

TECHNIQUES IN THE LIFE SCIENCES

Biochemistry – Volume B4/II

TECHNIQUES IN LIPID AND MEMBRANE BIOCHEMISTRY – PART II

Editorial Board for the Biochemistry Section:

T.R. Hesketh, H.L. Kornberg, J.C. Metcalfe, D.H. Northcote (Cambridge)

C.I. Pogson (Canterbury) and K.F. Tipton (Dublin)

B427

**NUCLEAR MAGNETIC RESONANCE TECHNIQUES IN LIPID AND
MEMBRANE BIOCHEMISTRY**

by

E. Oldfield

ELSEVIER BIOMEDICAL

© Elsevier Scientific Publishers Ireland Ltd., 1982

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior permission of the copyright owner.

Acknowledgements

Many of the illustrations come from other publications and the permission for their reproduction is gratefully acknowledged. The original sources are given in the captions and references.

Special regulations for readers in the U.S.A. This journal has been registered with the Copyright Clearance Center, Inc. Consent is given for copying of articles for personal or internal use, or for the personal use of specific clients. This consent is given on the condition that the Copier pays through the Center the per copy fee stated in the code on the first page of each article for copying beyond that permitted by Sections 107 or 108 of the U.S., Copyright Law. The appropriate fee should be forwarded with a copy of the first page of the article to the Copyright Clearance Center, Inc., 21 Congress Street, Salem, MA 01970, U.S.A. If no code appears in an article, the author has not given broad consent to copy and permission to copy must be obtained directly from the author. This consent does not extend to other kinds of copying such as for general distribution, resale, advertising and promotion purposes, or for creating new collective works. Special written permission must be obtained from the publisher for such copying.

Special regulations for authors in the U.S.A. Upon acceptance of an article by the journal, the author(s) will be asked to transfer copyright of the article to the publisher. This transfer will ensure the widest possible dissemination of information under the U.S. Copyright Law.

Published by:

Elsevier Scientific Publishers Ireland Ltd.
Bay 15, Shannon Industrial Estate
County Clare, Ireland

Sole distributors for the U.S.A. and Canada:

Elsevier Science Publishing Co., Inc.
52 Vanderbilt Avenue
New York, NY 10017

Published and printed in Ireland

Nuclear magnetic resonance techniques in lipid and membrane biochemistry*

ERIC OLDFIELD†

School of Chemical Sciences, University of Illinois at Urbana, 505 South Mathews Avenue, Urbana, IL 61801 (U.S.A.)

-
1. Introduction
 2. Instrumentation
 - 2.1. Spectrometers
 - 2.2. Sample volume
 - 2.3. Sample mass and data acquisition times
 - 2.4. New Fourier transform pulse methods
 3. Lipids
 - 3.1. Phospholipids
 - 3.2. Glycolipids
 4. Sterols
 - 4.1. NMR of sterols in membranes
 - 4.2. Sterol effects on membrane structure
 5. Polypeptides
 - 5.1. NMR of polypeptides in membranes
 - 5.2. Polypeptide effects on membrane structure
 6. Proteins
 - 6.1. NMR of proteins in membranes
 - 6.2. Protein effects on membrane structure
 7. Summary and view of the future
-

1. Introduction

In the last four or five years, there has been an impressive improvement in our ability to study intact membrane structure, due in no small part to the introduction of new solid-state nuclear magnetic resonance (NMR) techniques (Davis et al., 1976; Haberkorn et al., 1978) into the membrane area. As we will illustrate below, it is now possible to obtain high-resolution spectra of non-sonicated lipid membranes using rapid sample-rotation techniques (Haberkorn et al., 1978), or to study in detail the rates and types

*Part of this work was supported by the US National Institutes of Health (grants CA-00595, HL-19481), by the US National Science Foundation (grants PCM 78-23021, PCM 79-23170), and by the American Heart Association (grant 80-867), and has benefited from the use of facilities made available through the University of Illinois NSF Regional Instrumentation Facility (grant CHE 79-16100).
†USPHS Research Career Development Awardee, 1979-1984.

of motion of essentially any atom in a lipid molecule, by means of isotopic labelling and the quadrupole-echo pulse technique (Davis et al., 1976; Huang et al., 1980). In addition, all other stable nuclei (^{14}N , ^{17}O , ^{31}P) in phospholipids have now been investigated (Siminovich et al., 1980; Rajan et al., 1981, and Refs. therein; Tsai et al., unpublished data), enabling the investigator to focus on any part of the lipid bilayer membrane system he desires. Some representative studies and information on instrumentation required will be discussed below.

In addition to these rather detailed studies of lipid bilayer dynamics, and the effects of sterols, polypeptides and proteins on the dynamics, there have in addition quite recently been reported (Kinsey et al., 1981a; Kinsey et al., 1981b; Rice et al., 1981a) a number of studies which indicate that it is now possible to obtain solid-state spectra of proteins themselves in intact biological membranes. Results with *Halobacterium halobium* (an extreme halophile), *Escherichia coli*, and *Thermus thermophilus* (an obligate thermophile), will be outlined, indicating we hope, the ushering-in of a new era in which NMR methodologies may be used to determine the dynamic and static structures of those most interesting species, the membrane enzymes (Jost et al., 1973; Warren et al., 1974; Stockenius et al., 1979).

2. Instrumentation

2.1. Spectrometers

In the past 2–3 years, NMR spectrometers operating at fields of up to 140 kG (corresponding to a ^1H resonance frequency of 600 MHz) have become available. Unfortunately, complete high-field NMR spectrometers cost between \$350,000 (US) and \$600,000 (US) (perhaps best viewed as only 0.1c (US) per Hertz) so that access to such expensive instrumentation is naturally a factor in planning membrane NMR experiments, which may be quite lengthy (see below). As a means of ameliorating this situation, most workers in the membrane-NMR area have chosen to construct their own instruments using readily available components. Typical prescriptions have been reported previously (Oldfield et al., 1978a; Oldfield and Meadows, 1978). It is worth noting that we used the design of Meadows and Oldfield at 35, 60, 85 and 117 kG on four "homebuilt" spectrometers whose system performance is not exceeded by any commercial instrumentation. Notably, the cost of building such instruments is about one-half the cost of purchasing a complete commercial spectrometer.

For any very lengthy experiments, continued access to high field instrumentation is essential. In the United States such access is made available by a sizeable number of Regional NMR Facilities funded in part by the US National Science Foundation and the US National Institutes of Health. In these Facilities technical support personnel and highly sophisticated instrumentation are highly concentrated and provide the most advanced capabilities on a service basis, to qualified users. Interested readers are encouraged to consult the author for further details.

2.2. Sample volume

In our laboratory, we have standardized on a $\sim 0.7 \text{ cm}^3$ sample volume for the vast

majority of our NMR experiments. This size, of course, represents a trade-off between a variety of factors. In most of our experiments the most important factors are either a short 90° -pulse width (see below) in order to cover the entire spectral width of a ^2H powder pattern (~ 250 kHz width, 90° pulse $\sim 2\text{--}3\ \mu\text{s}$), or a strong ^1H decoupling field (for proton-decoupled ^{13}C -NMR studies). While larger sample sizes could in principle give improved signal-to-noise ratios and/or reduced data acquisition periods, there are unacceptable consequences, such as melting of probe components, associated with increasing radiofrequency (rf) power levels much above those we currently use ($\sim 1\text{--}2$ kW). The H_1 power in a sample coil is given, approximately, by (Clark, 1964)

$$H_1 \approx 3(PQ/\nu_0 V)^{1/2} \quad (1)$$

where P is the transmitter power in watts, Q is the quality factor of the probe circuit, ν_0 is the Larmor frequency in MHz, and V is the sample volume (in cm^3). Equation 1 shows, for example, that doubling the sample volume requires a corresponding doubling of rf power level to keep a constant H_1 field, or 90° pulse width (Farrar and Becker, 1971). For the very wide spectral widths obtained with ^2H , typically 200–300 kHz, it is not feasible to increase sample size in excess of $\sim 2\ \text{cm}^3$. Similar considerations apply to ^{13}C -NMR when using high-power proton decoupling to remove $^{13}\text{C}\text{--}^1\text{H}$ dipolar interactions (Pines et al., 1973; Schaefer and Stejskal, 1976; Haberkorn et al., 1978). By contrast, solution NMR experiments (or experiments with sonicated lipid vesicles) do not have these limitations since the static quadrupolar or dipolar interactions causing the linebroadening are removed by rapid particle tumbling, and conventional "high-resolution" spectra are obtained. For ^1H , ^2H , ^{13}C and ^{31}P solution NMR the range of spectral breadths are only at most ~ 10 kHz, so that rather large sample sizes may be used, up to perhaps 10–20 cm^3 , while still retaining a good rf power distribution across the complete spectrum (Farrar and Becker, 1971).

2.3. Sample mass and data acquisition times

The actual amount and physical state of a sample required for an experiment naturally varies a great deal, depending on the operating field of the spectrometer employed, the nucleus under investigation, the nature of the data required (e.g. a conventional Fourier Transform (FT) spectrum or relaxation measurements at a variety of temperatures), whether the sample is isotopically enriched (the general case with ^2H), and whether the sample is stable or not. In our laboratory, the general mode of operation is to carry out "short" runs of perhaps 1 min to several hours duration during the daytime, and to carry out larger data acquisitions, such as might typically be used in spin-lattice relaxation time measurements (Kinsey et al., 1981b) or in two-dimensional NMR spectroscopy (Aue et al., 1976) during the night. Such long data acquisitions are normally carried out under computer control, where the computer is programmed to increment the relevant timing instructions, acquire and store data, etc., in the absence of an operator.

Typical ^2H , ^{13}C , ^{14}N and ^{31}P spectra of pure non-sonicated liquid crystalline lipid systems may be obtained with a few minutes of data acquisition when large (~ 300 mg) samples are utilized. For ^2H -labelled samples, we have obtained spectra on as little as 0.5 mg of phospholipid, but data acquisition periods are then extended to several hours.

In the case of intact biological membranes, rapid data acquisition is of course mandatory, except perhaps in the case of the purple membrane of *Halobacterium halobium*, and extraordinarily stable structure (Crespi and Ferraro, 1979; Kinsey et al., 1981a,b). To date, the most rapid NMR spectrum obtained of any intact biomembrane appears to be one recorded of $[\gamma\text{-}^2\text{H}_6]\text{valine}$ -labelled purple membranes in only 1 ms (Kintanar et al., unpublished data). The spectrum is characterized by a quadrupole splitting ($\Delta\nu_Q$) of ~ 39 kHz, consistent with rapid methyl group rotation, and no other fast large-amplitude motions.

More typically, data acquisition periods are in the range approx. 10 min to 10 h. The actual time, as discussed above, depends on the nucleus, enrichment, field strength, and abundance of the residue in the system under investigation. For temperature-dependent spin-lattice relaxation studies data acquisition periods are at least an order of magnitude longer than conventional FT experiments. For such studies, in some instances, numerous membrane samples must be used in order to minimize artefacts arising from changes in sample structure during data acquisition. This will be a likely occurrence in rapidly metabolizing systems such as mitochondrial or *E. coli* membranes (unpublished results), while systems such as the *H. halobium* purple membrane remain quite intact over periods of days, if not longer. We should note that in almost all published studies of biomembrane structure, membranes were examined as "high-speed pellets", i.e. after centrifugation at $\approx 100,000 \times g$ for several hours, samples being transferred to the NMR spectrometer in minimal amounts of buffer, generally under anoxic conditions with no pH-statting, etc. Only a short lifetime, at the growth temperature, seems expected for most systems under such harsh treatment.

2.4. New Fourier Transform Pulse Methods

There have recently been introduced into the lipid and membrane area a number of new FT NMR techniques which now permit rapid acquisition of undistorted ^2H and ^{13}C NMR spectra of non-sonicated membrane systems, in some circumstances under resolution conditions comparable to those found in solution NMR spectroscopy.

The basic success of the FT pulse method lies in the simultaneous excitation of all spins in a molecule, due to application of a short radiofrequency pulse (Fig. 1A). When a

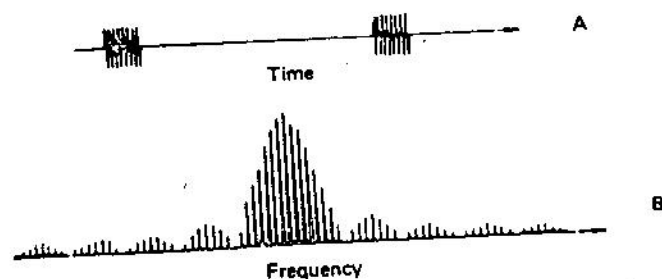


Fig. 1. Figure illustrating radiofrequency pulse (A) in the time-domain; (B) in the frequency-domain. The broad range of frequencies available in a short pulse (typically a few microseconds) permits excitation over a wide spectral breadth (typically tens or hundreds of kilohertz).

continuous sinusoidal radiofrequency signal of frequency ν_0 is switched (or gated) on and off for a time t_p , creating a pulse, additional frequency components covering the approximate range $\nu_0 \pm 1/t_p$ are created, as shown in the Fourier transform of the pulse in Fig. 1B. When applied to a collection of spins in the NMR spectrometer a transient response (or free induction decay) is elicited from the sample, as shown in Fig. 2A

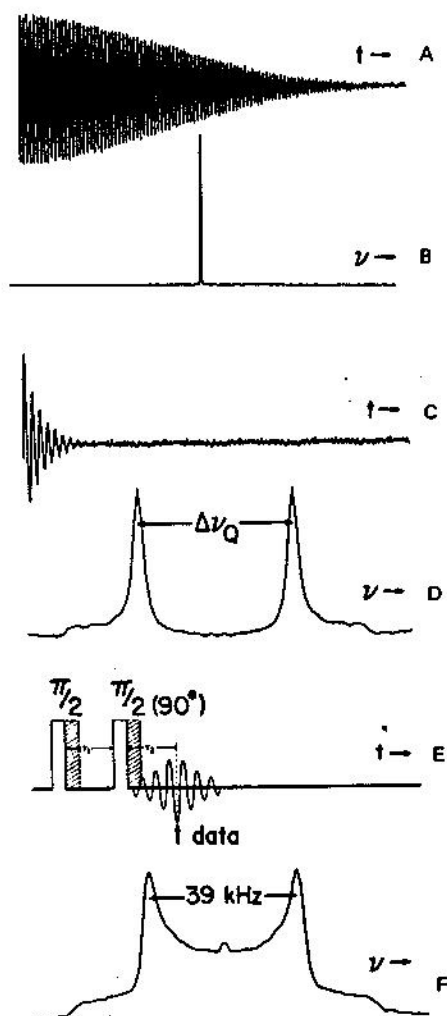


Fig. 2. 55.3 MHz deuterium NMR results on ^2H -labelled amino acids. (A) $[\gamma\text{-}^2\text{H}_3]\text{valine}$ in aqueous solution, time-domain spectrum. (B) same as (A) but after Fourier transformation. (C) $[\gamma\text{-}^2\text{H}_3]\text{valine}$ in the solid-state, time-domain spectrum after single 90° pulse excitation. (D) Sample as (C) but after Fourier transformation. Note lineshape distortion from ideal $I = 1$ ($\eta = 0$) powder pattern. $\Delta\nu_Q = 39$ kHz. (E) Quadrupole-echo pulse sequence ($90^\circ \rightarrow 90^\circ_{\text{echo}}$ -Echo) used to record distortion-free ^2H -NMR spectra; data acquisition begins at echo maximum after receiver recovers from pulse overload. (F) Quadrupole-echo Fourier transform NMR spectrum of solid $[\gamma\text{-}^2\text{H}_3]\text{valine}$: spectrum is not distorted as is spectrum in (D).

for $[\gamma\text{-}^2\text{H}_6]\text{valine}$ in solution. Fourier transformation of this time domain signal gives the conventional frequency-domain absorption spectrum (Fig. 2B). Unfortunately, membranes behave in the NMR experiment as do solids in that the static dipolar, chemical shift anisotropy and quadrupolar interactions are ineffectively averaged, due to the absence of rapid isotropic particle or molecular tumbling. As a result, very broad lines, or rapidly decaying time-domain signals, are obtained, Fig. 2C. When the time constants of such decays are similar to the response time of the instrument (typically 20–30 μs) then important data is "lost" or obscured at the beginning of the free-induction decay, and resultant Fourier transforms have severely distorted lineshapes containing either extreme baseline roll, or more typically spectra with relatively sharp lineshapes, due to loss of the rapidly decaying spectral components (Fig. 2D). Fortunately, however, use of a "spin-echo" pulse method, comprising a 90° pulse followed a few tens of microseconds later by a second, phase-shifted, 90° pulse (Davis et al., 1976) results in formation of a quadrupolar spin echo, as shown in Fig. 2E. The echo appears after the instrument response to the rf pulse has subsided, and the second half of the echo for the spin $I = 1$ ^2H nucleus may then be Fourier transformed to give the normal absorption lineshape, except in the case that the rate of motion of the residue of interest is $\sim (\Delta\nu_Q)^{-1}$, in which case distortions are again introduced (Spiess and Sillescu, 1981). Although this pulse method was first investigated in NMR as early as 1958 by Solomon, using K^{127}I crystals (Solomon, 1958) the first application to the membrane field was made by Davis et al. in 1976. We show in Fig. 2F the Fourier transform of the half echo of Fig. 2E. The resultant frequency-domain spectrum is an essentially undistorted spin $I = 1$, zero asymmetry-parameter powder pattern, having a quadrupole splitting ($\Delta\nu_Q$) of ~ 39 kHz. As we show below, the ability to obtain distortion-free spectral lineshapes in ^2H -NMR is an invaluable asset in deducing information on both the rates and types of motion undergone by the residues in question.

A second relatively new introduction to the membrane area is the application of so-called "magic-angle" sample-spinning methods (Lowe, 1959; Andrew and Eades, 1962) for ^{13}C -NMR, utilizing in addition cross-polarization and high-power proton decoupling (Pines et al., 1973; Schaefer and Stejskal, 1976). Rapid (100,000–200,000 rev./min) sample rotation at the appropriate orientation in the magnetic field has the effect of averaging various magnetic interactions which transform as second rank tensors, e.g. the chemical shift, dipolar, and quadrupolar interactions, with the result that lines are narrowed substantially (Cunningham and Day, 1966; Andrew et al., 1974; Schaefer and Stejskal, 1976; Waugh et al., 1978; Ackerman et al., 1979; Maricq and Waugh, 1979) together with in some instances the formation of well-resolved patterns of spinning sidebands (Waugh et al., 1978; Ackerman et al., 1979). Details may be found in the literature cited above.

We show in Fig. 3A the basic pulse sequence used for high-resolution high-sensitivity ^{13}C -NMR in solids. The method involves two steps: first, a transfer of polarization from the abundant ^1H spin system to the dilute ($\sim 1\%$ natural abundance) ^{13}C spins. This is brought about by means of a double-resonance step in which the energy levels of the ^1H and ^{13}C spins are matched to the so-called Hahn condition (Slichter, 1978), at which point energy may be exchanged between the two, coupled, spin systems. The result (Pines et al., 1973) in our case is a growth of the ^{13}C magnetization. After a suitable

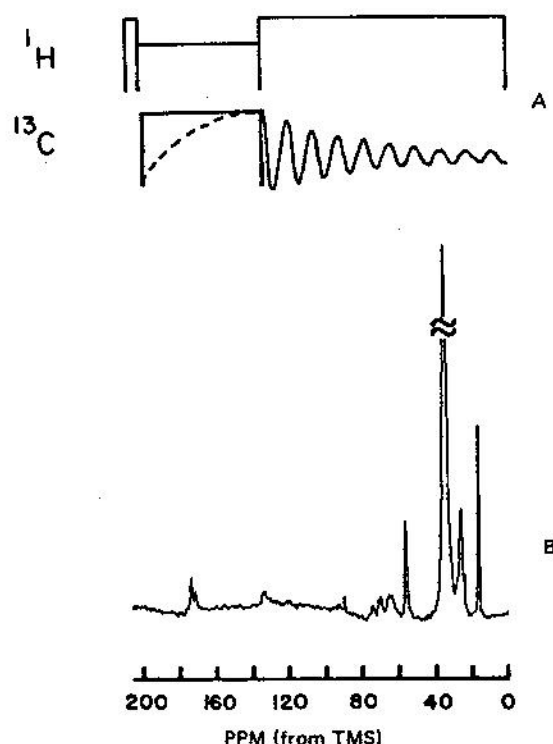


Fig. 3. Carbon-13 cross-polarization NMR pulse sequence and 37.7 MHz ^{13}C -NMR spectrum of DMPC as the anhydrous crystal at $22 \pm 2^\circ\text{C}$, with "magic-angle" sample spinning at 2.2 kHz.

period, typically $\sim 1\text{--}10$ ms, a maximum ^{13}C magnetization is reached, after which the ^{13}C signal begins to decay. The ^{13}C signal intensity, I , depends on the so-called mixing time (t_m , Fig. 3), the ^1H rotating frame spin-lattice relaxation time ($T_{1\rho}$) and the cross-polarization transfer rate constant (T_{CH}) as

$$I \propto \exp(-t_m/T_{1\rho})[1 - \exp(-t_m/T_{CH})] \quad (2)$$

as discussed elsewhere (Mehring, 1976). At the ^{13}C signal-intensity maximum, mixing is halted by turning off the ^{13}C rf field, but the ^1H rf field is kept on (or increased) in order to eliminate dipolar broadening of the ^{13}C resonance by the abundant ^1H spins. The result (Fig. 3A) is that an intense, relatively narrow ^{13}C free induction decay is obtained. The resultant Fourier-transformed frequency-domain spectrum is dominated by the effects of incompletely averaged chemical shift anisotropies, but these may readily be removed by carrying out the entire experiment under conditions of "magic-angle" sample rotation (Schaefer and Stejskal, 1976). A typical high-resolution solid-state ^{13}C -NMR spectrum of a lipid, in this case anhydrous 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), is shown in Fig. 3B, and similar results for hydrated specimens have been reported elsewhere (Haberkorn et al., 1978). Although this technique has not yet had widespread application to the lipid and membrane area, due to the limited

availability of appropriate NMR instrumentation, it is nevertheless quite clear from the results of Fig. 3 and Haberkorn et al. (1978) that it is now possible to obtain high-resolution ^{13}C -NMR spectra of lipids, and labelled biological membranes, without any perturbations other than that of the centrifugal force experienced in the rapidly-spun rotor system. It is therefore to be expected that considerable new insights into the static and dynamic structure of membranes will be made in the near future using this spinning method.

3. Lipids

3.1. Phospholipids

We have outlined above two new developments which permit analysis of phospholipid bilayer structure in some detail. One rather productive application has been in use of the ^2H -NMR spectroscopic method for determination of the conformation of lipid polar headgroups, the glycerol backbone region, and the fatty acyl chains in the liquid crystalline phase (Gally et al., 1975, 1976; Seelig and Gally, 1976; Seelig and Seelig, 1977, 1980; Brown and Seelig, 1977, 1978; Oldfield et al., 1978a; Seelig and Browning, 1978; Skarjune and Oldfield, 1979a,b; Seelig et al., 1980). Using samples of between 100 mg and 500 mg of pure phospholipid it has been shown that the fatty acyl chain conformations of saturated phospholipids are all rather similar, including a bend at the *m*-2' chain position in all phospholipids studied to date (Seelig and Browning, 1978). In addition there have been a number of studies of the lipid polar-headgroup region using a combination of ^2H - and ^{31}P -NMR spectroscopy, and several models for the static and dynamic structure of phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS), and phosphatidylglycerol (PG) headgroups have been presented (see for example Skarjune and Oldfield, 1979a; Seelig and Seelig, 1980). The following general conclusions have been reached: each phospholipid headgroup has its own characteristic set of spectroscopic parameters which is not influenced very much by the structure of the rest of the molecule; PC, PE and PG headgroups have rather similar structures while PS is more rigid, but all are undergoing very restricted motions; in PC and PE the headgroups are little affected by addition of cholesterol, but both are quite sensitive to the presence of di- and trivalent cations (Brown and Seelig, 1977).

In the last 1–2 years, there has been very considerable interest in using the powerful technique of ^2H -NMR spectroscopy to investigate the dynamic structure of the so-called gel phase of lipids. The gel phase of PC is characterized by a 4.1 \AA^{-1} sharp Bragg reflection in X-ray diffraction experiments. The chains in the gel phase are thought to be akin to those of crystalline hydrocarbons in that little off-axis molecular motion is occurring. This rigid nature of the gel phase has made it difficult to investigate using conventional ^1H - or ^2H -NMR methods since the broad lines which characterize this phase result in very poor signal-to-noise ratios, and when using pulse Fourier transform techniques, rapid free induction decays may easily be distorted by instrument response times, as outlined in §2. However, with the introduction of the quadrupole echo pulse technique, it is now possible to obtain undistorted spectra of gel-phase phospholipids. Representative examples are shown in Fig. 4. The spectra of gel phase PC, both in the

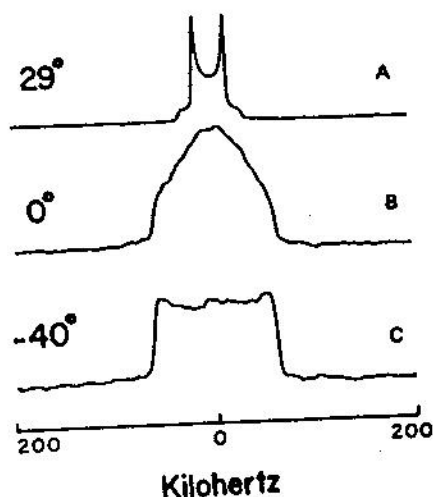
DMPC, $T_c = 23^\circ$ 

Fig. 4. 45.2 MHz deuterium Fourier transform NMR spectra of 1-myristoyl-2-[6',6'- $^2\text{H}_2$]myristoyl-*sn*-glycero-3-phosphocholine (50 wt.% in H_2O) at the temperatures indicated.

$\text{P}\beta'$ and $\text{L}\beta'$ forms, are characterized by rather broad featureless envelopes at or near the phase transition temperature. However, at lower temperatures, typical spin $I = 1$ zero asymmetry parameter spectra with the full rigid lattice breadth (corresponding to a quadrupole coupling constant e^2Qq/h of about 168 kHz) are obtained (Huang et al., 1980; Baianu et al., unpublished data). By contrast, spectra of PE just below the gel to liquid crystal phase transition temperature are characterized by sharp ^2H -NMR spectra having quadrupole splittings $\Delta\nu \sim 63$ kHz (Rice et al., 1981b). The spectra of the gel phase PE originate simply in fast axial hopping of the phospholipid molecule. By contrast, the deuterium spectra of the gel phase PCs are characterized by more complex modes of motion, involving in all probability rapid *gauche-trans* isomerization. A more clear example of such *gauche-trans* isomerization, in which the rate of motion is fast compared with the breadth of the powder pattern, is discussed in §3.2 where ^2H -NMR spectra of glycolipids are reported and analyzed.

Interestingly, similar spectra were reported some 10 years ago for perdeuterated phospholipid bilayers in the gel state, and for *Acholeplasma laidlawii* B membranes at their growth temperature (Oldfield et al., 1971, 1972) but in the earlier work the alternative explanation of a distribution of rotational motions along the sidechain, corresponding to a distribution of quadrupole splittings or order parameters, was put forward. Our current view is that these broad gel phase (or growth temperature) spectra arise from non-zero asymmetry parameter ($\eta \sim 1$) deuterium lineshapes, due to the occurrence of discrete *gauche-trans* isomerization over the tetrahedral angle (Soda and Chiba, 1969; Huang et al., 1980).

In addition, ^{13}C spectra of dipalmitoylphosphatidylcholine, ^{13}C -labelled at the carbonyl position of the *sn*-2 chain, have also been reported (Wittebort et al., 1981). In the

$L\beta'$ phase an axially symmetric spectrum of 112 ppm breadth is observed, and this transforms to an isotropic like line of ~ 7 ppm breadth in the $L\alpha$ phase. In the intermediate ($P\beta'$) phase, a temperature-dependent superposition of these spectra is observed, suggesting that the phase exhibits microscopic structural and dynamical properties of both the $L\beta'$ and $L\alpha$ phases. Analysis of the spectral lineshapes leads to the conclusion that the appearance of the isotropic-like line in the $P\beta'$ phase is primarily due to a conformational change at the *sn*-2 carbonyl, which is complete at the main transition. Increased rates of axial diffusion in the $P\beta'$ phase are also likely to contribute to the narrowing.

There have also been numerous reports of phospholipid hydrocarbon chain, glycerol backbone and headgroup organization in intact biological membranes and their lipid extracts (Metcalf et al., 1972; Burnell et al., 1980; Gally et al., 1980; Nichol et al., 1980; Kang et al., 1981). Typical sample sizes for these experiments are about 100–300 mg dry wt. membranes, which corresponds typically to a $\sim 1\text{-cm}^3$ sample size. In almost all instances reported ^{13}C - or ^2H -labelling has been necessary.

The principal result of these studies of phospholipid conformation in intact biological membranes is that there are very few differences between lipids in model (protein-free) membranes and in intact biological membranes, at least as viewed by the NMR spectroscopic technique.

There have also been a number of solid-state NMR relaxation studies of deuterated lipids using specifically isotopically labelled compounds (Stockton et al., 1977; Davis et al., 1978; Davis, 1979; Brown et al., 1979). Measurement of the spin-lattice relaxation time (T_1) and its frequency and temperature dependence, have allowed a more detailed interpretation of the dynamical structure of phospholipid bilayers, although it is still clear that a more detailed theoretical understanding of relaxation processes in such systems is necessary.

Relaxation experiments involve measurement of very many spectra and, in general, at different temperatures, and consequently this type of project is quite time-consuming.

For more widespread coverage of NMR studies of phospholipids, the reader is encouraged to consult Jacobs and Oldfield (1981).

3.2. Glycolipids

We have discussed above in some detail recent advances in our understanding of the static and dynamic nature of lipids in both model membrane systems and intact biological membranes themselves. Surprisingly, however, there have been very few such studies carried out on glycolipids, which in many instances make up a very sizeable portion of a cell membrane's structure. The likely importance of glycolipids in cell recognition naturally makes them desirable candidates for detailed investigation.

In the liquid crystalline phase, ^2H -NMR spectra of the glycolipid *N*-palmitoylgalactocerebroside, labelled both in the sugar headgroup and in the fatty acyl hydrocarbon chain, have been reported by Skarjune and Oldfield (1979b). Sample size was typically 50 mg, and data acquisition could be accomplished in a few minutes of signal averaging at a moderately high (85 kG) field strength. The results in the liquid crystalline phase were virtually indistinguishable from those obtained with phospholipids in their liquid crystal-

line phase, when compared at the same reduced temperature (Skarjune and Oldfield, 1979b). However, in the crystalline phase of *N*-palmitoylgalactocerebroside, which occurs below about 82°C (Skarjune and Oldfield, 1979b; Ruocco et al., 1981) rather unusual ^2H -NMR lineshapes have been obtained (Huang et al., 1980), which have been shown to originate from non-zero asymmetry parameters due to two (or more) site "hopping" in the gel phase. Such motions are not uncommon in NMR spectroscopy of salt hydrates, where water molecules have been shown to flip about their HOH bisector at very rapid rates (Ketudat and Pound, 1957; Soda and Chiba, 1969). Similarly, such two-site hopping processes have recently been unequivocally demonstrated for benzene ring "flipping" in the solid state in a variety of biological systems (Kinsey et al., 1981a; Schramm et al., 1981; Oldfield et al., 1981b; Rice et al., 1981a). Typical cerebroside gel state spectra are shown in Fig. 5B and C, and are compared with the more normal crystalline (-40°C) and liquid crystalline (85°C) spectra. The "triangular" lineshape seen at 55°C is characteristic of a non-zero asymmetry parameter, generated by the movement of the C-D vector from one site to another across a tetrahedral angle (Soda and Chiba, 1969; Huang et al., 1981) at a rate $\gg \Delta\nu_Q^{-1}$.

Although few spectra have been reported of the low temperature (gel phase) spectra of intact biological membranes (Oldfield et al., 1972; Smith et al., 1979) the broad lineshapes that have been observed may also originate in similar two-site *gauche-trans* hopping, as has been demonstrated in the case of galactocerebroside.

Spectra of a wide variety of headgroup ^2H -labelled galactocerebroside have recently been obtained by Skarjune and Oldfield (unpublished data). Such investigations have

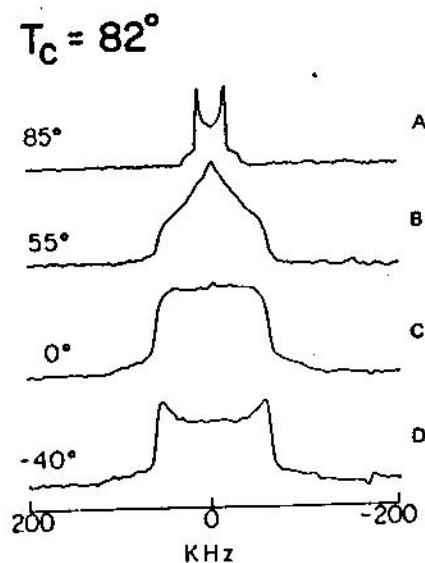


Fig. 5. 45.2 MHz deuterium Fourier transform NMR spectra of $\text{N}[6,6\text{-}^2\text{H}_1]\text{palmitoylgalactosylceramide}$ (50 wt.% in H_2O) at the temperatures indicated. The gel-to-liquid crystal phase transition is at $82 \pm 1^\circ\text{C}$.

involved extensive effort in synthesis of headgroup ^2H -labelled compounds, although spectra may be obtained (on ~ 10 – 20 mg of sample) with only a few minutes of signal averaging, at high field. Analysis of the wide body of ^2H -NMR data currently available has allowed a relatively unequivocal determination of the conformation of the glycolipid headgroup in the liquid crystalline lipid bilayer. The sugar group points out directly into the aqueous region. No corresponding studies have yet been performed on an intact biological membrane, where the effects of protein may modify this lipid headgroup conformation.

4. Sterols

4.1. NMR of sterols in membranes

Despite the wide occurrence of sterols in biological membranes there have been relatively few studies in either model or biological membrane systems aimed at determining the static and dynamic organization of the sterol in the bilayer. Early studies of sterols in membranes were performed by Opella et al. (1976) where the cross-polarization technique discussed above was used to enhance ^{13}C signal intensity. Spectra of $[4\text{-}^{13}\text{C}]$ - and $[26\text{-}^{13}\text{C}]$ cholesterol in lecithin bilayers were reported, and it was shown that the C-8 sidechain of the sterol molecule had considerable rotational freedom while the sterol ring underwent highly anisotropic motion. Similar results have been obtained for the sidechain and the sterol ring using ^2H -NMR. These results are consistent with a picture of sterols in lipid bilayers in which the sterol molecule undergoes rapid axial diffusion (greater than 10^6 s^{-1}), together with a slight off-axis "wobble" motion, which is a function of temperature and lipid bilayer composition (Gally et al., 1976; Oldfield et al., 1978a). Typical values of off-axis fluctuations are discussed by Oldfield et al. (1978a). To date, there have been no reports of cholesterol dynamics in an intact biological membrane, although such experiments are clearly quite feasible.

4.2. Sterol effects on membrane structure

By contrast to the lack of data on the dynamics of sterol molecules themselves in lipid bilayers, there has been an exceedingly large amount of literature published on the effects of sterols on the dynamics of lipid hydrocarbon chains in membranes, including studies of such effects in intact biological membranes themselves. As with most studies discussed above, the principal technique used has been ^2H -NMR spectroscopy. The sample requirements are, as with NMR of phospholipids and glycolipids, ~ 50 – 100 mg of ^2H -labelled material.

The "condensing" effect of cholesterol on lecithin bilayer structures has been investigated extensively by Oldfield et al. (1971, 1978a) and expressions for the condensing effect in terms of changes in hydrocarbon chain length have been presented (Oldfield et al., 1978a). In addition, these workers compared their results with those obtained using neutron diffraction data and showed that excellent agreement between the NMR and neutron data was obtained. For example, the NMR results give a bilayer thickness of about 31 \AA while the neutron results give D about 33 \AA (Oldfield et al.,

1978a), these thicknesses being determined at the level of the C-2 chain segment in a DMPC-30 mol% cholesterol bilayer.

^2H -NMR spectra demonstrating the large effects of cholesterol in intact biological membranes, in particular in the *Acholeplasma laidlawii* B system, have also been reported. Stockton et al. (1977) have shown that there is a dramatic increase in ^2H -NMR spectral breadth for ^2H -labelled lipid hydrocarbon chains in the *A. laidlawii* membrane system on incorporation of cholesterol into the membrane (at a 1 : 2 cholesterol/lipid ratio), corresponding to an increase in effective thickness of the lipid hydrocarbon chain region.

5. Polypeptides

5.1. NMR of polypeptides in membranes

In the last three or four years there have been a number of rather interesting developments in the area of NMR of polypeptides in membranes. Perhaps the most exciting has been the ability to investigate the molecule gramicidin in lipid bilayers. For example, using perdeuterated phospholipids, Feigenson et al. (1977) have shown that it is possible to obtain essentially high resolution proton spectra of some residues (e.g. Trp) of gramicidin A' in sonicated lipid vesicles. Perhaps even more exciting than this has been the work by Weinstein et al. (1980) aimed at determining unequivocally the organization of the gramicidin A dimer in lipid bilayer vesicles — is it single- or double-stranded, head-to-head, head-to-tail, or what (Urry, 1971; Urry et al., 1972; Glickson et al., 1972; Veatch et al., 1974)? By synthesis of a variety of selectively ^{13}C -labelled gramicidins, together with nitroxide and manganese-ion paramagnetic-broadening experiments, Weinstein et al. (1980) have shown unequivocally that it is the head-to-head configuration of the gramicidin dimer originally proposed by Urry that correctly describes gramicidin A organization in synthetic lipid vesicles. Such experiments were carried out using sonicated lipid vesicles, an approach which in the past has been criticized because of the possibility of subtle structural differences between small single-bilayer vesicles and multilamellar liposomes. Nevertheless, such ^{13}C -NMR studies could also probably be carried out using the "magic-angle" sample-spinning technique described above, with no need to produce sonicated vesicles. There is clearly therefore much further work to be done in investigating polypeptide structure in lipid membranes using such ^{13}C -labelling methods. In the studies described only relatively small (a few milligram) quantities of the labelled polypeptides (or ^1H polypeptide) were necessary to obtain data in relatively short periods (a few hours) of data acquisition.

5.2. Polypeptide effects on membrane structure

There have been relatively few recent advances in our understanding of the effects of polypeptides on lipid static and dynamic structure in model (or biological) membrane systems. Oldfield et al. have reported the effects of gramicidin A' incorporation into non-sonicated lipid bilayer systems using ^2H -NMR of selectively labelled lipids (Rice and Oldfield, 1979) and natural abundance N-14 (Rothgeb and Oldfield, 1981) and ^{31}P -NMR (Rajan et al., 1981) spectroscopy of the lipid constituents. The results are rather complex. Incorporation of gramicidin into a dimyristoylphosphatidylcholine

bilayer system results in a dramatic linebroadening in the ^2H -NMR spectra of chain-labelled species, together with a rapid increase in the rate of decay of the quadrupole-echo amplitude, consistent with the onset of additional large amplitude motions causing more effective relaxation. Methyl-group quadrupole-splittings were, however, collapsed, but this is again consistent with the onset of large amplitude motions in the hydrocarbon chain, perhaps due to the presence of a "rough" polypeptide surface. Similarly, collapse of a ^{31}P chemical shift powder pattern was obtained by Rajan et al. (1981), again consistent with the onset of a large amplitude motion in the lipid molecule. N-14 NMR spectra (Rothgeb and Oldfield, 1981) exhibited a loss of N-14 headgroup quadrupole coupling, with formation of an isotropic line, which was, however, far broader than that obtained with ^{31}P -NMR, due presumably to the large magnitude of the N-14 quadrupole interaction.

These experiments might also be interpreted in terms of formation of relatively small aggregates of molecules which could tumble rapidly, and thereby average the appropriate interactions, or by more rapid lateral diffusion of lipid molecules on addition of polypeptide. The formation of small particles seems, however, to be eliminated by analysis of freeze-fracture electron micrographs, which show large extended sheet structures (Chapman et al., 1977), together with the observation of sharp lamellar X-ray diffraction patterns from these gramicidin lipid systems (Chapman et al., 1977; Shipley and Oldfield, unpublished data). Clearly, more experiments are required in order to prove or disprove one or more possible mechanisms of linebroadening, and collapse, in the lipid-gramicidin system. Data acquisition periods in the ^2H -labelled lipid bilayer systems are very rapid, taking a few seconds or minutes when operating with ~ 200 mg of lipid at high-field (≈ 8.5 T).

6. Proteins

6.1. NMR of proteins in membranes

Although it is to be expected that the NMR of proteins in biological membranes is likely to be a rather demanding task experimentally, since individual types of amino acid will be very dilute (a single amino-acid resonances will be even more so), there has, nevertheless, been considerable interest in obtaining the NMR spectra of proteins in intact cell membranes. After all, it is the proteins which are the active catalysts, the enzymes, which are involved in all of the interesting reactions carried out by membranes, such as respiration, photosynthesis, vision, nerve impulse conduction and cell-cell recognition.

The first informative NMR studies of proteins in intact cell membranes have been reported recently (Oldfield et al., 1981a,b; Kinsey et al., 1981a,b; Schramm et al., 1981; Rice et al., 1981a). Despite fears to the contrary, we have shown that it is indeed relatively straightforward to obtain well-resolved high signal-to-noise ratio spectra of individual types of amino acid residue in intact biological membranes. As a first system, we and others have investigated the purple membrane of *Halobacterium halobium* R₁. This membrane is particularly exciting and suitable for study by NMR spectroscopy since there is only one protein in the purple membrane, bacteriorhodopsin, the protein has a known

sequence (Gerber et al., 1979; Ovchinnikov et al., 1979; Walker et al., 1979), a relatively low molecular weight of about 27,000, and its three-dimensional structure is beginning to become known (Henderson and Unwin, 1975; Engelman and Zaccai, 1980).

We show in Fig. 6 a typical ^2H -NMR spectrum of valine methyl groups in the purple membrane of *H. halobium*. In this case the sample size was again about 0.8 cm^3 , corresponding to a dry weight of $\sim 50\text{ mg}$, of which $\sim 80\%$ is the purple membrane protein, bacteriorhodopsin. Note that the spectrum of Fig. 6 was obtained in the rather short time period of 260 ms, and using more advanced instrumentation, we have recently obtained similar results in 1 ms. Unfortunately, the spectrum represents the superposition of resonances from all of the 26 valines in the purple membrane! Nevertheless, the purple membrane may be oriented by means of electric (Keszthelyi, 1980) and magnetic fields (Neugebauer et al., 1977; Rice et al., 1981a), or by mechanical sample ordering (Henderson and Unwin, 1975) so there are good reasons to believe that in the future it will be possible to obtain "single" crystal spectra of such oriented arrays, with concomitant resolution of individual sites in the membrane protein.

^2H -NMR results with the *H. halobium* purple membrane have been obtained by our group for a wide variety of deuterated amino acids, including glycine, alanine, valine, leucine, serine, threonine, phenylalanine, tyrosine, tryptophan and methionine (Kinsey et al., 1981a,b; Schramm et al., 1981; Oldfield et al., 1981a,b). The overall picture obtained of amino acid dynamics in this membrane protein is that the protein has a relatively rigid structure. Methyl groups in all cases rotate rapidly (10^{10} – 10^{11} s^{-1}) at room temperature. Motions about C^α – C^β in all instances (except for alanine) are slow on the timescale of the NMR experiment, say $\lesssim 10^4\text{ s}^{-1}$. Motion about C^β – C^γ in the case of the small sidechains threonine and valine is again fast, because of methyl rotation. However, motion about C^β – C^γ for the bulkier residues phenylalanine and tyrosine is much slower, $\gtrsim 10^6$ – 10^8 s^{-1} at the growth temperature of 37°C . In addition this motion clearly occurs as a discrete process whereby the benzene ring "flips" between one planar conformation and another (Schramm et al., 1981; Kinsey et al., 1981b; Oldfield et al., 1981a,b; Rice

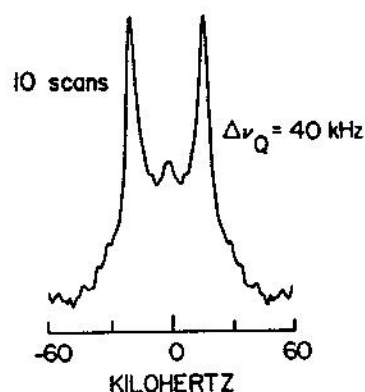


Fig. 6. 55.3 MHz deuterium Fourier transform NMR spectrum of $[\gamma\text{-}^2\text{H}_3]$ valine labelled bacteriorhodopsin in the purple membrane of *Halobacterium halobium* R₁, recorded in 260 ms.

et al., 1981a) i.e. it "flips" as a coin would between a "heads" and "tails" configuration. This type of rapid flipping process has been postulated previously in solution NMR spectra of proteins by Williams and coworkers and by Wuthrich and coworkers studying, amongst others, the proteins bovine pancreatic trypsin inhibitor (Wagner et al., 1976), cytochrome *c* and lysozyme (Williams, 1978). In the solid state, analysis of the ^2H -NMR lineshapes (Fig. 7) shows that the reorientational process is unequivocally a two-site flip, with the time taken to achieve the flip being much less than the actual residence time in a given conformational state. Theoretical studies of such "infrequent" motions have been carried out by Karplus and associates and a short account of the results has been reviewed recently (McCammon and Karplus, 1980).

On increasing the size of the hydrocarbon sidechain on going from phenylalanine to tryptophan, the increased bulk precludes any rapid, large-amplitude sidechain motion. As a result, motion about $\text{C}^\beta\text{--C}^\gamma$ in the tryptophan sidechain can reasonably be neglected, both $\text{C}^\alpha\text{--C}^\beta$ and $\text{C}^\beta\text{--C}^\gamma$ bonds of tryptophan being properly thought of as "rigid" in the purple membrane protein.

The ability to obtain, in a reasonable period of time (typically about 10 min) high quality spectra of individual types of amino acid residue in a defined membrane protein *in vivo* clearly ushers in a new era, in which very detailed studies of the static and dynamic structure of membrane enzymes may be carried out.

A particularly exciting area for future work involves the dissection of the exact nature of the effects of lipids on the dynamic structure of membrane proteins, determining for example the effects of lipid unsaturation, polar headgroup structure, and the presence of cholesterol, on the rates and types of motions of the various amino acid sidechains.

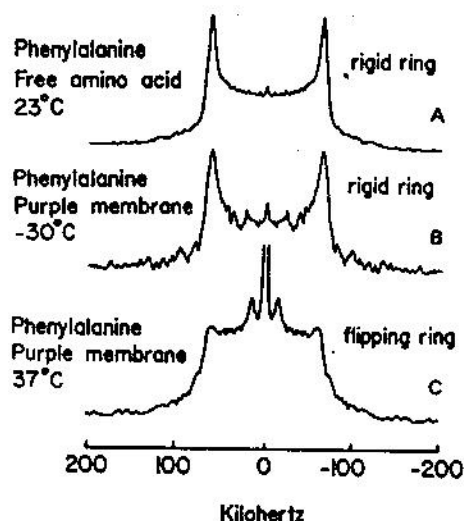


Fig. 7. 55.3 MHz deuterium Fourier transform NMR spectra of $[\delta_1, \delta_2, \epsilon_1, \epsilon_2, 5\text{-}^2\text{H}_1]$ phenylalanine: (A) free amino acid at 23°C; (B) labelled purple membrane at -30°C; (C) labelled purple membrane at 37°C (the growth temperature). The asymmetry parameters (η) in (A) (B) are ~ 0 and indicate that the Phe ring is immobile. The asymmetry parameter in (C) is ~ 0.66 , indicating that the Phe rings are undergoing rapid 2-fold "flipping" motions.

The availability of this type of information should also help interpret amino acid side-chains dynamics in crystalline proteins.

6.2. Protein effects on membrane structure

The nature of protein-lipid interaction in biological membranes, and in reconstituted lipid-protein systems, has been plagued by considerable controversy over the past 10 years. The original picture of protein-lipid interaction in biological membranes was put forward by Jost et al. (1973). Using electron spin resonance (ESR) spin-label techniques these authors showed, at least at high protein/lipid ratios, that lipid spin-labels were "immobilized" on the surface of several membrane proteins. To be "immobilized" in this instance means that there is no rapid large amplitude motion occurring on a timescale of $\sim 10^{-9}$ s. The interpretation of the ESR results is that the lipid molecules are "stuck" on the protein surface. Lipid titration and integrated intensity experiments indicated that only the first layer of lipid adjacent to protein is immobilized strongly in this way. These experiments were interpreted by numerous theoretical groups in terms of an "ordering" or "orienting" effect of protein on lipid-bilayer boundary lipid, the theoreticians treating the protein as a rigid rod or hexagonal cylinder plunging through the lipid bilayer, thereby prohibiting the normal flexing of the lipid hydrocarbon chains characteristic of liquid crystalline phase phospholipids. That is to say the proteins were assumed to have cholesterol-like condensing effects on lipid bilayer structure. However, more recent ^2H -NMR spectroscopic studies of these systems (Oldfield et al., 1978b; Seelig and Seelig, 1978) have given no evidence for such ordering of lipid hydrocarbon chains by any of the following proteins: myelin proteolipid apoprotein, cytochrome oxidase, cytochrome b_5 , bacteriophage coat protein, or sarcoplasmic reticulum ATPase. The observed spectra of protein-lipid recombinants are in almost all instances virtually identical to those of the pure lipid bilayer, whereas those of lipid-cholesterol systems are typically a factor of 2 wider, i.e. the lipid hydrocarbon chains in lipid-cholesterol systems are approximately twice as ordered as those in a pure lipid bilayer, or protein/lipid (2 : 1, w/w) complex. This has naturally led to a model in which there is essentially no strong lipid-protein interaction. In particular, there is no strong ordering of lipid hydrocarbon chains by protein surfaces. This, of course, seems to be a reasonable view given the observation that in no instance has a membrane protein been shown to resemble a hexagonal rod!

In many systems it is likely that there is rapid exchange between free bilayer lipid and lipid associated with the protein surface (Seelig and Seelig, 1978; Kang et al., 1979a,b; Bloom, 1980) and that the conformation of lipid hydrocarbon chains adjacent to the protein surface is only slightly different to that in the pure lipid bilayer. If anything, lipid associated with the protein surface is somewhat disordered by the rough or irregular nature of the protein surface, due to the wide variety of amino acids typically found in the interior of a membrane bilayer. However, it should be pointed out that only relatively few lipid-protein systems have been investigated to date, and undoubtedly future studies will show clear preferences of proteins for particular types of lipids, and that strong interaction (especially those involving Coulombic interactions between lipid headgroups and charged residues on protein surfaces) will be demonstrated to have

great significance in the mechanism of action of some membrane proteins. However, to date, the evidence for such interactions is relatively sparse, although Oldfield et al. (unpublished data) have obtained very broad spectra of cardiolipin in the presence of cytochrome *c* oxidase, indicating a strong interaction in this particular system. Interestingly, such a strong interaction was not observed for the phosphatidic acid/cytochrome *c* oxidase system.

The current picture, as obtained by ^2H -NMR spectroscopy, is that in most instances investigated there are no strong interactions between membrane proteins and membrane lipids. Indeed, it would be difficult to know, from a physical standpoint, what such interactions would be. There seems to be no compelling evidence to believe that van der Waals interaction between one lipid and another lipid or one lipid and a protein surface would be appreciably different, so that the most likely candidate forces for strong lipid-protein interaction are the Coulombic, or polar headgroup-protein interactions discussed above for the cardiolipin/cytochrome *c* oxidase system.

Some workers have suggested that trapping of lipids at high protein concentration may be important in affecting the dynamics of the lipid hydrocarbon chains (Chapman et al., 1979). However, in most biomembrane systems protein/lipid ratios of the order of about 1 : 1 are obtained, and using ^2H -NMR spectroscopy at 1 : 1 protein lipid ratios there is relatively little effect, on the average, of protein on lipid hydrocarbon chain organization. In short, the current view of protein lipid interaction by NMR spectroscopy is that proteins have little effect on lipid hydrocarbon chain or polar headgroup structure. The major effect seen is a slight increase in line broadening in ^2H and ^{31}P spectra together with a decrease in ^{31}P - and ^2H -NMR spin-lattice relaxation times. These two processes can be accounted for by a decrease in the rate of motion of the individual residues, but there is not necessarily any large change in the amplitude of the motion.

7. Summary and view of the future

In this short article we have tried to indicate some of the most exciting recent developments in the application of the NMR spectroscopic technique to the study of the static and dynamic structure of lipids and proteins in model and biological membrane systems. Most advances have been brought about by improvements in NMR instrumentation, and there is extraordinary promise for further developments in this area with the design and construction of new very-high-field superconducting magnets, which eventually will reach the 800–1000 MHz range for protons. Although this very high frequency may provide problems for proton NMR spectroscopists, there are few (if any) of these remaining in the membrane NMR area! The nuclei of choice are currently ^2H and ^{13}C , which will have resonance frequencies of about 150 MHz and 250 MHz at ~ 23 T, relatively simple frequencies with which to deal from an rf instrumentation standpoint.

The main interesting biochemical advances in the past 2–3 years are as follows. In the area of protein-lipid interaction, new models for the effects of proteins on lipid conformation have been put forward, and most workers in the NMR area now agree that there is no extensive ordering of lipid molecules caused by proteins, as does the small sterol cholesterol. There is now also considerable new insight into the motions of gel-state phospholipids and glycolipids, with the occurrence of two site *gauche-trans*

isomerization processes being essentially proven. In the area of NMR of membrane proteins themselves (and proteins in other condensed phases) there has been considerable activity by several groups in the last 1–2 years, and on a very rudimentary level we now have a picture of the dynamics of many of the naturally occurring amino acids found in membrane proteins. Such results are being analyzed and compared with results obtained on crystalline proteins (Schramm et al., 1981 and unpublished data) and proteins in solution, so that in the future it should be possible to make detailed comparisons between protein dynamics in solution, in membranes and in the crystalline solid state. Given the recent developments in X-ray crystallographic methods to study such dynamical processes in single protein crystals (Frauenfelder et al., 1979; Artymniuk et al., 1979), it is likely that in the next 5 years there will become available a most detailed knowledge of the static and dynamic structure of proteins in a wide variety of environments, including biological membranes.

Finally, I would again like to emphasize that these exciting new developments will only become possible with the introduction (and funding) of new high field superconducting magnets. This point is well illustrated by the following: in 1971 we obtained the first ^2H -NMR spectra of lipids (Oldfield et al., 1971). These spectra were obtained at 8 MHz and required large quantities (~ 100 mg) of perdeuterated phospholipids. Only moderate signal-to-noise ratios and spectral resolution were obtained. By contrast, 10 years later in 1981 we have obtained in only 1 ms, spectra of specifically labelled membrane proteins, a feat only made possible by the availability of much higher operating field strengths, in this case 8.5 T (360 MHz ^1H resonance frequency). Clearly, availability of even higher field instrumentation will permit acquisition of time-resolved spectral data, including, for example, studies of conformational changes upon illumination of photosynthetic membranes, and perhaps for some systems studies of the actual rates and types of motion of individual amino acid residues in membrane enzymes during the catalytic process.

References

- Ackerman, J.L., Eckman, R. and Pines, A. (1979) Experimental results on deuterium NMR in the solid state by magic angle sample spinning. *Chem. Phys.* 42, 423–428.
- Andrew, E.R. and Eades, R.G. (1962) Possibilities for high-resolution nuclear magnetic resonance of crystals. *Discuss. Faraday Soc.* 34, 38–42.
- Andrew, E.R., Hinshaw, W.S. and Tiffen, R.S. (1974) NMR in rapidly rotated metallic aluminium and cadmium. *J. Magn. Reson.* 15, 191–195.
- Artymniuk, P.J., Blake, C.C.F., Grace, D.E.P., Oatley, S.J., Phillips, D.C. and Sternberg, M.J.E. (1979) Crystallographic studies of the dynamic properties of lysozyme. *Nature* 280, 563–568.
- Aue, W.P., Bartholdi, E. and Ernst, R.R. (1976) Two-dimensional spectroscopy. Application to nuclear magnetic resonance. *J. Chem. Phys.* 64, 2229–2246.
- Bloom, M. (1980) In: 9th International Conference on Magnetic Resonance in Biological Systems, Abstract L22, Bender, France.
- Brown, M.F. and Seelig, J. (1977) Ion-induced changes in head group conformation of lecithin bilayers. *Nature* 269, 721–723.
- Brown, M.F. and Seelig, J. (1978) Influence of cholesterol on the polar region of phosphatidylcholine and phosphatidylethanolamine bilayers. *Biochemistry* 17, 381–384.
- Brown, M.F., Seelig, J. and Häberlen, U. (1979) Structural dynamics in phospholipid bilayers from deuterium spin-lattice relaxation time measurements. *J. Chem. Phys.* 70, 5045–5053.

- Burnell, E., Van Alphen, L., Verkleij, A. and de Kruijff, B. (1980) ^{31}P Nuclear magnetic resonance and freeze-fracture electron microscopy studies on *Escherichia coli*. I. Cytoplasmic membrane and total phospholipids. *Biochim. Biophys. Acta* 597, 492-501.
- Chapman, D., Cornell, B.A., Elias, A.W. and Perry, A. (1977) Interactions of helical polypeptide segments which span the hydrocarbon region of lipid bilayers. Studies of the gramicidin A lipid-water system. *J. Mol. Biol.* 113, 517-538.
- Chapman, D., Gómez-Fernández, J.C. and Goñi, F.M. (1979) Intrinsic protein-lipid interactions. Physical and biochemical evidence. *FEBS Lett.* 98, 211-223.
- Clark, W.G. (1964) Pulsed nuclear resonance apparatus. *Rev. Sci. Instrum.* 35, 316-333.
- Crespi, H.L. and Ferraro, J.R. (1979) Active site structure of bacteriorhodopsin and mechanism of action. *Biochem. Biophys. Res. Commun.* 91, 575-582.
- Cunningham, A.C. and Day, S.M. (1966) Pulsed nuclear magnetic resonance of Na^{23} in rapidly rotated NaCl. *Phys. Rev.* 152, 287-292.
- Davis, J.H. (1979) Deuterium magnetic resonance study of the gel and liquid crystalline phases of dipalmitoyl phosphatidylcholine. *Biophys. J.* 27, 339-358.
- Davis, J.H., Jeffrey, K.R., Bloom, M., Valic, M.I. and Higgs, T.P. (1976) Quadrupolar echo deuterium magnetic resonance spectroscopy in ordered hydrocarbon chains. *Chem. Phys. Lett.* 42, 390-394.
- Davis, J.H., Jeffrey, K.R. and Bloom, M. (1978) Spin-lattice relaxation as a function of chain position in perdeuterated potassium palmitate. *J. Magn. Reson.* 29, 191-199.
- Engelman, D. M. and Zaccai, G. (1980) Bacteriorhodopsin is an inside-out protein. *Proc. Natl. Acad. Sci. U.S.A.* 77, 5894-5898.
- Farrar, T.C. and Becker, E.D. (1971) Pulse and Fourier transform NMR. Academic Press, New York.
- Feigenson, G.W., Meers, P.R. and Kingsley, P.B. (1977) NMR observation of gramicidin A' in phosphatidylcholine vesicles. *Biochim. Biophys. Acta* 471, 487-491.
- Frauenfelder, H., Petsko, G.A. and Tsernoglou, D. (1979) Temperature-dependent X-ray diffraction as a probe of protein structural dynamics. *Nature* 280, 558-563.
- Gally, H.U., Niederberger, W. and Seelig, J. (1975) Conformation and motion of the choline head group in bilayers of dipalmitoyl-3-*sn*-phosphatidylcholine. *Biochemistry* 14, 3647-3652.
- Gally, H.U., Seelig, A. and Seelig, J. (1976) Cholesterol-induced rod-like motion of fatty acyl chains in lipid bilayers. A deuterium magnetic resonance study. *Hoppe-Seyler's Z. Physiol. Chem.* 357, 1447-1450.
- Gally, H.U., Pluschke, G., Overath, P. and Seelig, J. (1980) Structure of *Escherichia coli* membranes. Fatty acyl chain order parameters of inner and outer membranes and derived liposomes. *Biochemistry* 19, 1638-1643.
- Gerber, G.E., Anderegg, R.J., Herlihy, W.C., Gray, C.P., Biemann, K. and Khorana, H.G. (1979) Partial primary structure of bacteriorhodopsin: sequencing methods for membrane proteins. *Proc. Natl. Acad. Sci. U.S.A.* 76, 227-231.
- Glickson, J.D., Mayers, D.F., Settine, J.M. and Urry, D.W. (1972) Spectroscopic studies on the conformation of gramicidin A'. Proton magnetic resonance assignments, coupling constants, and H-D exchange. *Biochemistry* 11, 477-486.
- Haberkorn, R.A., Herzfeld, J. and Griffin, R.G. (1978) High-resolution ^{31}P and ^{13}C nuclear magnetic resonance spectra of unsaturated model membranes. *J. Am. Chem. Soc.* 100, 1296-1298.
- Henderson, R. and Unwin, P.N.T. (1975) Three-dimensional model of purple membrane obtained by electron microscopy. *Nature* 257, 28-32.
- Huang, T.H., Skarjune, R.P., Wittebort, R.J., Griffin, R.G. and Oldfield, E. (1980) Restricted rotational isomerization in polymethylene chains. *J. Am. Chem. Soc.* 102, 7377-7379.
- Jacobs, R. and Oldfield, E. (1981) NMR of membranes. *Prog. Nucl. Magn. Reson. Spectrosc.* 14, 113-136.
- Jost, P.C., Griffith, O.H., Capaldi, R.A. and Vanderkooi, G. (1973) Evidence for boundary lipid in membranes. *Proc. Natl. Acad. Sci. U.S.A.* 70, 480-484.
- Kang, S.Y., Gutowsky, H.S., Hsung, J.C., Jacobs, R., King, T.E., Rice, D. and Oldfield, E. (1979a) Nuclear magnetic resonance investigation of the cytochrome oxidase-phospholipid interaction: a new model for boundary lipid. *Biochemistry* 18, 3257-3267.

- Kang, S.Y., Kinsey, R.A., Gabridge, M.G., Rajan, S., Gutowsky, H.S. and Oldfield, E. (1981) High-field deuterium NMR of specifically labelled *Acholeplasma laidlawii* B and *Escherichia coli* membranes. *J. Biol. Chem.* 256, 1155-1159.
- Kang, S.Y., Gutowsky, H.S. and Oldfield, E. (1979b) Spectroscopic studies of specifically deuterium labelled membrane systems. Nuclear magnetic resonance investigation of protein-lipid interactions in *Escherichia coli* membranes. *Biochemistry* 18, 3268-3271.
- Keszthelyi, L. (1980) Orientation of membrane fragments by electric field. *Biochim. Biophys. Acta* 598, 429-436.
- Ketudat, S. and Pound, R.V. (1957) Electric quadrupole interactions of deuterons and molecular motion in $\text{Li}_2\text{SO}_4 \cdot \text{D}_2\text{O}$. *J. Chem. Phys.* 26, 708-709.
- Kinsey, R.A., Kintanar, A. and Oldfield, E. (1981a) Dynamics of amino acid side chains in membrane proteins by high field deuterium nuclear magnetic resonance spectroscopy. Phenylalanine, tyrosine and tryptophan. *J. Biol. Chem.* 256, 9028-9036.
- Kinsey, R.A., Kintanar, A., Tsai, M.-D., Smith, R.L., Janes, N. and Oldfield, E. (1981b) First observation of amino acid side chain dynamics in membrane proteins using high field deuterium nuclear magnetic resonance spectroscopy. *J. Biol. Chem.* 256, 4146-4149.
- Lowe, I.J. (1959) Free induction decays of rotating solids. *Phys. Rev. Lett.* 2, 285-287.
- Maricq, M.M. and Waugh, J.S. (1979) NMR in rotating solids. *J. Chem. Phys.* 70, 3300-3316.
- McCammon, J.A. and Karplus, M. (1980) Simulation of protein dynamics. *Annu. Rev. Phys. Chem.* 31, 29-45.
- Mehring, M. (1976) In: P. Diehl, E. Fluck and R. Kosfeld (Eds.), *NMR-Basic Principles and Progress. High Resolution NMR Spectroscopy in Solids*. Vol. 11, Springer-Verlag, New York.
- Metcalfe, J.C., Birdsall, N.J.M. and Lee, A.G. (1972) ^{13}C NMR spectra of *Acholeplasma* membranes containing ^{13}C labelled phospholipids. *FEBS Lett.* 21, 335-340.
- Neugebauer, D.-Ch., Blaurock, A.E. and Worcester, D.L. (1977) Magnetic orientation of purple membranes demonstrated by optical measurements and neutron scattering. *FEBS Lett.* 78, 31-35.
- Nichol, C.P., Davis, J.H., Weeks, G. and Bloom, M. (1980) Quantitative study of the fluidity of *Escherichia coli* membranes using deuterium magnetic resonance. *Biochemistry* 19, 451-457.
- Oldfield, E., Chapman, D. and Derbyshire, W. (1971) Deuteron resonance: A novel approach to the study of hydrocarbon chain mobility in membrane systems. *FEBS Lett.* 16, 102-104.
- Oldfield, E., Chapman, D. and Derbyshire, W. (1972) Lipid mobility in *Acholeplasma* membranes using deuteron magnetic resonance. *Chem. Phys. Lipids* 9, 69-81.
- Oldfield, E., Meadows, M., Rice, D. and Jacobs, R. (1978a) Spectroscopic studies of specifically deuterium labelled membrane systems. Nuclear magnetic resonance investigation of the effects of cholesterol in model systems. *Biochemistry* 17, 2727-2740.
- Oldfield, E., Gilmore, R., Glaser, M., Gutowsky, H.S., Hshung, J.C., Kang, S.Y., King, T.E., Meadows, M. and Rice, D. (1978b) Deuterium nuclear magnetic resonance investigations of the effects of proteins and polypeptides on hydrocarbon chain order in model membrane systems. *Proc. Natl. Acad. Sci. U.S.A.* 75, 4657-4660.
- Oldfield, E. and Meadows, M. (1978) Sideways-spinning 20-mm-tube probe for widebore superconducting magnet spectrometer systems. *J. Magn. Reson.* 31, 327-335.
- Oldfield, E., Janes, N., Kinsey, R., Kintanar, A., Lee, R.W.K., Rothgeb, T.M., Schramm, S., Skarjune, R., Smith, R. and Tsai, M.-D. (1981a) Protein crystals, membrane proteins and membrane lipids. Recent advances in the study of their static and dynamic structures using nuclear magnetic resonance spectroscopic techniques. *Biochem. Soc. Trans.* 46, 155-181.
- Oldfield, E., Kinsey, R.A. and Kintanar, A. (1981b) Recent advances in the study of bacteriorhodopsin dynamic structure using high-field nuclear magnetic resonance spectroscopy. *Methods Enzymol.*, in press.
- Opella, S.J., Yeznowski, J.P. and Waugh, J.S. (1976) Nuclear magnetic resonance description of molecular motion and phase separations of cholesterol in lecithin dispersions. *Proc. Natl. Acad. Sci. U.S.A.* 73, 3812-3815.
- Ovchinnikov, Y.A., Abdulaev, N.G., Feigina, M.Y., Kiselev, A.V. and Lobanov, N.A. (1979) The structural basis of the functioning of bacteriorhodopsin: an overview. *FEBS Lett.* 100, 219-224.
- Pines, A., Gibby, M.G. and Waugh, J.S. (1973) Proton-enhanced NMR of dilute spins in solids. *J. Chem. Phys.* 59, 569-590.

- Rajan, S., Kang, S.-Y., Gutowsky, H.S. and Oldfield, E. (1981) Phosphorus nuclear magnetic resonance study of membrane structure. Interactions of lipids with protein, polypeptide and cholesterol. *J. Biol. Chem.* 256, 1160-1166.
- Rice, D. and Oldfield, E. (1979) Deuterium nuclear magnetic resonance studies of the interaction between dimyristoylphosphatidylcholine and gramicidin A'. *Biochemistry* 18, 3272-3279.
- Rice, D.M., Herzfeld, J. and Griffin, R.G. (1981a) Solid state NMR studies of purple membrane. *Biophys. J.* 33, 103a.
- Rice, D.M., Blume, A., Herzfeld, J., Wittebort, R.J., Huang, T.H., DasGupta, S.K. and Griffin, R.G. (1981b) Solid-state NMR investigations of lipid bilayers, peptides and proteins. In: R.H. Sarma (Ed.), *Proceedings of the Symposium: Biomolecular Stereodynamics*, Vol. II, Adenine Press, New York, pp. 255-270.
- Rothgeb, T.M. and Oldfield, E. (1981) Nuclear magnetic resonance of heme protein crystals. *J. Biol. Chem.* 256, 1432-1446.
- Ruocco, M.J., Atkinson, D., Small, D.M., Skarjune, R.P., Oldfield, E. and Shipley, G.G. (1981) X-ray diffraction and calorimetric study of anhydrous and hydrated N-palmitoyl galactosyl ceramide (cerebroside). *Biochemistry* 20, 5957-5966.
- Schaefer, J. and Stejskal, E.O. (1976) Carbon-13 nuclear magnetic resonance of polymers spinning at the magic angle. *J. Am. Chem. Soc.* 98, 1031-1032.
- Schramm, S., Kinsey, R.A., Kintanar, A., Rothgeb, T.M. and Oldfield, E. (1981) Deuterium NMR of proteins in solution, in membranes and in the crystalline solid state. In: R.H. Sarma (Ed.), *Proceedings of the Symposium: Biomolecular Stereodynamics*, Vol. II, Adenine Press, New York, pp. 271-286.
- Seelig, A. and Seelig, J. (1977) Effect of a single cis double bond on the structure of a phospholipid bilayer. *Biochemistry* 16, 45-50.
- Seelig, A. and Seelig, J. (1978) Lipid-protein interaction in reconstituted cytochrome c oxidase/phospholipid membranes. *Hoppe-Seyler's Z. Physiol. Chem.* 359, 1747-1756.
- Seelig, J. and Browning, J.L. (1978) General features of phospholipid conformation in membranes. *FEBS Lett.* 92, 41-44.
- Seelig, J. and Gally, H.U. (1976) Investigation of phosphatidylethanolamine bilayers by deuterium and phosphorus-31 nuclear magnetic resonance. *Biochemistry* 15, 5199-5204.
- Seelig, J. and Seelig, A. (1980) Lipid conformation in model membranes and biological membranes. *Q. Rev. Biophys.* 13, 19-61.
- Seelig, J., Dijkman, R. and de Haas, G.H. (1980) Thermodynamic and conformational studies on *sn*-2-phosphatidylcholines in monolayers and bilayers. *Biochemistry* 19, 2215-2219.
- Siminovitch, D.J., Rance, M. and Jeffrey, K.R. (1980) The use of wide-line [^{14}N]nitrogen NMR as a probe in model membranes. *FEBS Lett.* 112, 79-82.
- Skarjune, R. and Oldfield, E. (1979a) Physical studies of cell surface and cell membrane structure. Determination of phospholipid head group organization by deuterium and phosphorus nuclear magnetic resonance spectroscopy. *Biochemistry* 18, 5903-5909.
- Skarjune, R. and Oldfield, E. (1979b) Physical studies of cell surface and cell membrane structure. Deuterium nuclear magnetic resonance investigation of deuterium-labelled *N*-hexadecanoylgalactosylceramides (cerebrosides). *Biochim. Biophys. Acta* 556, 208-218.
- Slichter, C.P. (1978) *Principles of Magnetic Resonance*, Springer-Verlag, New York.
- Smith, I.C.P., Butler, K.W., Tulloch, A.P., Davis, J.H. and Bloom, M. (1979) The properties of gel state lipid in membranes of *Acholeplasma laidlawii* as observed by ^1H NMR. *FEBS Lett.* 100, 57-61.
- Soda, G. and Chiba, T. (1969) Deuteron magnetic resonance study of cupric sulfate pentahydrate. *J. Chem. Phys.* 50, 439-455.
- Solomon, I. (1958) Multiple echoes in solids. *Phys. Rev.* 110, 61-65.
- Spiess, H.W. and Sillescu, H. (1981) Solid echoes in the slow-motion region. *J. Magn. Reson.* 42, 381-389.
- Stockton, G.W., Johnson, K.G., Butler, K.W., Tulloch, A.P., Boulanger, Y., Smith, I.C.P., Davis, J.H. and Bloom, M. (1977) Deuterium NMR study of lipid organization in *Acholeplasma laidlawii* membranes. *Nature* 269, 267-268.

- Stoeckenius, W., Lozier, R.H. and Bogomolni, R.A. (1979) Bacteriorhodopsin and the purple membrane of halobacteria. *Biochim. Biophys. Acta* 505, 215-278.
- Urry, D.W. (1971) The gramicidin A transmembrane channel: a proposed π (L_D)helix. *Proc. Natl. Acad. Sci. U.S.A.* 68, 672-676.
- Urry, D.W., Glickson, J.D., Mayers, D.F. and Haider, J. (1972) Spectroscopic studies on the conformation of gramicidin A'. Evidence for a new helical conformation. *Biochemistry* 11, 487-493.
- Veatch, W.R., Fessel, E.T. and Blout, E.R. (1974) The conformation of gramicidin A. *Biochemistry* 13, 5249-5256.
- Wagner, G., DeMarco, A. and Wuthrich, K. (1976) Dynamics of the aromatic amino acid residues in the globular conformation of the basic pancreatic trypsin inhibitor (BPTI). *Biophys. Struct. Mech.* 2, 139-158.
- Walker, J.E., Carne, A.F. and Schmitt, H.W. (1979) The topography of the purple membrane. *Nature* 278, 653-654.
- Warren, G.B., Toon, P.A., Birdsall, N.J.M., Lee, A.G., and Metcalfe, J.C. (1974) Reconstitution of a calcium pump using defined membrane components. *Proc. Natl. Acad. Sci. U.S.A.* 71, 622-626.
- Waugh, J.S., Maricq, M.M. and Cantor, R. (1978) Rotational spin echoes in solids. *J. Magn. Reson.* 29, 183-190.
- Weinstein, S., Wallace, B.A., Morrow, J.S. and Veatch, W.R. (1980) Conformation of the gramicidin A transmembrane channel: a ¹³C nuclear magnetic resonance study of ¹³C-enriched gramicidin in phosphatidylcholine vesicles. *J. Mol. Biol.* 143, 1-19.
- Wittebort, R.J., Schmidt, C.F. and Griffin, R.G. (1981) Carbon-13 nuclear magnetic resonance of dimyristoylphosphatidylcholine phase transitions. *Biochemistry*, in press.
- Williams, R.J.P. (1978) Energy states of proteins, enzymes and membranes, *Proc. R. Soc. London, Ser. B.* 200, 353-389.

See also: Techniques in Protein and Enzyme Biochemistry - Part I. B107, The study of protein structure and conformation using magnetic resonance.