no examples have yet been reported where the treatment gives diffusion coefficients which differ significantly from independent measurements.

Although T_2 is independent of α in the range of values observed for lipid systems, T_1 will be dependent on the chosen value for α. The value of $\alpha = 0.005$ was found to give the best fit of the data for relaxation in glycerol (Fiorito and Meister, 1972) and so will be the value used here.

Using Horwitz *et al.*'s (1972) T_2 value of 0.036 sec for the terminal CH_3 group at 20°, we obtain a correlation time τ_c of 5.2×10^{-8} sec for the translational motion, which corresponds to a self-diffusion coefficient of 9.5×10^{-9} cm^2/sec. Their T_2 value for the $CH_2C(=O)O$ protons of 0.008 sec (Horwitz *et al.*, 1972) would, if it were determined solely by translational motions, correspond to a diffusion coefficient of 2.1×10^{-9} cm^2/sec. However, for the end of the fatty acid chain near the glycerol group of the lipid, motion will be relatively anisotropic and slow, and effects of rotational motions (such as vesicle tumbling) would be expected to be important. Analysis of the data for the $(CH_2)_n$ protons is difficult, since the T_2 relaxation is nonexponential, but the reported values (Horwitz *et al.*, 1972) correspond to a self-diffusion coefficient of *ca.* 5×10^{-9} cm^2/sec.

Because of the nature of the dependence of T_2 on correlation time (see Figure 1), T_2 will be dominated by the slowest translational motion present in the system. We have considered two possibilities for this slow motion: (a) diffusion of the lipid molecules in the surface of the bilayer and (b) an oscillation of the fatty acid chains perpendicular to the plane of the bilayer. If the latter motion only involves movement of the hydrocarbon chains (which would be a reasonable motion to consider for the terminal methyl end of the chain), then it can be shown that it will probably be much too fast to be the motion which determines the T_2 measurements. Self-diffusion in liquid paraffins is thought to occur by the relative translation of two hydrocarbon chains with parallel long axes in the direction parallel to the long axes. In liquid n-$C_{18}H_{38}$ the self-diffusion coefficient for this motion is 0.5×10^{-5} cm^2/sec at 50° and in n-$C_{32}H_{66}$ it is 0.2×10^{-5} cm^2/sec at *ca.* 70° (Douglass and McCall, 1958). Since diffusion in liquid paraffins probably occurs by the relative displacement of only short sections of chain, the rate of motion of the terminal methyl end of the fatty acid chains of lipids in bilayers in the direction perpendicular to the plane of the bilayer is likely to have a similar value. These values are several orders of magnitude greater than the minimum self-diffusion coefficients calculated from T_2 measurements for egg lecithin bilayers.

If this analysis is correct, then the slow motion detected by ^1H nmr will be due to lateral diffusion of the lipids. *In any case*, since ^1H T_2 values for the terminal methyl group are determined by the slowest translational motion, *the rate of surface diffusion of lipids in the bilayer cannot be less than the experimentally determined diffusion coefficient.* This value is 9×10^{-9} cm^2/sec calculated for a value of $d = 5$ Å or 1.8×10^{-8} cm^2/sec for a value of $d = 2.5$ Å, as suggested by studies of relaxation in glycerol (Fiorito and Meister, 1972). A value of *ca.* 1.8×10^{-8} cm^2/sec has been obtained by Devaux and McConnell (1972) for the rate of diffusion of a spin-labeled lipid in phosphatidylcholine multilayers at 40°.

Because of its greater dependence on details of the diffusive motion and because of its dependence on motions with very different correlation times (see Figure 1), the interpretation of the T_1 data is not so straightforward. Equation 5 leads to the prediction that the intermolecular relaxation time T_1 due to a single diffusive motion cannot be less than about 1.5 sec at 100 MHz. We observe a T_1 value for the terminal methyl group at 40° and 100 MHz in egg lecithin of 0.54 sec (Lee *et al.*, 1972). The most plausible explanation of this low value is that as well as the slowest motion contributing to T_2, there are several other faster intermolecular motions contributing to T_1. Similar observations have also been made in the plastic crystalline phase of pivalic acid, where T_1 is also less that the expected value for a single translational diffusive motion (Jackson and Strange, 1971).

For the fatty acid protons nearer the glycerol backbone we

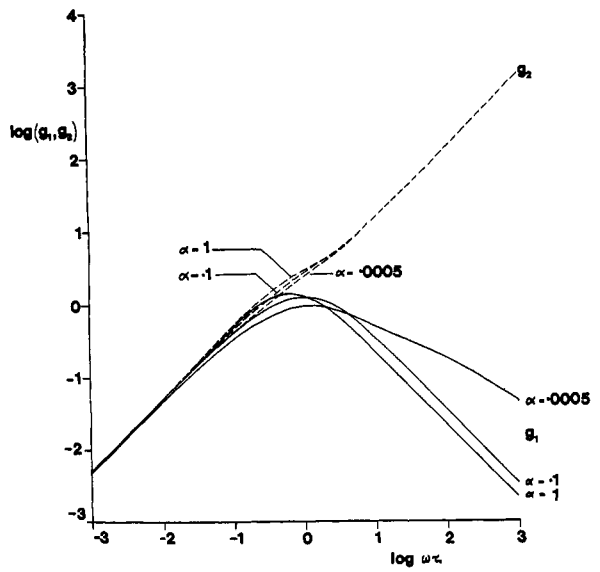


FIGURE 1: $G_1(\alpha,\chi)$ and $G_2(\alpha,\chi)$ *vs.* χ for $\alpha = 0.0005$, 0.1, and 1.0.

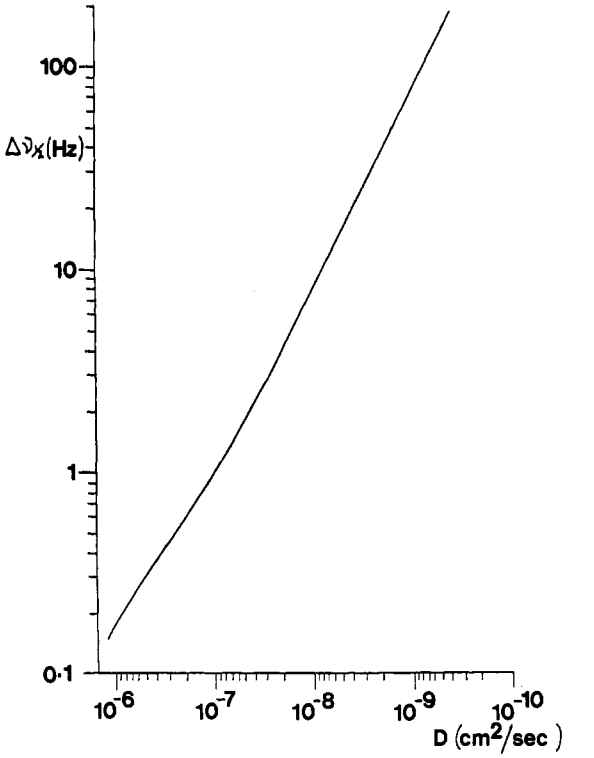


FIGURE 2: The contribution to line width due to T_2 relaxation *vs.* diffusion coefficient calculated for intermolecular relaxation in lipid bilayers for a distance of closest approach of $d = 5$ Å. The dependence is linear for $\Delta\nu_{1/2} > 3$ Hz.

can show that intramolecular contributions to T_1 will become of increasing importance. The ^{13}C T_1 data for dipalmitoyllecithin has been analyzed (Levine *et al.*, 1972b) to show that the effective correlation times for rotation about C–C bonds increases from *ca.* 0.02×10^{-10} sec for the terminal methyl group to 1.9×10^{-10} sec for carbon two. Whereas a motion with the former correlation time will result in a long intramolecular T_1 value, the latter motion will produce an intramolecular T_1 value of less than 0.5 sec, close to the observed T_1 values.

Estimation of a Lower Limit for D from Line-Width Experiments. In the preceding discussion we have shown how a value for the self-diffusion coefficient can be obtained from measurements of transverse relaxation time T_2. Values of T_2 can be determined by a Fourier-transform spin-echo technique, but there may be experimental difficulties in such determinations (Vold, 1972). However, a time T_2^* can be defined by $T_2^* = 1/\pi\Delta\nu_{1/2}$, where $\Delta\nu_{1/2}$ is the measured line width of the resonance. If the line width is determined solely by transverse relaxation, then $T_2^* = T_2$. If there are contributions to line width other than those due to transverse relaxation, the $T_2^* \leq T_2$. Further, T_2^* must always be equal to or less than the T_2 due to intermolecular dipolar relaxation. As a consequence, since T_2 is determined by the slowest motion, the self-diffusion coefficient D calculated from T_2^* must be less than or equal to the true self-diffusion coefficient D which would be calculated from T_2. Proton line widths can therefore be used to estimate a lower limit for the rate of diffusion.

Sheard (1969) has reported a $(CH_2)_n$ line width of 14 Hz at 100 MHz for egg lecithin vesicles at 34°. This line width corresponds to a self-diffusion coefficient of 6×10^{-9} cm²/sec (see Figure 2). The line width is, however, field dependent, and the line width extrapolated to zero field strength is *ca.* 8 Hz (Sheard, 1969) corresponding to $T_2^* = 0.039$ sec. This value corresponds to a self-diffusion coefficient of 9.7×10^{-9} cm²/sec, in excellent agreement with that obtained from the spin-echo T_2 data. The reason for the field dependence of the line width is not yet established, but part, at least, could be due to chemical shift heterogeneity within the fatty acid chain; some of the small field dependence of the N^+Me_3 line width is due to this cause (Levine *et al.*, 1972c). Since it has been shown that very similar diffusion coefficients are calculated from line width and T_2 data, an estimate of the activation energy E_D of surface diffusion can be obtained from the variation of $(CH_2)_n$ line width with temperature, since $D = D_0 \exp(-E_D/RT)$. Figure 3A shows a plot of D *vs.* T for dipalmitoyllecithin from which a value of $E_D = 7$ kcal/mol is obtained. The data obtained below the transition temperature (42°) is from the line width of the *residual* $(CH_2)_n$ resonance of chains which have not crystallized. The resonances from chains of lipid molecules which have crystallized are too broad to be observed. This explains why no discontinuity appears in plots of diffusion constant against temperature measured in this way.

Effects of chemical shift heterogeneity are likely to be smaller for the resonance from a single group than for a $(CH_2)_n$ chain. The observed proton line width for the HC=CH group in the fatty acid chains of dioleyllecithin vesicles at 40° and 100 MHz corresponds to $T_2^* = 0.014$ sec and can be compared with the T_2 value of 0.020 sec obtained by Horwitz *et al.* (1972) for egg lecithin at 20° and 220 MHz. The variation of diffusion coefficient with the temperature estimated from the line width data is shown in Figure 3B and a value of $E_D = 3$ kcal/mol is obtained. This value is perhaps surprisingly low and it is important that a value should be obtained from spin-echo T_2 measurements.

Addition of cholesterol to sonicated aqueous dispersions of unsaturated lipids up to a molar ratio of 1:2 causes line broadening of all proton resonances. At a molar ratio of 1:1, there is a considerable further broadening and an apparent loss in area for the fatty acid $(CH_2)_n$ peak. If the line broadening observed for 1:4 and 1:2 cholesterol egg lecithin and 1:2

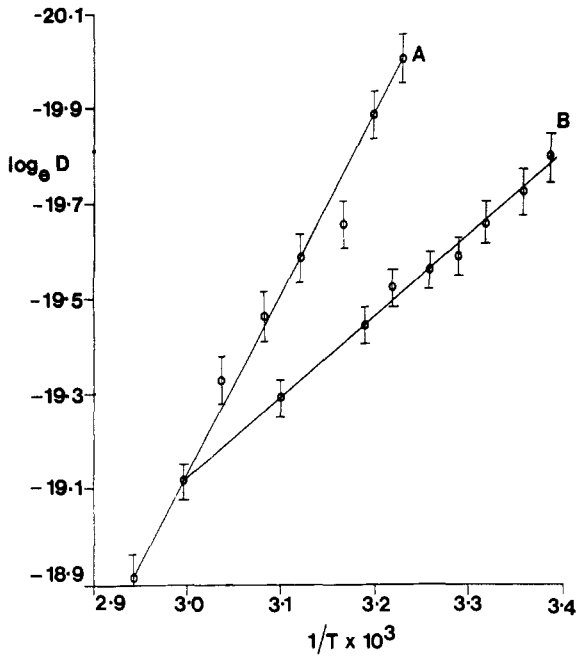


FIGURE 3: Variation of diffusion coefficient with temperature for (A) dipalmitoyllecithin and (B) dioleyllecithin.

cholesterol dioleylecithin mixtures corresponds to a change in T_2, then it reflects a considerable reduction in the coefficients of self-diffusion. The values calculated making this assumption are listed in Table III. The activation energies estimated from the variation of line width with temperature are 3 kcal/mol both for a 1:2 and a 1:4 molar ratio of cholesterol to egg lecithin.

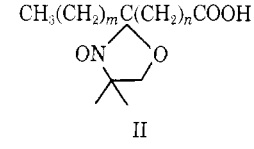
Estimates of self-diffusion coefficients can also be made for intact biological membranes. Some observed line widths and estimated lower limits for the self-diffusion coefficients are given in Table IV.

Effect of Paramagnetic Species on Nuclear Magnetic Relaxation. Because of the large magnetogyric ratio of the electron (over 600 times greater than that of the proton), the incorporation of spin-labeled molecules into a lipid bilayer produces considerable reductions in nuclear magnetic relaxation times. It has been shown elsewhere (Levine *et al.*, 1972c) that the reduction in lipid ^{1}H relaxation times caused by nitroxide-labeled lipids and fatty acids cannot be satisfactorily explained in terms of rotational motions. However, both the ^{1}H and ^{13}C data (Levine *et al.*, 1972a, c) are consistent with surface diffusion. Only an approximate estimation of a spin density due to the unpaired electrons of the nitroxide molecules in the lipid bilayer can be made, and the estimation of a distance of closest approach between a given proton and a nitroxide at some position in a fatty acid chain is also difficult.

For a 60:1 molar ratio of lipid to fatty acid labelled with a nitroxide group at carbon n, the electron spin density in the bilayer at the level below the bilayer surface corresponding to carbon n is very approximately 3×10^{19} spins/cm^3. That part of the observed relaxation time T_1^{obsd} in the presence of spin label which is due to the spin label (T_1^{SL}) is given by

$$\frac{1}{T_1^{obsd}} = \frac{1}{T_1^{SL}} + \frac{1}{T_1^0}$$

where T_1^0 is the relaxation time in the absence of spin label. The value of T_1^{SL} at 52° for the terminal methyl group in a 60:1 molar ratio of dipalmitoyllecithin to a 12-labeled stearic acid (II, n = 12) is 0.10 sec. This corresponds (eq 12) to a



II

diffusion coefficient of approximately 2×10^{-8} cm^2/sec with a distance of closest approach of 5 Å. The T_1^{SL} value of 0.19 sec for the terminal methyl carbon for a 1:60 molar ratio of a 7-labeled stearic acid (I, n = 7) corresponds to a diffusion coefficient of approximately 4×10^{-8} cm^2/sec with a distance of closest approach between the methyl group and the nitroxide of 8 Å.

The ^{13}C data (Levine *et al.*, 1972a) can be analyzed similarly in terms of a diffusion coefficient of approximately 5×10^{-9} cm^2/sec. Any more detailed analysis of the data

TABLE III: Estimated Lower Limits for Diffusion Coefficients in Egg Lecithin–Cholesterol Bilayers.

Molar Ratio Cholesterol–Egg Lecithin	Temp (°C)	Lower Limit for Self-Diffusion Coef × 10^9 (cm^2/sec)
1:4	28	1.6
	40	1.9
	48	2.2
1:2	28	1.0
	40	1.3
	48	1.4
	52	1.6
1:2 (cholesterol–dioleyllecithin)	52	1.1

TABLE IV: Lower Limits to Lipid Self-Diffusion Coefficient Estimated from Proton Line Widths.

System	Line Width $\Delta\nu_{1/2}$ (Hz)	Lower Limit for Self-Diffusion Coef D × 10^9 (cm^2/sec)
Sarcoplasmic reticulum		
Membrane, 8°	15	6
50°	8	10
Extracted lipid, 31°	22	4
Rabbit sciatic nerve		
Membrane, 31°	15	5
Extracted lipid, 31°	10	8
Electroplax membrane[a]		
Membrane, 33°	<75	1

[a] Data from J.-P. Changeux, M. Weber, N. J. M. Birdsall, A. G. Lee, and J. C. Metcalfe, unpublished observations.

does not seem justified, but the results from both the ^1H and ^{13}C paramagnetic experiments are certainly not inconsistent with the more precise rates of surface diffusion of lipids estimated from line width and T_2 data in the absence of spin labels.

Conclusions

Estimates of the rates of surface diffusion of lipid and steroid molecules in lipid bilayers and monolayers have recently been made from esr line-broadening measurements (Devaux and McConnell, 1972; Trauble and Sackmann, 1972). These determinations have a number of inherent theoretical and practical limitations, and it is of importance that estimates of rates of diffusion should be made using independent techniques, involving a different set of approximations. The extension of the esr line-broadening method to the study of biological membranes necessitates the prior incorporation of spin-labeled molecules into that membrane, which may perturb the lipid structure of the membrane.

In this paper we have argued that estimates of the rate of diffusion of lipid molecules both in lipid bilayers and in intact biological membranes can be obtained from ^1H T_2 relaxation times and that line-width measurements provide a lower limit to the rate of diffusion. The value of the lateral diffusion coefficient D which is obtained will depend on the relationship which is assumed between D and the correlation time τ_c. This has been the subject of some debate (see Kruger, 1969). Using the relationship given in eq 4 we obtain values of D for egg lecithin bilayers at 20° of 0.9–1.8 $\times$ 10^{-8} cm^2/sec, in excellent agreement with the value of 1.8 $\times$ 10^{-8} cm^2/sec for the diffusion of a spin label in didihydrosterculoylphosphatidylcholine multilayers at 25° (Devaux and McConnell, 1972) and with the value of *ca.* 10^{-8} cm^2/sec for the diffusion of spin-labeled androstane in dipalmitoyllecithin monolayers (Trauble and Sackmann, 1972). These diffusion coefficients can be compared with those of 7 $\times$ 10^{-7} cm^2/sec for hemoglobin in aqueous solution (Singer and Nicolson, 1972) and 4 $\times$ 10^{-8} cm^2/sec for glycerol at 30° (Fiorito and Meister, 1972). The effects of spin labels (fatty acids and lipids) on proton T_1 values are also consistent, within better than an order of magnitude, with the rate of surface diffusion estimated in the absence of spin labels. Changes in relaxation times caused by addition of spin labels must, of necessity, be largely intermolecular.

The estimates for lipid diffusion are in broad agreement with data on the surface viscosity of lipid monolayers. The surface viscosity of a dipalmitoyllecithin monolayer at the air–water interface has been measured as between 10^{-3} and 10^{-4} g per sec at 25° depending on the surface pressure (Vilallonga, 1968). This corresponds (see, for example, Li and Chang, 1955) to a self-diffusion coefficient of *ca.* 10^{-10}–10^{-9} cm^2/sec, in reasonable agreement with the value for the dipalmitoyllecithin bilayer estimated from nmr measurements.

The observation of an important intermolecular contribution to ^1H relaxation is of relevance to a number of other nmr studies which have been reported. For example, Horwitz *et al.* (1972) have attempted to explain ^1H T_1 and T_2 data for lipid fatty acid chains in bilayers in terms of intramolecular dipolar relaxation. This leads them to the conclusion that the "relatively small displacements due to rotations of individual methylene carbon atoms that occur at high frequency" are "roughly constant along the fatty acid chains." This conclusion is inconsistent with our ^{13}C data (Levine *et al.*, 1972a,b) and the deuterated lipid experiments reported here. ^1H relaxation in the lipid head-group region also probably contains a large intermolecular contribution: the T_2 value of 0.075 sec obtained by Horwitz *et al.* (1972) for the N^+Me_3 protons of egg lecithin at 20° and 220 MHz is much smaller than the corresponding T_1 value of 0.41 sec. It seems likely that intermolecular ^1H relaxation could also be important in other similar systems. Thus Tiddy (1972) reports ^1H and ^{19}F T_1 relaxation times for the lamellar phase of $CF_3(CF_2)_2$-$(CH_2)_{10}COONa$ in D_2O and concludes that "there is little or no distribution of CH_2 or CF_3 rotational motions as distance from the head group increases." We also find for a series of dipalmitoyllecithin molecules containing fluorine-labeled fatty acids that the changes in ^{19}F T_1 relaxation times along the chain are smaller than the corresponding changes in ^{13}C T_1 values in unlabeled lipids (N. J. M. Birdsall, A. G. Lee, and J. C. Metcalfe, unpublished observations) but believe it to be more likely due to an important intermolecular relaxation mechanism for ^{19}F, than to a major difference in molecular motion for the fluorine-labeled lipids (Levine *et al.*, 1972a).

Similarly, Chan *et al.* (1972) interpret proton line-width and T_1 data for unsonicated egg lecithin in terms of intramolecular relaxation and come to conclusions which appear to conflict with data from esr spin labeling (Hubbell and McConnell, 1971) and ^{13}C experiments (Metcalfe *et al.*, 1971). However, it has recently been shown that the very broad lines observed in the spectrum of unsonicated egg lecithin are due largely to static dipolar broadening (Charvolin and Rigny, 1972), and there is no *a priori* reason for neglecting intermolecular contributions to T_1 and T_2 relaxation in this system. The diffusion coefficient calculated from the T_2 data of Charvolin and Rigny (1972) for an unsonicated egg lecithin dispersion is *ca.* 2 $\times$ 10^{-8} cm^2/sec, in close agreement with that calculated for sonicated egg lecithin.

The determination of a value for the rate of diffusion from ^1H nmr experiments requires a T_2 value obtained by a Carr–Purcell spin-echo method, but such measurements can be difficult, particularly if the system exhibits homonuclear scalar coupling (Vold, 1972). However, it is possible to estimate at least a lower limit for the rate of diffusion from line-width measurements. For sonicated aqueous dispersions of egg lecithin, the line width and T_2 values correspond quite closely, and the lower limit for the diffusion coefficient calculated from the line width is only a factor of two lower than the value calculated from T_2.

The change in line width for the proton resonances of the lipid fatty acid chains with temperature can be used to estimate the change in diffusion coefficient with temperature, if the changes in any other contributions to line width with temperature are small. The activation energy for the diffusion process in dipalmitoyllecithin bilayers can be estimated as *ca.* 7 kcal/mol, and for dioleyllecithin bilayers as *ca.* 3 kcal/mol. Interestingly, the activation energies for surface diffusion estimated in this way for 4:1 and 2:1 molar ratios of egg lecithin–cholesterol are also approximately 3 kcal/mol, although the diffusion coefficients are seemingly reduced by addition of cholesterol. The effect of addition of cholesterol on the rate of diffusion would then seem to be largely an entropy effect. This would be consistent with data (Engelman and Rothman, 1972; Hinz and Sturtavent, 1972) which suggest that the cholesterol–lipid phase is highly ordered.

The values for the lower limits to the rates of surface diffusion in egg lecithin–cholesterol bilayers are appreciably less than the value of 12 $\times$ 10^{-8} cm^2/sec estimated by Scandella *et al.* (1972) for a 4:1 molar ratio at 40° by a spin-label tech-

nique. This value seems high by comparison with a value of 1.8×10^{-8} cm²/sec (Devaux and McConnell, 1972) for the rate of surface diffusion of lipids in the absence of cholesterol also estimated from esr studies. However, further discussion of the effect of cholesterol is deferred until we have obtained spin-echo T_2 values for these systems.

Fairly sharp ^{1}H resonances with $T_2^* < T_1$, corresponding to rapid surface diffusion of lipids, have been observed for a number of biological membranes. Thus the sarcoplasmic reticulum membrane gives an ^{1}H nmr spectrum in which the intensity of the sharp resonances corresponds to up to 25% of the membrane lipids, although the chemical identity of this component is uncertain. The lower limit to the rate of diffusion of the lipids in this fluid component is $D > 6 \times 10^{-9}$ cm²/sec at 8° and $D > 1 \times 10^{-8}$ at 50°. Very recently Scandella *et al.* (1972) have reported a diffusion rate of $D = 7.5 (\pm 1.5) \times 10^{-8}$ cm²/sec at 50° for a spin-labeled phosphatidylcholine inserted into the sarcoplasmic reticulum membrane. This value was obtained by esr line shape analysis in the temperature range 50–70°.

A comparison can be made between the self-diffusion coefficients of the lipids giving rise to the ^{1}H spectrum in sarcoplasmic reticulum, and that of the lipids surrounding the protein rhodopsin in the retinal rod membrane. Cone (1972) has reported that the rhodopsin molecule is in a membrane environment of viscosity *ca.* 2 g/cm sec. If the surroundings of the rhodopsin molecule approximate a lipid bilayer, then this viscosity would correspond to a lipid self-diffusion coefficient of approximately 10^{-7} cm²/sec.

The line width of the broad component due to the lipid fatty acid chains in the ^{1}H spectrum of the sarcoplasmic reticulum membrane is *ca.* 200 Hz, corresponding to a diffusion coefficient of $D > 4 \times 10^{-10}$ cm²/sec. However, although the proton line widths can be used to calculate a lower limit for the rate of diffusion, when the line is very broad this lower limit may be very much less than the actual value of the rate of diffusion, because very broad lines can be caused by dipolar broadening. For example, the broad line of width *ca.* 500 Hz observed in the spectrum of unsonicated egg lecithin is due largely to static dipolar broadening and T_2 has, in fact, been estimated as only *ca.* 0.02 sec (Charvolin and Rigny, 1972). In this case, therefore, the lower limit of the rate of diffusion calculated from the line width ($D > 2 \times 10^{-10}$ cm²/sec) is very much less than the value calculated from the estimated value of T_2 (D = *ca.* 2×10^{-8} cm²/sec).

Well-resolved ^{1}H spectra are also obtained from rabbit sciatic nerve and the intensities of the $(CH_2)_n$ and CH_3 resonances correspond to a major proportion of the lipids in the nerve. The proton T_1 values in the nerve are similar to those in the sonicated aqueous lipid extract: $(CH_2)_n$ T_1 = 0.33 and 0.27 sec; CH_3 T_1 = 0.64 and 0.42 sec, respectively, at 31° (J. D. Robinson, N. J. M. Birdsall, A. G. Lee, and J. C. Metcalfe, unpublished observations). The lower limit to the rate of surface diffusion is $D > 5 \times 10^{-9}$ in the nerve and $D > 8 \times 10^{-9}$ in the extracted lipid.

The last possibility which we would like to mention is that of estimating the rates of diffusion of small molecules within a membrane by ^{1}H measurements. At low concentrations, the intermolecular relaxation of a small molecule bound in the bilayer will be dominated by the lipid protons. Line-width and T_2 measurements for the small molecule then give the rate of diffusion of the small molecule relative to the lipid.

Taken together, recent studies of the rotational diffusion of rhodopsin in retinal rod membranes (Cone, 1972), the aggregation of immunoglobulins bound to the surface of lymphocyte membranes (Taylor *et al.*, 1971) and the interdiffusion of antigenic components of mouse cells and human cells fused by viruses (Frye and Edidin, 1970), all suggest that static models of membrane structure may be inadequate to describe the important functional characteristics of membranes. It is now possible to study by nmr the dynamic aspects of unperturbed and unmodified biological membranes, gradients of motion within lipids by ^{13}C nmr and diffusion of lipid molecules in the surface of membranes by ^{1}H nmr. Both motions appear to have useful sensitivity to the influence of membrane proteins.

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The Binding of a Naphthalene Dye Associated with J-Chain Attachment in an Immunoglobulin A Mouse Immunoglobulin†

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ABSTRACT: Native multimeric protein 460, a γ A mouse myeloma protein, binds 8-anilinonaphthalene-1-sulfonate (ANS) with a $K_0 = 3 \times 10^5$ l./mol. Neither the 7S monomers nor the isolated J chain derived from this protein bind ANS. When 7S monomers and J chains are recombined, ANS binding is partially restored. The evidence is consistent with ANS binding to the site of the attachment of the J chain rather than to the J chain itself. Dimeric and trimeric forms of protein 460 bind 1 mol of ANS/mol of 7S monomer. ANS shows a blue shift in its fluorescence emission maximum and a 200-fold increase in fluorescence quantum yield on binding to the protein. This suggests that the binding site for ANS is relatively hydrophobic.

A new immunoglobulin polypeptide chain, designated the J or joining chain, has recently been described (Halpern and Koshland, 1970; O'Daly and Cebra, 1971; Morrison and Koshland, 1972; Parkhouse, 1972). Its role appears to be to join the subunits of IgA and IgM immunoglobulins together, to form the naturally occurring IgA dimers and trimers as well as the IgM pentamers (Morrison and Koshland, 1972). This polypeptide component has been characterized by its mobility in alkaline–urea polyacrylamide gels (Halpern and Koshland, 1970; O'Daly and Cebra, 1971; Parkhouse, 1972), and its size (O'Daly and Cebra, 1971; Morrison and Koshland, 1972). It has a different amino acid composition when isolated from human or rabbit immunoglobulins (O'Daly and Cebra, 1971; Morrison and Koshland, 1972).

We have identified a similar polypeptide chain in native protein 460 synthesized and excreted by a mouse plasmacyte tumor, MOPC 460. We have employed the technique of fluorescence enhancement (Stryer, 1965, 1968; Parker *et al.*, 1967; Green *et al.*, 1972) using the naphthalene dye 8-anilinonaphthalene-1-sulfonate as the fluorescent probe, to study the nature of the site of attachment of the J chain to protein 460.

Materials and Methods

Reagents. Reagent grade ANS[1] as the magnesium salt (Eastman) was used without further treatment. Tritium-labeled ANS was synthesized by the method of Parker and Osterland (1970) and had a specific radioactivity of 0.70 Ci/mol. ANS concentration was calculated assuming $\epsilon_M = 4.99 \times 10^3$ at 350 nm (Parker and Osterland, 1970). Dithiothreitol (Sigma Chemical Co., St. Louis) was used without further treatment. Iodoacetic acid (Aldrich Chemical Co.) was recrystallized twice from water. [^{3}H]Iodoacetic acid was supplied by New England Nuclear. The specific radioactivity was adjusted by the addition of unlabeled iodoacetic acid to 7.8 Ci/mol.

Preparation of Proteins. MOPC 460 and 315 tumor lines were generous gifts of Drs. Herman Eisen and Ernest Simms and were maintained in BALB/c mice as previously described (Rosenstein *et al.*, 1972).

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[1] Abbreviation used is: ANS, 8-anilinonaphthalene-1-sulfonate.