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Nitrogen-14 Nuclear Magnetic Resonance Spectroscopy as a Probe of Lipid Bilayer Headgroup Structure*

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We have obtained nitrogen-14 NMR spectra at 26 MHz (corresponding to a magnetic field strength of 8.5 Tesla) of a variety of lipid bilayer systems, using a quadrupole echo Fourier transform method. Spectra of lecithins in the liquid crystalline phase are characterized by sharp absorption spectra having quadrupole splittings of about 10 kHz. The axis of motional averaging of the nitrogen-14 quadrupole tensor is the bilayer normal. Cooling from the liquid crystalline to the crystalline gel phase results in a rapid broadening of the absorption lineshape, but no significant increase in quadrupole splitting. Incorporation of cholesterol at 1:1 mole ratios below the pure lipid $P\beta'$-$L\alpha$ phase transition temperature (T_c) prevents formation of the solid phase and spectra which are virtually indistinguishable from high temperature liquid crystal phase spectra are obtained. Our results indicate that the rigid lattice quadrupole coupling of nitrogen-14 in the lecithin bilayer is about 130 kHz. Apparent discrepancies between deuterium and nitrogen-14 NMR data are resolved by means of a model in which motion about the directly bonded methylene C_β—N bond becomes slow at low temperatures in the absence of cholesterol, and only at high temperatures or in the presence of cholesterol is motion about this bond fast enough to cause complete averaging of the deuterium quadrupole tensor. We have also investigated the effects of a variety of ions, drugs, antibiotics, and proteins on phospholipid bilayer structure using nitrogen-14 NMR quadrupole splittings. In no cases have we found any increase in the phospholipid N-14 quadrupole splitting upon addition of the impurity molecule: the tricyclic antidepressant drug desipramine reduces the quadrupole splitting by a factor of 2 at 50 mole % levels; the proteins cytochrome oxidase (ferrocytochrome c:O_2 oxidoreductase, EC 1.9.3.1) and sarcoplasmic reticulum ATPase (ATP phosphohydrolase, EC 3.6.1.3) cause small (about 10%) decreases in the quadrupole splitting at 70 weight % levels, suggesting that the most ordered state of the lecithin bilayer headgroup is that found in the pure bilayer itself.

In order to fully understand the function or mechanism of action of cell membranes, it seems likely that a full knowledge of their molecular structure will be required. A great increase in our knowledge of the static and dynamic aspects of the organization of the lipid constituents of membranes has been obtained over the past 10 years, in both model membranes and in biological membranes themselves, through the use of nuclear magnetic resonance spectroscopy. The use of the nuclei ^1H, ^2H, ^{13}C, and ^{31}P has been particularly prevalent (1–4), using, in many instances, isotopic labeling techniques to provide improved sensitivity, and selectivity. Since such efforts have been expended using rather lengthy chemical labeling methods, it is therefore rather surprising that there have only recently been reports of the use of the naturally abundant nucleus, ^{14}N, in nonsonicated systems (5), although there have been additional results reported using small single bilayer vesicles (6, 7). Presumably, some workers have been dissuaded by the relatively low gyromagnetic ratio of ^{14}N, but with modern Fourier transform instrumentation, as we show below, this is a minor problem and is easily offset by the very large natural abundance (>99.6%) of the ^{14}N nucleus.

Similarly, although it is well known that the electric quadrupole coupling constants (e^2qQ/h) of many organic compounds are very large, say 6 MHz (8), the highly symmetric electronic environments of several of the phospholipids, in particular of course the RN^+Me_3 groups in the diacylglycerophosphocholines and sphingolipids, strongly suggest that ^{14}N NMR[1] of these systems should be relatively straightforward. This view is supported by the observation that the quadrupole coupling constants of the related species *n*-hexadecyltrimethylammonium bromide, and of betaine, as determined by solution NMR techniques, must be in the range 110 to 160 kHz (9, 10), suggesting that the ^{14}N resonances of phospholipids would be particularly simple to detect, as was indeed shown recently by Siminovitch *et al.* (5). In this publication, we present the results of a broad survey designed to test the idea that ^{14}N NMR of phospholipids will be useful in determining the static and dynamic structures of membrane systems.

EXPERIMENTAL PROCEDURES

Nitrogen-14 NMR spectra were obtained at 26.0 MHz (corresponding to a magnetic field strength of 8.5 Tesla), using a "home-built" spectrometer and data system based on a LSI-11 minicomputer. A two-pulse echo technique (11) using 90° pulse widths of ~9 μs was used to record the ^{14}N spectra. We used the "home-built" medium field spectrometer described previously (12) to record deuterium spectra. Single phase detection and a spectrum reverse technique ((Nicolet software package FT-74) was used to obtain an effective 100 kHz spectral width. The deuterium 90° pulse width was 6 to 7 μs, and

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[1] The abbreviations used are: NMR, nuclear magnetic resonance; CHOL, cholesterol; DLPC, 1,2-dilauroyl-*sn*-glycero-3-phosphocholine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine.

again a two-pulse sequence was used (11) to minimize spectral lineshape distortions.

The deuterated lipid used, 1,2-dioleyl-*sn*-glycero-3-phospho[*N-methyl*-$^2H_3$]choline (DOPC-d_3), was purchased from Lipid Specialties, Boston, MA, while all other lipids were from Sigma Chemical Co. Gramicidin A′ (a mixture of gramicidins A, B and C) was obtained from Nutritional Biochemicals Co. (Cleveland, OH) and used without further purification.

Phospholipid purity was monitored by thin layer chromatography on Merck Silica Gel 60 F-254 plates (EM Laboratories, Inc., Elmsford, NY), using, in most cases, a $CHCl_3$/MeOH/7 M NH_4OH (230:90:15, v/v/v) solvent system. Visualization was with one or more of the following reagents: I_2, rhodamine 6G, Mo phosphate reagent (13), or Dragendorff choline reagent (14). Phospholipid purity was also checked periodically during a given series of NMR experiments on representative samples. In the latter cases, samples were freeze dried and then extracted with $CHCl_3$/MeOH (2:1, v/v) and the lipid extract examined as described above.

Cytochrome oxidase and sarcoplasmic reticulum ATPase were the samples whose isolation has been described previously (15, and references cited therein), as have our methods for reconstitution of both these proteins and gramicidin A′ with phospholipid (15–18). Cholesterol admixture with lecithin was as described (12). The oriented sample of egg yolk-lecithin was produced by loading a concentrated sample of egg yolk-lecithin in chloroform onto microscope cover slides which had been cut to ≈20 × 7 mm, followed by solvent removal *in vacuo*, after which the plates were gently stacked into a "sandwich" and equilibrated in a sealed vessel over H_2O for 24 h.

RESULTS AND DISCUSSION

The theoretical background appropriate for consideration of the ^{14}N NMR spectra of lipid membranes is discussed in detail elsewhere (5, 19, 20) so we shall be content to briefly quote the principal result that for a ^{14}N nucleus (with spin I = 1 and assuming an asymmetry parameter $\eta = 0$) the allowed transitions correspond to $+1 \leftrightarrow 0$ and $0 \leftrightarrow -1$, and give rise to a "quadrupole splitting" of the NMR absorption line with separation between peak maxima of

$$\Delta\nu_Q = \frac{3}{2}\frac{e^2qQ}{h}\frac{3\cos^2\theta - 1}{2} \qquad (1)$$

e^2qQ/h is the nitrogen quadrupole coupling constant and θ is the angle between the principal axis of the electric field gradient tensor at the nitrogen nucleus, and the magnetic field, H_0. For a rigid polycrystalline solid all values of θ are possible and one obtains a so-called "powder pattern" lineshape in which the separation between peak maxima is $3e^2qQ/4h$ and the separation between the outer edges (Fig. 1, *B* to *E*), is twice this value. In general, in systems undergoing motion, these quadrupole splittings of the absorption lines will be reduced by the motion, and the degree of the reduction and the actual shape of the NMR line may be used to deduce information about the rates and types of motion undergone by the group in question.

As mentioned above, the range of quadrupole coupling constants (e^2qQ/h) for ^{14}N compounds is very large (8), the actual values depending on the magnitude of the electric field gradient at the respective ^{14}N nucleus. For systems having cubic symmetry, for example the NH_4^+ ion in ammonium chloride, there is no quadrupole interaction and a narrow "isotropic" absorption line is obtained, Fig. 1*A*, even in the solid state. Note, however, that when this perfect cubic symmetry is removed, for example, by introducing successively larger halide counterions to the tetramethylammonium species (Me_4N^+, Fig. 1, *B* to *D*), or by having differing alkyl substituents, then values of e^2qQ/h increase dramatically. We have, for example, been unable to detect well defined signals from $MeNH_3^+Cl^-$, $Me_2NH_2^+Cl^-$, or $Me_3NH^+Cl^-$, and have obtained only poor spectra from choline phosphate ($Me_3N^+CH_2CH_2OPO_3^{2-}$), solid anhydrous lecithins, and cetyltrimethyl ammonium bromide. Fortunately, however, in the liquid crystal phase of lecithin, and in the hydrated gel state, well resolved spectra are obtained (Fig. 1*E* and Ref. 5) indicating significant averaging of the quadrupole interaction due to molecular motion. However, in the liquid crystal phase of phosphatidylethanolamine we have been unable to obtain good quality ^{14}N spectra suggesting that ^{14}N NMR of intact cell membranes will be dominated by the membrane phosphorylcholine constituents, of lecithin and sphingomyelin.

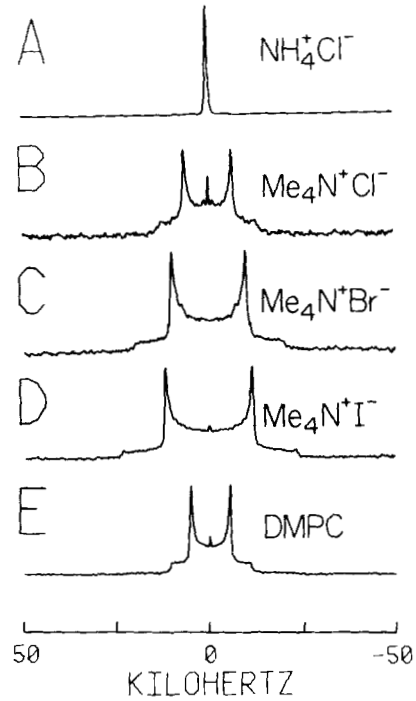

FIG. 1. **Nitrogen-14 NMR spectra of solid ammonium salts and DMPC.** *A*, ammonium chloride; *B*, tetramethylammonium chloride; *C*, tetramethylammonium bromide; *D*, tetramethylammonium iodide; *E*, DMPC, in excess water. Spectra were obtained using the Fourier transform method at 26 MHz (corresponding to a magnetic field strength of 8.5 Tesla) at ~23 °C using a two-pulse quadrupole echo method. The quadrupole splittings ($\Delta\nu_Q$, kHz) and linewidths (2δ, Hz, Ref. 15) obtained from spectral simulations were *A*, 0, 600; *B*, 13.2, 300; *C*, 19.9, 250; *D*, 23.5, 250; *E*, 10.7, 250. Spectral conditions were typically $\tau_1 = \tau_2 = 80$ μs, and a recycle time of 200 ms (*A*, *B*, and *E*), 10 s (*C*), or 120 s (*D*). The number of scans varied from 37 to 8192.

The ^{14}N NMR spectrum of the liquid crystalline sample of DMPC (Fig. 1*E*), is considerably narrower than that of the anhydrous gel[2] or of the model systems betaine and cetyltrimethylammonium bromide (9, 10), having a quadrupole splitting ($\Delta\nu_Q$) of ~10.7 kHz (5). The results of Fig. 2 indicate that averaging of the quadrupole interaction is achieved by fast axial diffusion about the bilayer normal, as has been demonstrated previously in the case of ^{31}P and ^2H NMR of lecithins (21, 22). We show in Fig. 2 the ^{14}N NMR spectra of a random powder sample of DMPC, hand-dispersed in excess water at 24 °C, having $\Delta\nu_Q = 10.7$ kHz, together with that of a sample of egg yolk lecithin which had been oriented on glass slides using the methods discussed under "Experimental Procedures." The oriented sample had the perpendicular to the glass plates (the bilayer normal or director axis) oriented along the static magnetic field (H_0) direction. In this orientation, a quadrupole splitting twice that observed for the singularities in the random powder distribution pattern (Fig. 2*A*) is expected. However, we used a sample of egg lecithin for the sample of Fig. 2*B* to facilitate ordering of the bilayers at room temperature (the gel-to-liquid crystal phase transition tem-

[2] Unpublished results.

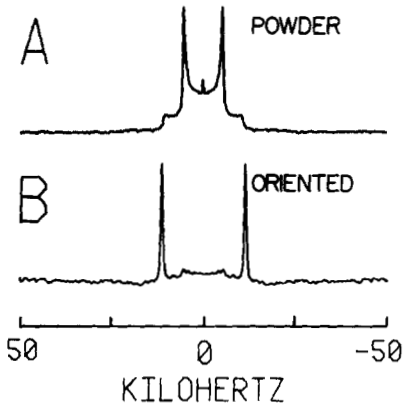

FIG. 2. **Nitrogen-14 NMR spectra of random-powder and mechanically oriented lecithins obtained at 26 MHz.** *A*, DMPC, about 40 weight % dispersion in H_2O, 24 °C, 200 ms recycle time, 11 μs 90° pulse widths, $\tau_1 = \tau_2 = 80$ μs, 100 kHz spectral width, 4096 real data points, 8192 scans, $\Delta\nu_Q = 10.7$ kHz. *B*, egg-yolk lecithin oriented and hydrated on glass slides, 25 °C, other conditions as in *A* except 6.5 μs 90° pulse widths and 961 scans. $\Delta\nu_Q = 22.5$ kHz.

perature of DMPC is 23 °C (23), while that of egg lecithin is about −10 °C (23)). Nevertheless, the 22.5-kHz quadrupole splitting of the oriented sample (Fig. 2*B*), is approximately twice the 10.7 kHz observed in the random powder distribution of DMPC (Fig. 2*A*). Also, a spectrum of a random powder sample of Fig. 2*B* at lower signal to noise ratios did exhibit[3] the 11.3-kHz quadrupole splitting predicted. As expected, high signal-to-noise ratio spectra of the mechanically ordered samples may be obtained with only a few minutes signal averaging at high field on samples as small as 10 mg.

We explore in Fig. 3 the effects of the gel-to-liquid crystal phase transition on the observed nitrogen-14 NMR spectra of lecithin together with the effects of cholesterol on the phase transition. We show in Fig. 3, *A* to *C*, the spectra of the pure lipids, 1,2-dilauryl-*sn*-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), all obtained at 25 °C. The results of Fig. 3, *A* and *B*, clearly indicate that the DLPC and DMPC are in the liquid crystalline (Lα) phase (23) while DPPC is below its gel-to-liquid crystal phase transition temperature of approximately 41 °C (23). As observed previously by Siminovitch *et al.* (5), the principal effect of transferring from the liquid-crystal to the gel phase is to increase the ^{14}N NMR linewidths while the quadrupole couplings remain approximately constant (Fig. 3, *C* and *F*). Addition of cholesterol, however, removes the gel-to-liquid crystal phase transition (24) and at both high and low temperature, Fig. 3, *D* and *E*, sharp, well resolved quadrupole splittings and narrow line spectra are obtained. In addition, the Gaussian decays of the quadrupole echo intensity in $90°\text{-}\tau\text{-}90°_{90}$ experiments on both samples have the same time constants.[3] As viewed by ^{14}N NMR, therefore, cholesterol appears to have very little effect on the static or dynamic structure of the lecithin polar headgroup and presumably, therefore, acts simply as an inert spacer molecule.

Previous workers (5) have attempted to use ^{14}N NMR results such as those shown in Figs. 1 to 3 in combination with previously published ^2H NMR data to deduce the magnitude of the ^{14}N rigid lattice electric quadrupole coupling constant (e^2qQ/h) for lecithin. In principle it is, of course, straightforward to compute this if the appropriate order parameters are known (5). That it may not be straightforward to transform the ^2H data into ^{14}N couplings is, however, suggested by the results of Figs. 3 and 4. In Fig. 4 we show two sets of data; one for ^2H NMR of 1,2-dioleyl-*sn*-glycero-3-phosphocholine labeled as *N*-[$^2H_3$]*methyl* in the choline ammonium group, both in the absence and presence of cholesterol as a function of temperature, and second we show ^{14}N NMR data of these samples together with additional results obtained using DPPC and DPPC-cholesterol (1:1).

The deuterium NMR results clearly show that incorporation of cholesterol at 1:1 mole ratio into the DOPC bilayer above the phase transition temperature (−22 °C, Ref. 23) has a small effect which is increasingly important at low temperature. For example, at 25 °C (47 °C above the gel-to-liquid crystal phase transition temperature) cholesterol reduces the deuterium quadrupole splitting from 1.2 to 1.0 kHz, a 17% effect, while at −18 °C (4 °C above the gel-to-liquid crystal phase transition temperature) the effect of cholesterol incorporation is to reduce the observed quadrupole splitting by 31%. If it were a straightforward matter to transform the deuterium data into N-14 NMR quadrupole splittings, then we would predict the same reductions in N-14 NMR quadrupole splittings caused by cholesterol as are evidenced in the ^2H NMR data. The results of Fig. 4 indicate that this does not occur. For example, incorporation of equimolar quantities of cholesterol to a DOPC bilayer at 25 °C results in a 6% reduction in the N-14 quadrupole splitting, from 10.9 to 10.3 kHz, while at about −15 °C the effect remains the same

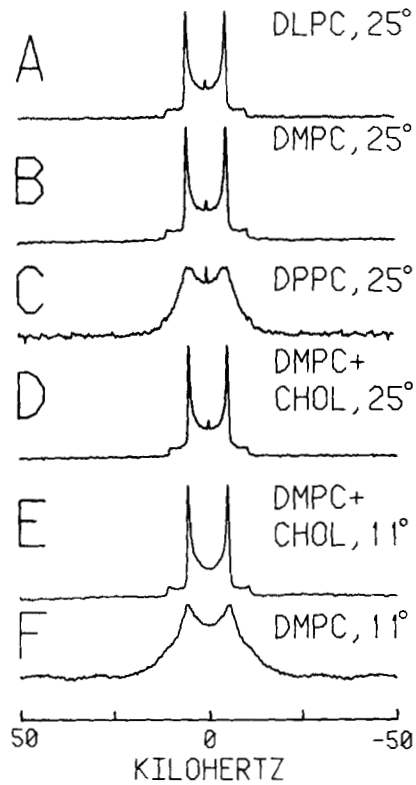

FIG. 3. **Nitrogen-14 NMR spectra at 8.5 Tesla of lecithins showing the effects of cholesterol incorporation into the bilayer.** *A*, DLPC, ~40 % weight % dispersion in H_2O, 25 °C, 65 ms recycle time, 11 μs 90° pulse widths, $\tau_1 = \tau_2 = 250$ μs, 100 kHz spectral width, 4096 real data points, 8192 scans, $\Delta\nu_Q = 10.4$ kHz, line width $w = 250$ Hz. *B*, DMPC, other conditions as *A* except 200 ms recycle time, $\tau_1 = \tau_2 = 80$ μs, $\Delta\nu_Q = 10.7$ kHz. *C*, DPPC, other conditions as in *A* except $\Delta\nu_Q = 11.1$ kHz and $w = 2500$ Hz. *D*, DMPC-cholesterol (CHOL), 1:1 mole ratio, in excess water, other conditions as in *B*. *E*, DMPC-cholesterol, 1:1 mole ratio at 11 °C, in excess water, other conditions as in *B*. *F*, DMPC, 11 °C, in excess water, other conditions as in *B*, $\Delta\nu_Q = 12.5$ kHz.

[3] T. M. Rothgeb and E. Oldfield, unpublished results.

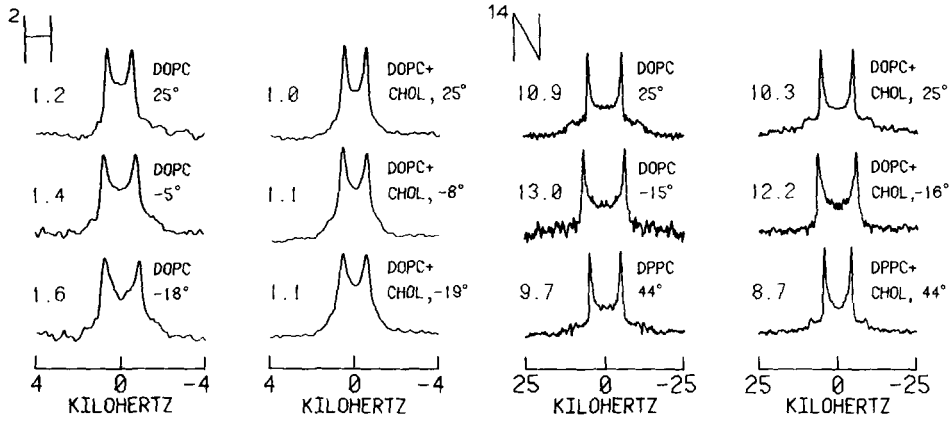

FIG. 4. **Deuterium and nitrogen-14 NMR spectra of DOPC, DPPC, and the effects of cholesterol at several temperatures.** Deuterium spectra were obtained at 5.2 Tesla and nitrogen-14 spectra at 8.5 Tesla. The ^2H spectra are shown on the left half of the figure and the ^{14}N spectra on the right half. All samples were dispersions in excess water at the temperatures indicated. The experimentally measured quadrupole splittings are given in kilohertz on the figure.

corresponding to a reduction from 13 to 12.2 kHz. Similarly, immediately above the gel-to-liquid crystal phase transition temperature of DPPC (41 °C) incorporation of equimolar quantities of cholesterol into the DPPC bilayer reduces the observed ^{14}N quadrupole splitting again by a factor of about 10%, from 9.7 to 8.7 kHz; considerably less than the percentage indicated by previous workers using ^2H NMR spectroscopy (25, 26). Comparison of the ^2H and ^{14}N NMR results of Fig. 4 thus suggests that it may not be straightforward to simply extrapolate from ^2H to ^{14}N results. The apparent discrepancies of Fig. 4 have considerable relevance for determinations of ^{14}N quadrupole coupling constants from ^2H NMR data as outlined by Siminovitch *et al.* (5). Perhaps the most likely origin of these effects is that only at high temperature or in the presence of cholesterol is motion about the directly bonded methylene carbon-nitrogen (C_β—N) bond fast in the phospholipids we have studied. This possibility is suggested by the large difference in activation energy for the motion of the two sets of choline methylene deuterons (6, 27) which suggests that the $N^+(CH_3)_3$ group may be susceptible to an appreciable motional restriction from the phosphate group network (6). Our results could indicate that motional averaging of the methyl-deuteron quadrupole coupling is complete only at high temperature in the presence of cholesterol, where interactions between the lecithin molecules are minimal. This could explain the large temperature dependence of the DOPC-^2H data of Fig. 4 (1.2 to 1.6 kHz) while the DOPC-CHOL (1:1) data remain essentially constant at 1.1 kHz over the same 46 °C range in temperature. We suggest that the ^{14}N quadrupole coupling is not affected by fast motions about the C_β—N bond since this is likely to be the principal axis of the ^{14}N electric field gradient tensor. It may therefore be possible to generate relatively large effects in ^2H NMR spectra (1.6 → 1.1 kHz, DOPC → DOPC-CHOL at −18 °C) with very minor changes being seen in the corresponding ^{14}N NMR spectra (13 → 12.2 kHz, DOPC → DOPC-CHOL at −15 °C). If this is correct, then extrapolation of the results of Fig. 4 indicates that for a 1.1-kHz deuterium quadrupole coupling (DOPC-CHOL, ~20 °C) and a 10.3 kHz ^{14}N coupling (DOPC-CHOL, ~20 °C), using the bond angles reported previously (27), a rigid-lattice quadrupole coupling constant of about 131 kHz is expected for the ^{14}N nucleus. This is in good agreement with the value determined previously by Siminovitch *et al.* (5) and is close to the average value for betaine and cetyl trimethylammonium bromide reported by other workers (9, 10).

We show in Fig. 5 the results of a series of experiments designed to test the sensitivity of the ^{14}N nucleus in lecithin bilayers to the perturbing effects of a variety of drugs, ions, antibiotics, and proteins. We show in Fig. 5*A* the effect of the antidepressant drug, desipramine, which has been shown previously to have large effects on lipid bilayer structure using both nuclear magnetic resonance spectroscopic and scanning calorimetric studies (28, 29). Desipramine carries a positive charge in our nonbuffered system and as such it apparently intercalates into the phospholipid polar headgroup region causing a considerable disordering of the lecithin headgroup and hydrocarbon chain region (28). The results of Fig. 5*A* show that increasing desipramine concentration in the bilayer from 0 to 50 mole % (about 25 weight %) results in a monotonic decrease in the observed ^{14}N quadrupole splitting from the lipid bilayer polar headgroup region. Notably, neither of the N-14 nuclei in the tricyclic antidepressant drug are observed under our experimental conditions. Control experiments using ^1H NMR spectroscopy have shown that all drug is membrane-associated at these drug/lipid ratios or at least is in fast exchange with bound drug.[4] The results of Fig. 5*A* indicate, in addition to disordering the bilayers, that a second phase of unknown structure appears at high drug concentration, but further studies will be required in order to fully characterize its nature.

We show in Fig. 5*B* the effect of two different metal ions on the ^{14}N NMR spectra of DMPC lipid bilayers. Addition of the anionic ferricyanide ion has a relatively small effect on lipid bilayer structure as determined by ^{14}N NMR spectroscopy, the observed quadrupole splitting decreasing from 10.7 to 9.6 kHz, due presumably to a relatively weak interaction with the positively charged lecithin trimethylammonium group. By contrast, however, addition of the trivalent cation europium (III) causes an extreme broadening of the NMR line, similar to that observed on transition from a liquid crystal to gel state phospholipid bilayer. This result is that expected on the basis of previous calorimetric studies (30) and probably corresponds to cross-linking of the polar headgroups by the multivalent cation, with formation of a gel phase structure. At high temperature (43 °C, Fig. 5*B*), there is apparently a change in the static conformation of the polar headgroup, as evidenced by the decrease in quadrupole splitting from 10.3 to 7.5 kHz.

Addition of the polypeptide antibiotic gramicidin A at low concentrations causes a slight reduction in the observed quadrupole splitting (from 10.7 kHz at 0 mole % gramicidin to 8.6 kHz at 33 mole % gramicidin) consistent with effects observed previously using ^2H NMR (17) while incorporation of high levels of gramicidin results in formation of a broad isotropic spectral component, again paralleling the effects observed previously with ^2H and ^{31}P NMR (17, 31). Addition of the much larger membrane proteins cytochrome oxidase and sarcoplasmic reticulum ATPase (Fig. 5*D*) does not show the severe line-broadening evident in the case of the gramicidin-lecithin complex. The results we have obtained with cytochrome oxidase and sarcoplasmic reticulum ATPase are con-

[4] E. Oldfield, unpublished results.

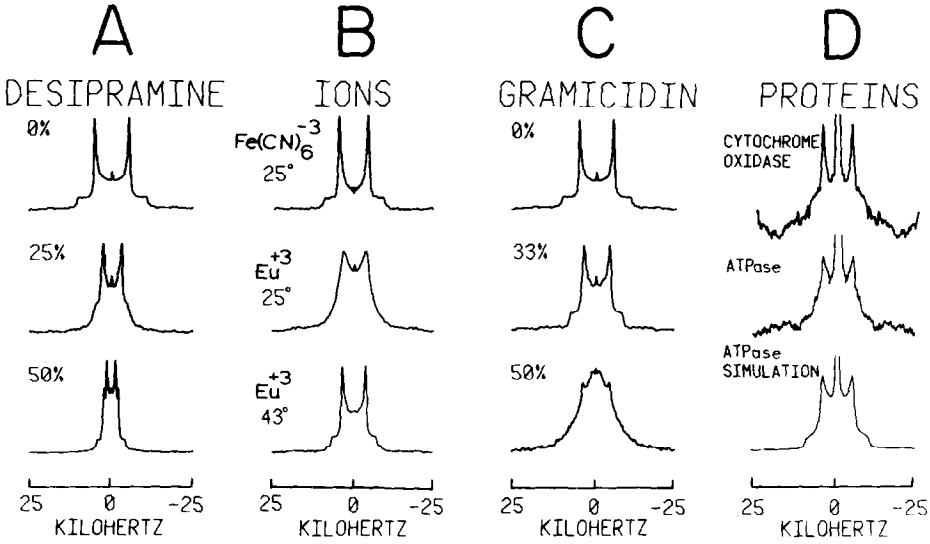

FIG. 5. **Nitrogen-14 NMR spectra of DMPC in the presence of a variety of perturbing species.** *A*, desipramine at the mole per cent indicated, in excess H_2O at 25 °C. *B*, potassium ferricyanide or europium (III) chloride, 25 mole % of the ionic species, at the temperatures indicated in the figure. The spectral simulation parameters ($\Delta\nu_Q$, kHz; W, Hz) for $Fe(CN)_6^{3-}$, Eu(III) 25 °C and Eu(III) 43 °C are 9.6, 250; 8.0, 1600; and 7.5, 300, respectively. *C*, gramicidin A′ at the weight per cent indicated, dispersed in H_2O at 25 °C. The spectral simulation parameters ($\Delta\nu_Q$, kHz; W, Hz) for both the 33% and 50% samples are 8.6, 400. The 33% gramicidin simulation contained a 40% contribution of an isotropic component having a width of 12,000 Hz, the 50% gramicidin had a 93% contribution of the same isotropic component. *D*, cytochrome oxidase, 32 °C, ~70 weight % protein, $\Delta\nu_Q$ = 10 kHz; sarcoplasmic reticulum ATPase, 25 °C, ~70 weight % protein, $\Delta\nu_Q$ = 9.0 kHz. Both samples were in excess water. The spectral simulations included an isotropic component which probably arises from Tris buffer. We cannot absolutely rule out the possibility that highly mobile lipids or membrane fragments contribute to these small signals, however, the intensities are <10 to 20% of the main lipid quadrupole doublet.

sistent with the idea that these large proteins have only a small perturbing effect on the lipid bilayer structure, reducing the ^{14}N quadrupole splitting from about 10.7 to 10 (sarcoplasmic reticulum ATPase) or 9 kHz (cytochrome oxidase), presumably due to their decreased surface area/volume ratio compared with the polypeptide gramicidin. These results were obtained from spectral simulations as shown in Fig. 5*D*.

The results of Fig. 5 indicate that ^{14}N NMR is a sensitive indicator of the perturbing effects of a variety of drugs, antibiotics, proteins, and ions on phospholipid bilayer headgroup structure. When taken in combination with ^{31}P, ^{13}C, and ^{2}H NMR, these results should give a better indication of the types of interaction undergone by the membrane lipid headgroups. It is important to note, however, that there are rather stringent requirements for the observation of the ^{14}N NMR spectra of membrane components. In particular, the environment of the ^{14}N nucleus must be highly symmetric, or highly ordered or oriented arrays of lipid components must be utilized if systems with large quadrupole couplings are to be investigated. To date, it therefore seems most likely that the technique will be restricted to investigations of phosphatidylcholine and sphingomyelin lipids in both model and intact cell membranes, although in model systems it may be possible to investigate oriented drug or polypeptide constituents. The results of Fig. 2 strongly suggest that sufficient spectral sensitivity is now available for observation of such dilute components if samples may be oriented near the "magic-angle." Similar restrictions will likely apply to the observation of ^{17}O NMR spectra of lipid bilayers, where quadrupole coupling constants may be even larger than those of ^{14}N, a view substantiated by preliminary results obtained using ^{17}O-labeled soap lipid bilayers.[5]

Finally, perhaps even more interesting than the observation of lipid resonances will be the eventual detection of the protein constituents of cell membranes. We have recently detected[3] weak but reproducible signals of about 100 kHz quadrupole splitting from the microsomal membranes of rat liver, which apparently do not originate in the membrane lipid constituents. If oriented arrays of such systems can be obtained (32), then it should be possible in the future to investigate both protein and lipid components in biological membranes by means of ^{14}N NMR spectroscopy.

[5] M. D. Tsai and E. Oldfield, unpublished results.

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