[18] Nuclear Magnetic Resonance Studies of Biological and Model Membrane Systems

By D. CHAPMAN and E. OLDFIELD

Nuclear magnetic resonance (NMR) spectroscopy is a technique in which nulcei with spin $I \geq 1/2$ absorb radiofrequency energy of a particular frequency, in a magnetic field. The phenomenon was first successfully detected by Bloch[1] and Purcell.[2] Physical aspects of the technique are well described in texts by Abragam,[3] Andrew,[4] Mansfield,[5] and Slichter,[6] and

[1] F. Bloch, W. W. Hansen, and M. Packard, *Phys. Rev.* 70, 474 (1946).
[2] E. M. Purcell, H. C. Torrey, and R. V. Pound, *Phys. Rev.* 69, 37 (1946).
[3] A. Abragam, "The Principles of Nuclear Magnetism." Oxford Univ. Press, London, 1961.
[4] E. R. Andrew, "Nuclear Magnetic Resonance." Cambridge Univ. Press, London and New York, 1955.
[5] P. Mansfield, Pulsed NMR in solids. *In* "Progress in Nuclear Magnetic Resonance Spectroscopy" (J. W. Emsley, J. Feeney, and L. H. Sutcliffe, eds.), Vol. 8. Pergamon, Oxford, 1971.
[6] C. P. Slichter, "Principles of Magnetic Resonance." Harper & Row, New York, 1963.

more chemical and biochemical aspects in the works of Emsley, Feeney, and Sutcliffe,[1] Farrar and Becker,[2] and Levy and Nelson.[3]

For a given external magnetic field strength, different nuclei absorb energy at different frequencies. Thus it is possible to study, for example, carbon-13 or phosphorus-31 nuclei in a complex biochemical system, all other nuclei in the system remaining "transparent." The shape and width of an NMR absorption line, and the magnitude of its relaxation times, can all give interesting information on the mobility of the group containing the nucleus under observation, and this can lead to a better understanding of molecular mobility in complex systems.

NMR experiments may be carried out by applying radiofrequency excitation in a *pulsed* mode (pulsed NMR), or in a *continuous wave* mode (cw NMR). The pulsed mode is more suitable for measurements of nuclear-spin relaxation times, which are directly related to the rates and types of motion of the nuclei under observation.

In systems where motion is particularly slow or anisotropic, the actual width of an NMR absorption line is likely to be great. Under these circumstances electromagnets providing magnetic fields of only moderate homogeneity (e.g., 1 part in 10^6) may be adequate. This lack of necessity for extreme homogeneity represents a great reduction in cost of a spectrometer system and has led to the development of so-called "wide-line" NMR.

In some circumstances, however, electromagnets or superconducting solenoid magnets with homogeneities of ~ 1 in 10^8 are desirable, and this has led to the development of "high resolution" NMR.

The principal limitation of NMR as a technique with respect to, for example, electron spin resonance (ESR) is the poor sensitivity of NMR. Sensitivity in NMR is often improved by resorting to one or more of the following:

Signal Averaging. Here, analog signals are generally converted to digital form and successive signals are added digitally in the memory of a small computer (typically for ^{13}C NMR experiments, a 4096 18-bit word device). *Signal* increases in direct proportion to the number of scans, n, whereas *noise* increases as $\sqrt{n}$. The net increase in *signal-to-noise* (S/N) after n accumulations is thus $n/\sqrt{n} = \sqrt{n}$. Thus, to increase a

[1] J. W. Emsley, J. Feeney, and L. H. Sutcliffe, "High Resolution Nuclear Magnetic Resonance Spectroscopy." Pergamon, Oxford, 1965.
[2] T. C. Farrar and E. D. Becker, "Pulse and Fourier Transform NMR." Academic Press, New York, 1971.
[3] G. C. Levy and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists." Wiley (Interscience), New York, 1972.

given S/N by a factor of, e.g., 100, $(100)^2 = 10{,}000$ times as many scans would be necessary. This may become prohibitive so that additional techniques are often resorted to.

Large Sample Tubes. For high-resolution proton NMR, sample tubes of 5 mm o.d. containing 500–600 μl of sample are of general use. However, for study of systems having broad lines, where S/N is low, or for study of nuclei that have low natural abundance, e.g., ^{13}C or ^{15}N, or for nuclei that are "dilute" in the system of interest, e.g., ^{31}P in lipids and membranes, it is preferable to use the largest sample size possible. Most commercial ^{13}C Fourier transform (FT) NMR spectrometers utilize sample tubes with 13 mm o.d. Even larger sample tubes, with an o.d. of 20 mm have now been developed.[10] These give time savings of 200–300 over "conventional" 5-mm tubes. Even larger tubes are currently under development, and one may thus confidently expect time savings of two to three orders of magnitude over 5–12-mm tubes in the near future.

Overhauser Enhancement. For ^{13}C NMR, irradiation of ^1H nuclei can lead to a maximal nuclear Overhauser enhancement (NOE) of the ^{13}C signal, by a factor of 2.988 when the ^{13}C relaxation is purely dipolar and the "extreme narrowing" condition $(\omega_H + \omega_C)\tau_R \ll 1$ is satisfied, where ω_H and ω_C are the Larmor frequencies of ^1H and ^{13}C respectively, and τ_R is the rotational correlation time. For $(\omega_H + \omega_C)\tau_R \gg 1$ the NOE = 1.153.

Sample Spinning. In high-resolution NMR, the sample is spun in a gas turbine. This spinning has the effect of averaging out some laboratory magnetic field (H_0) inhomogeneities, and was first demonstrated by Anderson and Arnold.[11] As a result of a more homogeneous H_0, resonance lines are narrower and hence signal-to-noise is improved.

In this chapter, we outline some of the considerations necessary for studying molecular mobility in natural and model membrane systems, using the techniques of wide line and high-resolution NMR in both pulsed and continuous-wave modes.

Wide-Line NMR

Continuous Wave

Proton NMR. Continuous wave (or frequency domain) NMR studies on protons in both model lipid membrane and biological membrane systems, have been reported.[12-15] For studies on lipids or membranes, ca. 100 mg dry weight material is required for a single-scan spectrum. When

[10] A. Allerhand, R. F. Childers, R. A. Goodman, E. Oldfield, and X. Ysern, *Amer. Lab.* 4, 19 (1972).

[11] W. A. Anderson and J. T. Arnold, *Phys. Rev.* 94, 497 (1954).

aqueous dispersions are to be studied, extreme care should be taken to exchange residual ^1H_2O in the sample for ^2H_2O, otherwise much detail will be obscured by overlap from the water peak. Because the observed linewidths are of a *dipolar* origin,[16,17] these linewidths are a good measure of the molecular mobility of a given group.

Deuterated compounds[12,13] are often a necessary aid in interpreting the resultant spectra, since the proton absorption lines are generally several kHz wide, and overlap. Deuteration removes selective resonances, so that specific assignments can be made. Methods are available for breakdown of complex line shapes,[18,19] and curve analyzers may also be of use.

Deuteron NMR. Continuous-wave NMR studies have also been reported on deuterium nuclei (deuteron magnetic resonance, DMR).[20,21]

In this method, protons in a given group of interest are chemically substituted by deuterons.[22] For example, completely deuterated fatty acids may be readily synthesized by simple exchange reactions such as

$$CH_3(CH_2)_{14}CO_2H \xrightarrow[250^\circ\ 30\ \text{hours}]{D_2O,\ Pt/D_2,\ Na_2O_2} CD_3(CD_2)_{14}CO_2H$$

The "labeled" fatty acids may then be incorporated into model systems[20]; or can be *biosynthetically* incorporated into natural membranes. For ex-

$$CD_3(CD_2)_{12}CO_2H \xrightarrow{(COCl)_2} CD_3(CD_2)_{12}COCl \xrightarrow[\text{pyridine}]{\text{glycerophosphoryl choline-cadmium chloride adduct}}$$

$$\begin{array}{l} CH_2\text{—}OCO(CD_2)_{12}CD_3 \\ | \\ CHOCO(CD_2)_{12}CD_3 \\ | \\ CH_2\text{—}OPO_2OCH_2CH_2NMe_3 \end{array}$$

di(perdeuteromyristoyl)-Lα-lecithin

[16] Z. Veksli, N. J. Salsbury, and D. Chapman, *Biochim. Biophys. Acta* 183, 434 (1969).
[17] A. Darke, E. G. Finer, A. G. Flook, and M. C. Phillips, *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 18, 326 (1971).
[18] T. J. Jenkinson, V. B. Kamat, and D. Chapman, *Biochim. Biophys. Acta* 183, 427 (1969).
[19] N. J. Salsbury and D. Chapman, *Biochim. Biophys. Acta* 163, 314 (1968).
[20] S. I. Chan, G. W. Feigenson, and C. H. A. Seiter, *Nature (London)* 231, 110 (1971).
[21] E. Oldfield, J. Marsden, and D. Chapman, *Chem. Phys. Lipids* 7, 1 (1971).
[22] C. W. Wilson and G. E. Pake, *J. Polymer Sci.* 10, 503 (1953).
[23] C. W. Wilson and G. E. Pake, *J. Chem. Phys.* 27, 115 (1957).
[24] E. Oldfield, D. Chapman, and W. Derbyshire, *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 16, 102 (1971).
[25] E. Oldfield, D. Chapman, and W. Derbyshire, *Chem. Phys. Lipids* 9, 69 (1972).
[26] N. Dinh-Nguyen and E. A. Stenhagen, Patent (France) No. 1,466,088 *Chem. Abstr.* 67, 63814 (1967).

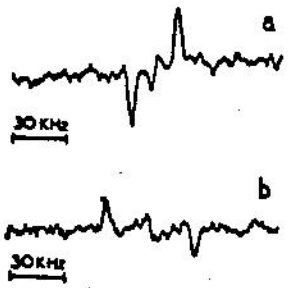

FIG. 1. Deuteron magnetic resonance spectra of di(perdeuteromyristoyl)-L-α-lecithin in H_2O obtained at 8 MHz on an extensively modified Varian wide-line spectrometer. (a) Lecithin, 50 mg in 1 ml of H_2O, 30° (liquid crystalline state. The gel → liquid crystal transition temperature for deuterated dimyristoyl lecithin is the same as for the protonated species, i.e., 23°). Spectrum shown is the result of ca. 6 hours signal averaging. (b) Same conditions as in (a) except that sample contains in addition 25 mg of cholesterol, i.e., is a 1:1 lecithin–cholesterol "complex" in excess water. The central narrow component of both spectra does not arise from natural abundance $^2H^1HO$ but represents, presumably, rapidly reorienting terminal CD_3-groups [E. Oldfield, D. Chapman, and W. Derbyshire, *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 16, 102 (1971)].

ample, we have successfully incorporated lauric acid-d_{23} into the plasma membrane of *Acholeplasma laidlawii* B.[21] Typically, 2–4 hours of signal averaging are required for 100 μmoles of lipid, at a field strength of 1.4 T.

In lipids, the *quadrupole splitting* of the absorption line gives information on the molecular motion of the particular group in question.[20] Figure 1a shows a typical deuteron resonance spectrum of a liquid crystalline lipid system, di(perdeuteromyristoyl)-L-α-lecithin-H_2O. The maximal quadrupole splitting of the absorption line is given by[23]

$$\Delta\nu_{max} = \frac{3}{4}\frac{e^2qQ}{h}(3\cos^2\theta - 1)$$

where e^2qQ is the deuteron quadrupole coupling constant, which is about 170 kHz for CD_2 groups,[24] and θ is the angle between the electric field gradient tensor at the nucleus, and H_0. $(3\cos^2\theta - 1)$ is a function of the rate and type of motion undergone by the CD_2 group. Addition of cholesterol to the liquid crystalline system (Fig. 1b) causes an increase in $\Delta\nu_{max}$ from 27 kHz to 49.4 kHz, implying an increase in $(3\cos^2\theta - 1)$, i.e., an increase in restriction and/or a decrease in the rate of motion of the CD_2 group.

[23] M. H. Cohen and F. Reif, *In* "Solid State Physics" (F. Seitz and D. Turnbull, eds.), Vol. 5. Academic Press, New York, 1957.
[24] L. J. Burnett and B. H. Muller, *J. Chem. Phys.* 55, 5829 (1971).

In the natural biomembrane system, very broad absorption lines were found for isotopically enriched *A. laidlawii* B, and it was thus concluded that the lipids were in a *crystalline gel state.*[21,22]

An advantage of deuteron resonance over, for example, ^{13}C NMR, is that it is not necessary to remove scalar coupling by the use of noise decoupling.[26]

Phosphorus NMR. Phosphorus-31 occurs in all phospholipids, and ^{31}P NMR spectra have recently been reported by Davis and Inesi.[27] Interestingly, Sheetz and Chan[28] have recently observed an anisotropic chemical shift powder patter for ^{31}P in lecithin, in both gel and liquid crystalline bilayers. It appears that the ^{31}P nucleus is relatively rigid in these systems, as expected. High fields and signal averaging are generally required for adequate S/N from ~100 mg of phospholipid.

Pulsed NMR

Spin-Spin Relaxation. There has been some controversy over the mechanism of spin-spin relaxation (or linebroadening) in lipids,[29-31] though this now appears to be resolved[16,17] at least for protons in fields up to ~2.0 T. Adequate sensitivity can generally be obtained by use of 8-mm sample tubes containing ~100 mg of lipid or membranes, at 60 MHz, from a single pulse.

For relatively wide lines, Data Laboratories DL 101 S or DL 102 S signal averagers can improve S/N significantly, and for very broad lines (i.e., very short T_2s, as would be expected from solid, crystalline lipids), Biomation transient recorders can be used. Of course, wideline pulsed (or CW) techniques are applicable only to unsonicated model or natural membrane systems, where T_2s are short (~1 ms). Since the shape of the signal decay after the pulse (free induction decay, or FID) is the inverse Fourier transform of the continuous-wave absorption spectrum,[32] the two can be interrelated. Typical free induction decays for gel and liquid crystalline dipalmitoyl lecithin in D_2O are given in Figs. 2a,b. It is possible by use of radiofrequency *pulse sequences* to remove some of

[21] E. Oldfield and D. Chapman, *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 23, 285 (1972).
[22] R. R. Ernst, *J. Chem. Phys.* 45, 3845 (1966).
[27] D. G. Davis and G. Inesi, *Biochim. Biophys. Acta* 282, 180 (1972).
[28] S. I. Chan, private communication.
[29] S. A. Penkett, A. G. Flook, and D. Chapman, *Chem. Phys. Lipids* 2, 273 (1968).
[30] S. Kaufman, J. M. Steim, and J. H. Gibbs, *Nature (London)* 225, 743 (1970).
[31] J. R. Hansen and K. D. Lawson, *Nature (London)* 225, 542 (1970).
[32] I. J. Lowe and R. E. Norberg, *Phys. Rev.* 107, 46 (1957).

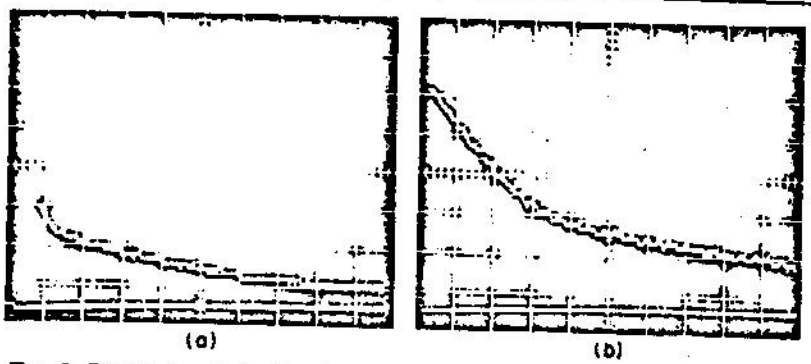

FIG. 2. Proton free induction decays obtained on dipalmitoyl-L-α-lecithin (Koch-Light Laboratories, Colnbrook, U.K.) in 99.8% deuterium oxide (Prochem Ltd., Carolyn House, Croydon, U.K.) at 60 MHz on a Bruker 322s pulsed nuclear magnetic resonance spectrometer. Polaroid photographs were taken of the FID which was stored in the memory of a Data Laboratories DL 102s signal averager. (a) Dipalmitoyl lecithin, 100 mg in 400 μl of D_2O, 32.5°, gel state, single 90° pulse of <2 μs width. Horizontal scale is 40 μs per division. (b) As in (a) except that temperature is 61° (liquid crystalline state) (E. Oldfield, J. Marsden, and D. Chapman, unpublished results).

the magnetic field inhomogeneities imposed by use of a low-resolution magnet system.[33,34] Care is necessary in interpretation of results.[16,17]

Spin-Lattice Relaxation (in the Laboratory Frame). Spin-lattice relaxation (T_1) measurements of ^1H, of lecithins, have been reported.[16,17,35]

Generally, again, about 100 mg of lipid are required for proton T_1 measurements in unsonicated systems. The method is capable of giving information on high frequency ($\sim 10^5$ Hz) molecular motions, though the possibility of dipolar spin-diffusion[16,17,35,36] can complicate interpretation of results. This difficulty is not expected in systems giving high-resolution chemically shifted signals, since static dipolar coupling is absent —this being the reason for observing high-resolution signals. This lack of spin-diffusion has been confirmed experimentally.[37,38]

It is often difficult to analyze a multicomponent T_1 decay into its individual components, and this difficulty should be borne in mind in interpreting data.

Spin-Lattice Relaxation (in the Rotating Frame). Spin-lattice relaxa-

[33] H. Y. Carr and E. M. Purcell, *Phys. Rev.* 94, 630 (1954).
[34] S. Meiboom and D. Gill, *Rev. Sci. Instrum.* 29, 688 (1958).
[35] J. T. Daycock, A. Darke, and D. Chapman, *Chem. Phys. Lipids* 6, 205 (1971).
[36] J. E. Anderson and W. P. Slichter, *J. Phys. Chem.* 69, 3099 (1965).
[37] N. J. M. Birdsall, A. G. Lee, Y. K. Levine, and J. C. Metcalfe, *Chem. Commun.* 1171 (1971).
[38] A. G. Lee, N. J. M. Birdsall, Y. K. Levine, and J. C. Metcalfe, *Biochim. Biophys. Acta* 255, 43 (1972).

tion measurements in the rotating frame ($T_{1\rho}$) have been reported for lecithins, deuterated lecithins, and phosphatidylethanolamines, and the effect of water has been noted.[39] The method again requires ca. 100 μmoles of lipid (or ~70–100 mg membranes), and is capable of giving information on *low frequency* molecular motions (ca. 50 kHz). Spin-diffusion is again an important factor in interpretation of data.[39,40]

High-Resolution NMR

Continuous Wave

Unsonicated Systems. Use of a high field (≥ 5.0 T) NMR spectrometer is preferable for acceptable signal to noise, since resonances

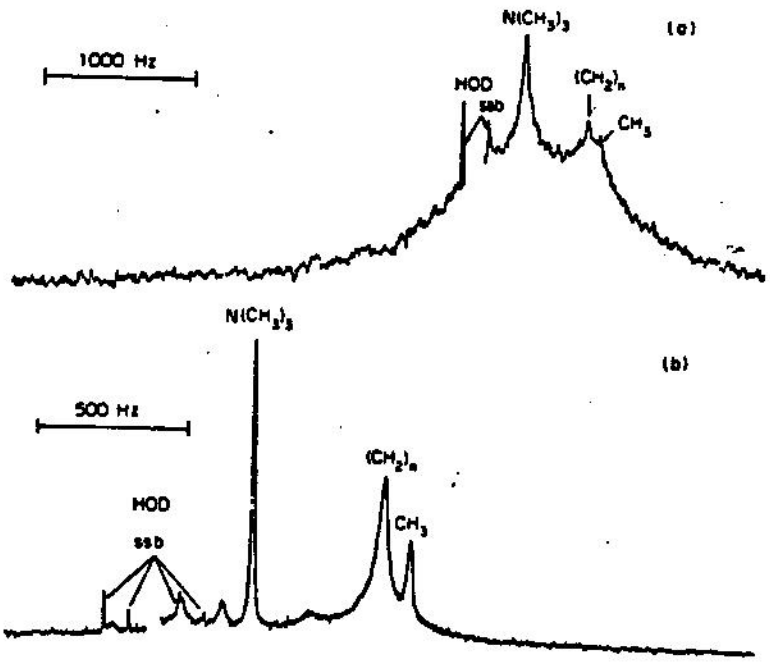

FIG. 3. Proton nuclear magnetic resonance spectra of dimyristoyl-La-lecithin (Koch Light Laboratories, Colnbrook, U.K.) in 99.8% deuterium oxide (Prochem Ltd., Carolyn House, Croydon, U.K.) recorded on a Varian HR-220 spectrometer at 220 MHz in a continuous-wave mode at 30°. (a) Unsonicated dimyristoyl lecithin, 30 wt% and (b) 10 wt% dimyristoyl lecithin after 20 minutes sonication, under nitrogen at 4°, using a Kerry Ultrasonics sonicator. Spectra recorded approximately 4 hours after sample preparation, single sweep (J. Marsden and D. Chapman, unpublished results).

[39] N. J. Salsbury, D. Chapman, and G. Parry Jones, *Trans. Faraday Soc.* 66, 1554 (1970).

[40] D. C. Douglass and G. P. Jones, *J. Chem. Phys.* 45, 956 (1966).

in membranes or lipid dispersions are generally broad ($\gtrsim$100 Hz)[18] (Fig. 3a).

High-resolution spectra have been obtained from sarcoplasmic reticula,[41,42] sciatic nerve,[43] and erythrocyte membranes.[44,45] Generally $\gtrsim$ 20 mg of membrane or lipid are required. Care should be taken to assay the amount of high-resolution spectrum obtained from the material (by analysis of the total proton content, and the spectrum integral, using an external standard, e.g., benzene in a small capillary), and for biomembranes, dissolution of membranes in water[46,47] should be checked by centrifugation of membrane from the dispersion. Reversible dissociation of 6000 molecular weight polypeptides is known to occur from red cell ghosts at high temperatures.[46]

Recently, it has been shown[49] that "high-resolution" spectra may be obtained from unsonicated model membrane systems by spinning the sample about an axis inclined at an angle $\beta = \sec^{-1}\sqrt{3}$, the "magic angle," to the magnetic field H_0. This causes reduction of direct nuclear dipole–dipole interactions, and a consequent narrowing of the absorption lines (Fig. 4a,b). This technique would appear to be extremely promising in the study of intact biomembranes as well as model-membrane systems.

Sonicated Systems. Ultrasonication of lipids or membranes causes formation of a high-resolution spectrum,[50-52] which has been suggested to be due to averaging of dipolar interactions by rapid particle rotation,[53] or to a change in packing in the small unsonicated vesicles compared with that found in nonsonicated systems,[28] or even a combination of both effects.[52] Because of the well resolved spectra obtainable (Fig. 3b), less material is required, e.g., 5–10 mg at 220 MHz for ^{1}H. Sonication of

[41] D. G. Davis and G. Inesi, *Biochim. Biophys. Acta* 241, 1 (1971).
[42] J. D. Robinson, N. J. M. Birdsall, A. G. Lee, and J. C. Metcalfe, *Biochemistry* 11, 2903 (1972).
[43] P. Dea, S. I. Chan, and F. Dea, *Science* 175, 206 (1972).
[44] V. B. Kamat and D. Chapman, *Biochim. Biophys. Acta* 163, 411 (1968).
[45] M. Glaser, H. Simpkins, S. J. Singer, M. Sheetz, and S. I. Chan, *Proc. Nat. Acad. Sci. U.S.* 65, 721 (1970).
[46] S. I. Chavin, *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 14, 269 (1971).
[47] D. Mazia and A. Ruby, *Proc. Nat. Acad. Sci. U.S.* 61, 1005 (1968).
[48] M. P. Sheetz and S. I. Chan, *Biophys. Soc. Abstr.* 46a (1970).
[49] D. Chapman, E. Oldfield, D. Doskočilová, and B. Schneider, *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 25, 251 (1972).
[50] D. Chapman and S. A. Penkett, *Nature (London)* 211, 1304 (1966).
[51] D. Chapman, V. B. Kamat, J. de Gier, and S. A. Penkett, *J. Mol. Biol.* 31, 101 (1968).
[52] D. Chapman, *Ann. N.Y. Acad. Sci.* 195, 179 (1972).
[53] E. G. Finer, A. G. Flook, and H. Hauser, *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 18, 331 (1971).

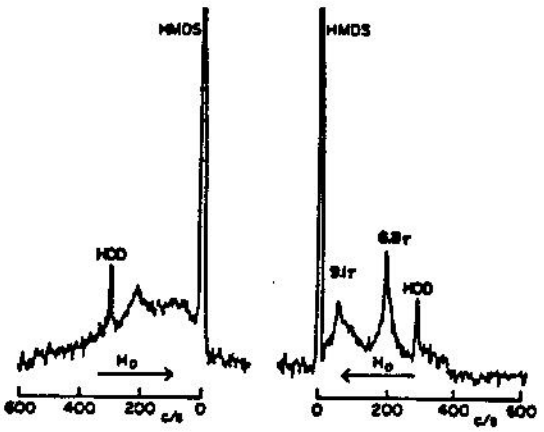

FIG. 4. Proton nuclear magnetic resonance spectra obtained on egg-yolk lecithin (Lipid Products Ltd., South Nutfield, Surrey, U.K.) as 33 wt% hand-dispersions in 99.8% D_2O (Prochem Ltd., Carolyn House, Croydon, U.K.) at 60 MHz on a modified JEOL JNM-3-60 spectrometer, at 25°. (a) Sample spun in a glass rotor in a gas-driven turbine at 500 Hz with β, the angle between the axis of rotation and H_0, equal to 90°. (b) as in (a), except $\beta = \sec^{-1}\sqrt{3}$, the "magic angle." Spectra were calibrated using audiofrequency modulation side bands, and the reported tau values are the average of several consecutive runs.

The spectra shown in (a) and (b) were obtained within a few seconds of each other, so that sample "homogenization" effects possibly caused by the high centrifugal forces involved, are negligible. In addition, line broadening was observed on the transition from (b) → (a) [D. Chapman, E. Oldfield, D. Doskočilová, and B. Schneider, *FEBS* (*Fed. Eur. Biochem. Soc.*) *Lett.* 25, 261 (1972)].

phospholipids can be carried out with a variety of ultrasonic devices, and these include the Dawe Instruments Sonitype Probe 1130, the Kerry Vibrason System 150, and the sonifier cell disintegrator TW 140D Heat Systems Ultrasonics Inc. One milliliter of the coarse phospholipid (1–5 wt%) dispersion is sonicated in a glass tube (ca. 7 × 1 cm) surrounded by iced water. When unsaturated lipids are examined, manipulations should be carried out under nitrogen. Chemical degradation of unsaturated lipids can occur, and care should be taken to check for possible sample breakdown[54] or membrane "hybridization"[55] effects. The saturated lipids appear to show less degradation than the unsaturated lipids. Interesting experiments utilizing Eu^{3+} induced pseudo-contact shifts have been reported in model systems[56] and may be applicable to some biomembranes.

[54] H. O. Hauser, *Biochem. Biophys. Res. Commun.* 45, 1049 (1971).
[55] N. Tsukagoshi and C. F. Fox, *Biochemistry* 10, 3309 (1971).
[56] V. F. Bystrov, N. I. Dubrovina, L. I. Arsukov, and L. D. Bergelson, *Chem. Phys. Lipids* 6, 343 (1971).

Since heterogeneity in packing among sonicated vesicles themselves exists,[26,27] care should be exercised in interpreting results on "bulk" sonicated systems.

^{19}F fatty acid probes have been used to study mobility in ultrasonically dispersed lecithin.[58] ^{1}H noise decoupling is necessary for moderate resolution of the ^{19}F line. Care should be taken in assessing the effect of the large CHF moment of inertia on mobility of the chain at the substituent site.

Pulsed (Fourier Transform) NMR

As implied above in the section on pulsed NMR, the unsaturated slow passage steady-state absorption lineshape of a nuclear resonance is the Fourier transform of the free induction decay.[32] Ernst and Anderson have used this fact to introduce the technique of Fourier transform NMR.[59]

The technique has the advantage over continuous-wave methods, in almost all cases, of greater sensitivity. In addition, numerous individual relaxation times in multispin systems may be determined simultaneously, using partially relaxed Fourier transform NMR.[60,61]

Sonicated Systems. Both ^{13}C and ^{1}H NMR T_1 measurements using partially relaxed Fourier transformation have been made on sonicated systems.[37,38] Because of the lengthy time required for data acquisition, samples should be ca. 10 wt% lipid.

Unsonicated Systems. ^{13}C pulsed Fourier transform NMR studies have been reported on liquid crystalline lecithins[62-64] (Fig. 5a), on sphingomyelin[62] (Fig. 5c), on lecithin–cholesterol interactions[62] (Fig. 5b), and on erythrocyte and mitochondrial membranes[62,64] (Fig. 6). Poor signal-to-noise ratios are a problem with natural abundance ^{13}C studies of lipids and membranes, so suspensions should be as concentrated as possible, e.g., for natural membranes, prepared by high speed centrifugation, or for model systems, 1:1 lipid-water hand-dispersions.

[26] D. Marsh, A. D. Phillips, A. Watts, and P. F. Knowles, *Biochem. Biophys. Res. Commun.* 49, 641 (1972).
[27] N. J. M. Birdsall, A. G. Lee, Y. K. Levine, and J. C. Metcalfe, *Biochim. Biophys. Acta* 241, 693 (1971).
[32] R. R. Ernst and W. A. Anderson, *Rev. Sci. Instrum.* 37, 93 (1966).
[58] R. L. Vold, J. S. Waugh, M. P. Klein, and D. E. Phelps, *J. Chem. Phys.* 48, 3831 (1968).
[59] A. Allerhand, D. Doddrell, and R. Komoroski, *J. Chem. Phys.* 55, 189 (1971).
[60] K. M. Keough, E. Oldfield, D. Chapman, and P. Beynon, *Chem. Phys. Lipids* 10, 37 (1973).
[61] E. Oldfield and D. Chapman, *Biochem. Biophys. Res. Commun.* 43, 949 (1971).
[62] J. C. Metcalfe, N. J. M. Birdsall, J. Feeney, A. G. Lee, Y. K. Levine, and P. Partington, *Nature (London)* 233, 199 (1971).

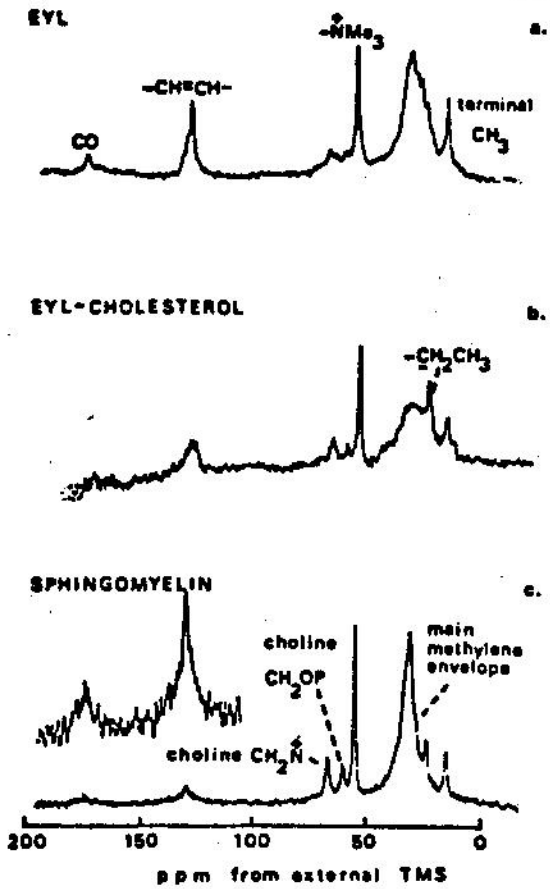

FIG. 5. Carbon-13 Fourier transform nuclear magnetic resonance (NMR) spectra of some model membrane systems, obtained at 25.1 MHz on a JEOL PFT-100 FT NMR system, under conditions of maximal proton decoupling power. (a) Egg yolk lecithin (EYL) (Lipid Products, South Nuttfield, Surrey, U.K.) in 99.8% D_2O (Prochem Ltd., Carolyn House, Croydon, U.K.), 50 wt% hand-dispersion, ca. 1 g of lipid, 38°, 8 hours signal accumulation. (b) Egg-yolk lecithin cholesterol (B.D.H. Ltd., Poole, Dorset, U.K.) 1:1 hand-dispersion in D_2O, 38°, 8 hours accumulation. (c) Ox-brain sphingomyelin (Koch-Light Ltd., Colnbrook, Bucks., U.K.), 800 mg in 1.2 ml D_2O, 75°, 8 hours signal accumulation [K. M. Keough, E. Oldfield, D. Chapman, and P. Beynon, *Chem. Phys. Lipids* 10, 37 (1973)].

An important consideration in ^{13}C NMR of lipids is the adequacy of proton decoupling. For sonicated systems this is unlikely to be a problem, though for unsonicated systems, where ^1H absorption lines are relatively wide, incomplete decoupling will lead to multiplet formation and a decreased NOE, as will deviations from the "extreme narrowing condition."

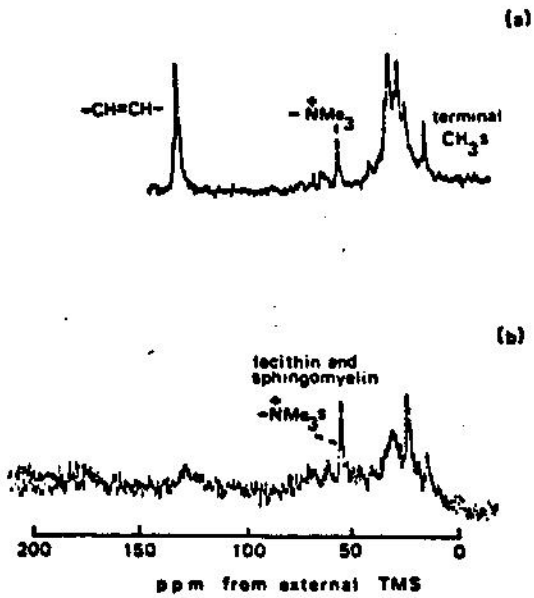

FIG. 6. Carbon-13 Fourier transform nuclear magnetic resonance (NMR) spectra of mitochondrial membranes (a) and erythrocyte membranes (b) obtained at 25.1 MHz on a JEOL PFT-100 FT NMR system [K. M. Keough, E. Oldfield, D. Chapman, and P. Beynon, *Chem. Phys. Lipids* 10, 37 (1973)].

Future Work

Of the numerous nuclei that could be studied in biological membranes, e.g., ^{1}H, ^{2}H, ^{13}C, ^{14}N, ^{15}N, ^{17}O, and ^{31}P, it would appear that the spin-1/2 nucleus, carbon-13, shows greatest promise. ^{2}H, ^{14}N, and ^{17}O are quadrupolar and hence give broad absorption lines, which are difficult to detect. In addition, all are far less sensitive than ^{13}C. ^{15}N has a negative gyromagnetic ratio, so that, when relaxation is of a dipolar nature, it is likely that under some circumstances, if proton decoupling is used, Overhauser effects may cause loss of all signal. ^{1}H NMR, of course, has tremendous sensitivity, but it is not readily amenable to isotopic enrichment techniques. ^{31}P also has high sensitivity, but it is relatively restricted in its distribution, and no suitable other nuclei exist for isotopic substitution experiments. It is possible that pulsed double resonance of the type used by Pines *et al.*[65] may be applicable to some biomembrane systems, especially with the use of isotopic enrichment.

Among the future studies of direct importance to the biochemist on lipid and membrane systems which the NMR methods make available are the following:

[65] A. Pines, M. G. Gibby, and J. S. Waugh, *J. Chem. Phys.* 56, 1776 (1972).

1. A determination of the detailed molecular motion of all the various groups in lipid and membrane systems should soon become possible. With such a molecular map of the membrane, biochemical changes involving permeability characteristics may be followed.

2. Interaction experiments of lipids with other biologically important molecules can be studied, e.g., interactions of cholesterol, chlorophyll, polypeptides.[50]

3. Interaction experiments with membranes using detergents, proteases, lipases[44,51] are possible.

4. Determination of lipid asymmetry using transition metal titration methods[54] should enable the lecithin plus sphingomyelin content of inner and outer biological membranes to be compared.

Acknowledgments

The Science Research Council is thanked for general support and for a Postgraduate Studentship to E. O.