tivity of whole cell bioselective membrane electrodes.

At present, significant research on bacterial and tissue-based potentiometric membrane electrodes is being carried out in just three or four laboratories around the world. This situation is likely to change, however, as improved techniques are found to improve the characteristics of such electrodes and to demonstrate their practical utility for bioanalytical measurements. In view of the great number and range of biological materials that might be used in conjunction with ion- or gas-sensing membrane electrodes to make potentiometric sensors, future research is almost certain to result in some exceptionally attractive new measurement devices. The advantages of simplicity and low cost that can be realized with such electrodes are already fully apparent.

References and Notes

1. G. H. Fricke, *Anal. Chem.* **52**, 259R (1980).
2. P. Bergveld, N. F. DeRooij, J. N. Zemel, *Nature (London)* **273**, 438 (1978); J. Janata, in *Ion-Selective Electrodes in Analytical Chemistry*, H. Freiser, Ed. (Plenum, New York, 1980), p. 107.
3. G. A. Rechnitz, *Chem. Eng. News* **53**(4), 29 (1975).
4. G. Eisenman, *Glass Electrodes for Hydrogen and Other Cations* (Dekker, New York, 1967).
5. M. S. Frant and J. W. Ross, Jr., *Science* **154**, 1553 (1966).
6. J. W. Ross, *ibid.* **156**, 1378 (1967).
7. L. A. R. Pioda, V. Stankova, W. Simon, *Anal. Lett.* **2**, 665 (1969).
8. R. P. Scholer and W. Simon, *Chimia* **24**, 372 (1970).
9. N. Lakshminarayanaiah, *Membrane Electrodes* (Academic Press, New York, 1976).
10. P. L. Bailey, *Analysis with Ion-Selective Electrodes* (Heyden, London, 1976).
11. P. W. Carr and L. D. Bowers, *Immobilized Enzymes in Analytical and Clinical Chemistry* (Wiley-Interscience, New York, 1980).
12. J. W. Ross, J. H. Riseman, J. A. Krueger, *Pure Appl. Chem.* **36**, 473 (1973).
13. ———, *Chem. Eng. News* **56**(41), 16 (1978).
14. G. A. Rechnitz, M. A. Arnold, M. E. Meyerhoff, *Nature (London)* **278**, 466 (1979); U.S. Patent 4,216,065 (5 August 1980).
15. M. A. Arnold and G. A. Rechnitz, *Anal. Chem.* **52**, 1170 (1980).
16. S. Updike and I. Treichel, *ibid.* **51**, 1643 (1979).
17. M. A. Arnold and G. A. Rechnitz, *ibid.* **53**, 515 (1981).
18. ———, *Chem. Eng. News* **59**(9), 24 (1981).
19. M. E. Meyerhoff, *Anal. Lett.* **13**(B15), 1345 (1980).
20. C. Divies, *Ann. Microbiol. (Paris)* **126A**, 175 (1975).
21. ———, *Chem. Eng. News* **54**(44), 23 (1976).
22. R. K. Kobos, in *Ion-Selective Electrodes in Analytical Chemistry*, H. Freiser, Ed. (Plenum, New York, 1980), vol. 2, pp. 69–84.
23. S. R. Grobler and G. A. Rechnitz, *Talanta* **27**, 283 (1980).
24. G. A. Rechnitz, T. L. Riechel, R. K. Kobos, M. E. Meyerhoff, *Science* **199**, 440 (1978).
25. R. R. Walters, B. E. Moriarty, R. P. Buck, *Anal. Chem.* **52**, 1680 (1980).
26. R. K. Kobos and G. A. Rechnitz, *Anal. Lett.* **10**, 751 (1978).
27. M. A. Jensen and G. A. Rechnitz, *Anal. Chim. Acta* **101**, 125 (1978).
28. R. K. Kobos, D. J. Rice, D. S. Flournoy, *Anal. Chem.* **51**, 1122 (1979).
29. T. L. Riechel and G. A. Rechnitz, *J. Membr. Sci.* **4**, 243 (1978).
30. R. K. Kobos and H. Y. Pyon, *Biotechnol. Bioeng.* **23**, 627 (1981).
31. K. Matsumoto, H. Seijo, T. Watanabe, I. Karube, I. Satoh, S. Suzuki, *Anal. Chim. Acta* **105**, 429 (1979).
32. T. Matsunaga, I. Karube, S. Suzuki, *ibid.* **99**, 233 (1978).
33. C. L. Di Paolantonio, M. A. Arnold, G. A. Rechnitz, *ibid.* **128**, 121 (1981).

Recent Developments in Nuclear Magnetic Resonance Spectroscopy

George C. Levy and David J. Craik

From modest but promising beginnings in the 1940's and 1950's, nuclear magnetic resonance (NMR) spectroscopy has developed into an important research tool (*1*). Early in its history, NMR spectroscopy was adapted from sole use by physicists, who had first discovered it, to the realm of chemists, who saw the potential of the so-called chemical shift phenomenon as a structural probe. This first useful parameter has now been supplemented by many other experimentally accessible quantities. In this article we outline some of these new developments in NMR spectroscopy.

In a review of this scope it is not possible to cover all developments in an area. We have attempted to provide a brief overview of the advances in technology during the past several years together with a discussion of some of the many new applications. We have stressed applications in chemistry and biology, as these are the areas in which most of the new uses of NMR have occurred. Of course, there are also many applications in physics, and the future holds promise for the further expansion of NMR spectroscopy into other disciplines. For example, routine medical diagnosis through NMR seems possible in the foreseeable future. Geological applications are also under development, largely as a result of advances in the technology associated with NMR of solid samples. We hope that this review will demonstrate some of the strengths of NMR to scientists not familiar with the technique and perhaps stimulate some further new applications.

Instrumentation

In broad terms, most new applications of NMR in recent years have derived from parallel improvements in instrumentation and methods. The instrumental improvements may be categorized as follows:

1) The development and use of spectrometers operating at higher magnetic fields, in some cases with large and versatile probe (sample) geometries.

2) The development of multinuclear spectrometers.

3) Improved spectrometer design for Fourier transform techniques and higher sensitivity for NMR with protons and other nuclei.

4) Advances in computer capabilities.

Magnetic field strength. One of the primary instrumental improvements has been the use of stronger magnetic fields. In the early 1960's it was rare for an NMR spectrometer to be other than a 60-megahertz instrument, capable of observing only sensitive nuclei such as protons. Commercial spectrometers of the day were based on iron permanent or electromagnets with fields of about 1.4 tesla (14 kilogauss). In the middle 1960's electromagnets with fields of 2.3 tesla (equivalent to a resonant frequency of 100 MHz for protons) became available, and they are still in extensive use. This field represents about the limiting strength of a conventional NMR magnet. However, since the early 1970's there has been increasing use of superconducting solenoid–based systems, which are capable of much higher magnetic fields.

George Levy is a professor in the Department of Chemistry and director of the NIH Biotechnology Research Resource for Multi-Nuclei NMR and Data Processing at Syracuse University, Syracuse, New York 13210. David Craik is a CSIRO (Australia) postdoctoral research associate at Syracuse University.

0036-8075/81/1016-0291$01.00/0

Summary. Nuclear magnetic resonance spectroscopy is a powerful and versatile technique that yields information related to molecular structure, interactions, and dynamics. Methods are currently being developed for real-time monitoring of metabolic processes in vivo and for noninvasive detection of disease and abnormality in living animals. Other nuclear magnetic resonance techniques are providing entirely new approaches for analyses of complex chemical systems. The increased power and popularity of nuclear magnetic resonance spectroscopy today are due to many developments in instrumentation and methods that have occurred over the 35-year history of the technique. The most important single advance, particularly in recent years, has been increased sensitivity. Concurrent improvements in effective spectral resolving power and an array of new methods and applications have also contributed to elevating nuclear magnetic resonance spectroscopy to its present position as one of the premier analytical techniques.

The highest fields in use today (11.7 to 14.4 tesla) correspond to proton NMR frequencies of 500 to 600 MHz.

The main advantage of high-field spectrometers is their greater spectral dispersion. For example, two peaks with a chemical shift difference of 10 Hz on a 100-MHz instrument have a separation of 50 Hz on a 500-MHz spectrometer. The proton spectra in Fig. 1 show the effect of field strength on chemical shift dispersion. High dispersion is often necessary to aid in the resolution and assignment of individual peaks in ^{1}H or ^{13}C studies of complex biomolecules. Furthermore, complicated second-order ^{1}H spectra of coupled-spin systems are often greatly simplified at higher fields. As an additional bonus, higher fields bring about an inherent increase in sensitivity, giving the spectra a much improved signal-to-noise ratio.

The development of wide-bore superconducting magnets has also made possible many new applications. The first important benefit is an increased sample volume and thus increased sensitivity (the more nuclei within the receiver coil the stronger the signal). Second, a larger sample volume allows more flexibility; for example, it is now possible to place organs or even living animals into the probe of a high-resolution magnet.

Multinuclear NMR. The earliest nuclei studied by NMR were those with the highest susceptibility to detection. Studies of less accessible nuclei awaited improvements in instrument sensitivity, and it was not until the 1970's that multinuclear spectrometers dramatically expanded the range of potential applications of NMR. The initial trend was to have a separate radio-frequency (RF) circuit and probe for each nucleus, but this is being changed with the development of broadband spectrometers and tunable probes, which allow rapid changeover between nuclei. NMR is no longer just associated with a limited number of nuclei such as ^{1}H, ^{19}F, and ^{13}C, but has been applied to many others, including ^{2}H, ^{15}N, ^{17}O, ^{23}Na, ^{113}Cd, and ^{195}Pt. Harris and Mann (*2*) have surveyed NMR applications for many of the elements of the periodic table.

Fourier transform NMR. Before 1966 all NMR spectra were recorded in the continuous wave (CW) mode, in which either the magnetic field or the frequency was swept slowly. In 1966 Ernst and Anderson (*3*) introduced the pulsed NMR Fourier transform (FT) technique, in which the sample is irradiated with an intense pulse of RF energy for a short time (1 to 100 microseconds) and the resulting electrical signal, termed a free induction decay (FID), is collected, digitized, and stored in a computer (*4*). Subsequent Fourier transformation produces a normal spectrum. The technique was commercialized around 1970 and brought an immediate 10- to 15-fold increase in sensitivity for ^{13}C NMR (as well as a smaller increase for ^{1}H NMR). Since then, improvements in spectrometer design (for instance, optimization of receiver coil and electronic circuits for pulsed operation) have produced an additional order of magnitude gain in sensitivity, essentially matching the initial advantage of FT NMR. Table 1 summarizes the dramatic improvements in sensitivity over the last 20 years.

Computer developments. The modern NMR computer system and software can control multipulse experiments in which factors such as delay times, decoupler levels, pulse widths, or RF phase may be varied systematically. In fact, virtually all spectrometer functions are now under computer control. This not only expands the range and types of experiments but also increases throughput, as several experiments can be preprogrammed and run consecutively without operator intervention. The processing capabilities of modern computers have also been greatly expanded, and the ability to efficiently store and manipulate large data arrays is central to many of the new methods discussed below. Another significant advance has been the use of improved hardware and software to allow foreground/background operation, in which data acquisition can occur simultaneously with operator interaction (to process stored data or set up new experiments). Some designs even incorporate multiple computers within a single spectrometer, and it is also possible to connect spectrometers to general-purpose minicomputers in network configurations, allowing more sophisticated off-line spectral processing.

Methods

The second factor responsible for many recent applications is that of conceptually new NMR methods. Some of these are listed below.

1) Two-dimensional FT NMR.
2) High-resolution NMR in solids.
3) New kinds of pulse sequences.
4) Chemically induced dynamic nuclear polarization.
5) Multiple quantum NMR.
6) NMR imaging.

This breakdown represents a somewhat arbitrary division of subjects and techniques, as there is a great deal of overlap among the various categories. Before discussing them, however, we summarize the most important parameters obtained from NMR experiments and briefly indicate their applications: (i) chemical shifts (δ) provide information about the structural and electronic environment of a nucleus; (ii) coupling constants (J) also provide structural information, particularly with respect to the relative orientations of neighboring bonds; (iii) relaxation parameters [T_1, T_2, and NOE (nuclear Overhauser effect)] are related to the time response of the nuclear magnetization and thus yield information related to molecular motions as well as structure.

Two-dimensional FT NMR spectroscopy. Although this technique was first proposed in 1971, its widespread application has occurred only in the last 5 years. Briefly, the technique involves the collection of data as a function of two independent time domains, t_1 and t_2, followed by a double Fourier transformation. The resulting two-dimensional spectrum contains one intensity axis and two frequency axes. A large variety of experiments are possible (*5*) depending on the perturbations (frequency or phase of RF irradiation, decoupler level, and so on) that are applied to the nuclear spins

during the intervals t_1 and t_2. We will not discuss exactly how these two-dimensional experiments are carried out, but it is important to recognize that different types of information can be derived from different types of two-dimensional spectra.

The term two-dimensional correlated spectroscopy refers to one general class of experiments in which it is possible to probe the connectivity among coupled nuclei. For example, in nucleotide studies, two-dimensional correlated spectra in which one axis represents ^{31}P chemical shifts and the other axis ^1H chemical shifts allow the ready identification of protons coupled to phosphates. Most applications of this technique to date have involved ^{13}C and ^1H studies. Often it is possible to only partially assign ^{13}C and ^1H spectra of complex organic molecules. A two-dimensional spectrum in which one axis represents ^{13}C chemical shifts and the other ^1H chemical shifts yields cross peaks only for coupled nuclei (that is, there is a degree of correlation between the ^{13}C and ^1H spectra). This information often allows additional assignments to be made in the individual ^{13}C or ^1H spectra.

While two-dimensional correlated spectroscopy provides information that is not directly obtainable from a one-dimensional experiment, another class of experiments, collectively called two-dimensional resolved spectroscopy, simplify complex spectra by spreading the lines in a one-dimensional spectrum into a second dimension. Typical spreading parameters include scalar couplings, dipolar couplings, or chemical shifts. When scalar coupling constants are used as the spreading parameter the technique is called *J*-resolved two-dimensional spectroscopy, and chemical shift and coupling information can be effectively separated. This is a very powerful feature of two-dimensional FT NMR spectroscopy, as it is often very difficult to obtain coupling constants from normal (one-dimensional) spectra of complex molecular systems because of the overlap of many multiplets. In this type of spectroscopy it is possible, for example, to obtain spectra in which one axis represents ^{13}C chemical shifts and another axis scalar C-H coupling constants (the third axis represents peak intensity). Also, in ^1H studies of large biological molecules (*6*), spreading the overlapping peaks in a complex ^1H spectrum into a second dimension often greatly simplifies the spectrum and allows complete peak assignments to be made. By using appropriate projections in this type of *J*-resolved two-dimensional proton spectrum it is, in fact, possible to produce a simulated broadband homonuclear decoupled proton spectrum. Such a spectrum cannot be achieved by other means.

Table 1. Nuclear magnetic resonance sensitivity increases, based on the approximate signal-to-noise ratio (*S/N*) obtained for a particular peak in the ^1H spectrum of a 1 percent sample of ethylbenzene. [Courtesy of E. D. Becker]

Year	Spectrometer	*S/N*
1961	Varian A-60	6
1965	Varian HA-100	30
1969	Varian HR-220	80
1978	Varian XL-200	300
1978	Bruker WH-360	800
1980	Bruker WM-500	2000

The value of two-dimensional spectroscopy in studies of biological systems is evident from Fig. 2, which shows a spectrum of the basic pancreatic trypsin inhibitor. The two-dimensional technique used to obtain this spectrum, called SECSY (for spin-echo correlated spectroscopy) (*7*), is an extremely useful method for determining the connectivity of the various proton resonances; the positions of the cross peaks provide information on which protons are spin-coupled.

NMR spectroscopy of solid samples. At least as much NMR information can be obtained from a solid sample as from a liquid, but it is much more difficult to extract. Hence the development of methods for obtaining high-resolution spectra of solids (*8*) has greatly enhanced the range of potential NMR applications. Here we discuss ^{13}C applications, but the techniques may be readily extended to a number of other nuclei.

Under the conditions normally used to obtain NMR spectra of liquids, a solid sample would yield an extremely broad, featureless spectrum, with line widths up to 20 kHz. Much of the broadening arises from static dipolar interactions, which average to zero in a liquid because of the fast random molecular motion. Interactions between magnetic nuclei (for example, ^{13}C and nearby protons) produce a characteristic dipolar splitting, which depends on the angle between the internu-

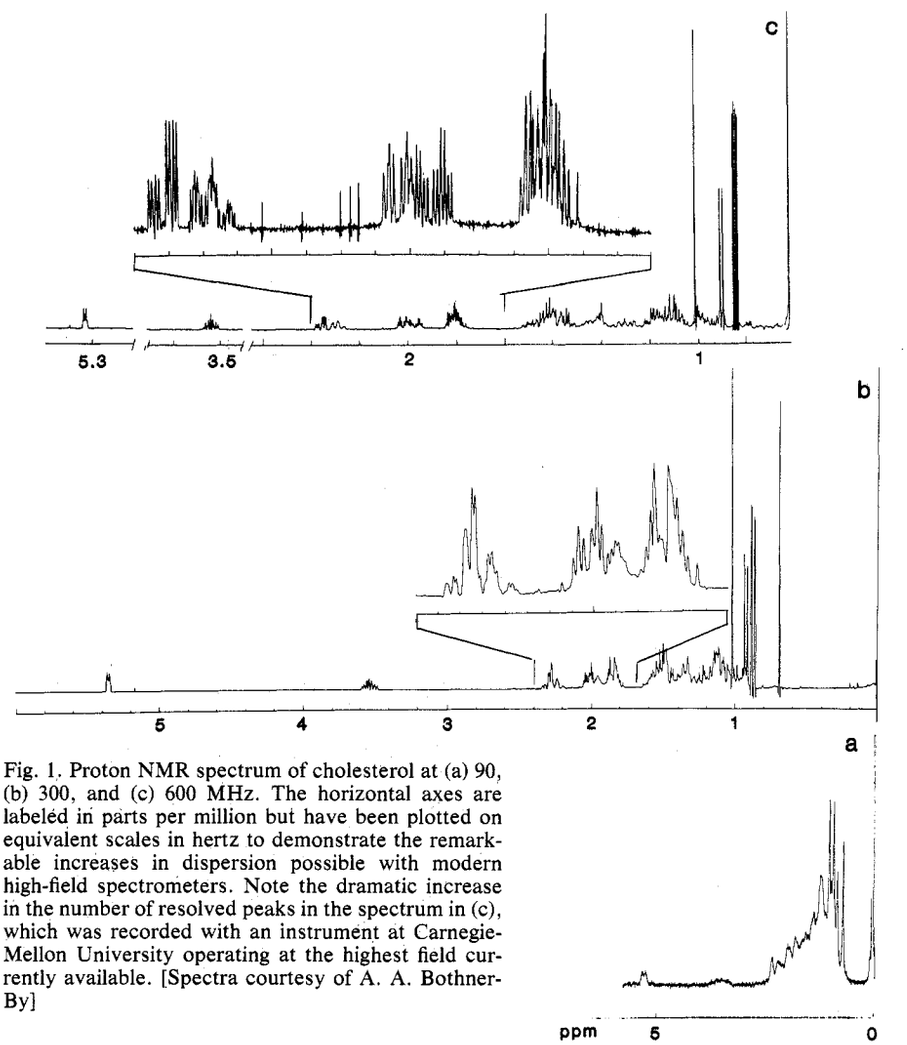

Fig. 1. Proton NMR spectrum of cholesterol at (a) 90, (b) 300, and (c) 600 MHz. The horizontal axes are labeled in parts per million but have been plotted on equivalent scales in hertz to demonstrate the remarkable increases in dispersion possible with modern high-field spectrometers. Note the dramatic increase in the number of resolved peaks in the spectrum in (c), which was recorded with an instrument at Carnegie-Mellon University operating at the highest field currently available. [Spectra courtesy of A. A. Bothner-By]

clear C-H vector and the external magnetic field. In an amorphous solid sample there are a large number of possible orientations of a given internuclear vector and hence a large number of dipolar splittings. The observed broad spectrum is a summation of the many sharper lines arising from individual dipolar interactions. It is possible to remove these dipolar splittings by observing the ^{13}C signal in the presence of broadband irradiation at proton frequencies. This is similar to the method used to remove C-H scalar couplings in ^{13}C spectra of liquids, but a much higher power level is required for the ^{1}H irradiation.

Spectra of solids obtained by high-power proton irradiation (often termed dipolar decoupling) can have much of the initial broadening removed but still have line widths of 5 to 10 kHz or more. This broadening is due to chemical shift anisotropy. The observed broad envelope is produced by the many individual chemical shifts of nuclei in molecules oriented differently within the sample. An examination of the appropriate equations reveals that the anisotropy can be removed if the sample is spun rapidly at an angle of 54.7° with respect to the external magnetic field—a technique called magic angle spinning (MAS). In effect, the solid sample is made to act like a liquid by physically spinning it at the magic angle.

By use of dipolar decoupling and MAS it is possible to produce spectra of solids with nearly as much resolution as spectra of liquids. However, there is one further complication. Since an experiment conducted with ^{13}C in natural abundance has an inherently low sensitivity, it is usually necessary to accumulate many scans so that coherent signals will be increased and random noise averaged out. This can be very time-consuming if many scans are required, so in a FT experiment it is necessary to pulse as quickly as possible. The factor that determines how fast this can be done is the time taken for the ^{13}C magnetization to recover to its equilibrium position, or the spin-lattice relaxation time (T_1), which is generally much longer in solids than in liquids. This restriction severely limits the signal-to-noise ratio that can be obtained in a given time for a solid sample.

The sensitivity problem in ^{13}C studies of solid samples is overcome by the technique of cross polarization (CP) (*9*). This method not only brings about an increase in sensitivity by allowing the magnetization from the abundant ^{1}H nuclear spins to be transferred (cross polarized) to the dilute ^{13}C nuclei, but also allows signal accumulation to be repeated at intervals related to the shorter ^{1}H relaxation times. The method by which this cross polarization is carried out (*8–10*) involves a pulse technique requiring high-power amplifiers and irradiation coils. Because of the way the CP experiment is done, dipolar decoupling is also usually achieved simultaneously, allowing both sensitivity and line widths to be improved. In fact, it is also usual to perform the unrelated MAS experiment in conjunction with cross polarization

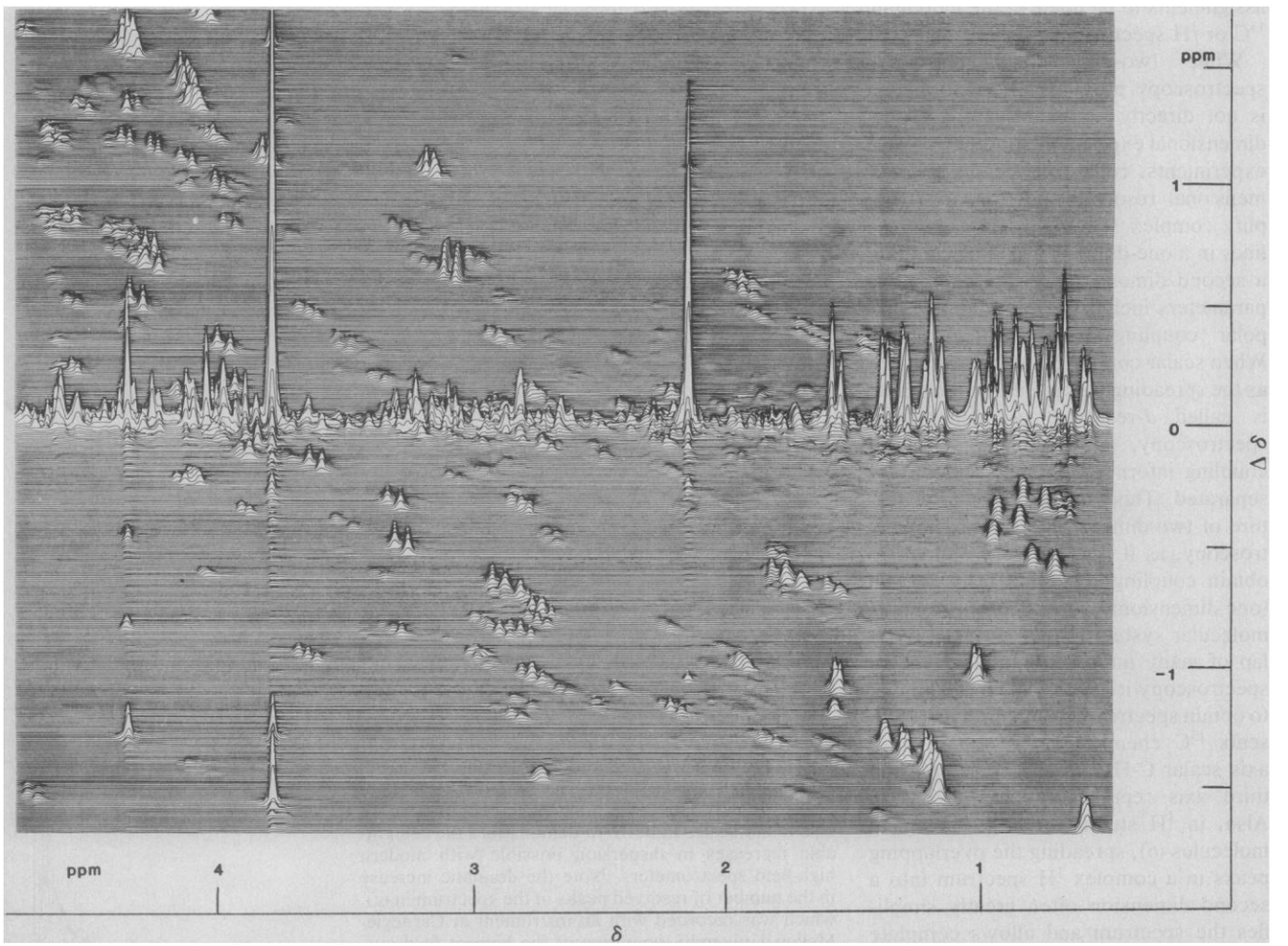

Fig. 2. Section of a 360-MHz two-dimensional spin-echo correlated ^{1}H NMR spectrum of a 0.01*M* solution of the globular protein BPTI (basic pancreatic trypsin inhibitor). The chemical shift δ corresponds to that in a conventional one-dimensional spectrum; Δδ represents difference frequencies for correlated nuclei. Cross peaks between spin-coupled nuclei are at $\pm 1/2\ \Delta\delta$. [Spectrum courtesy of R. R. Ernst]

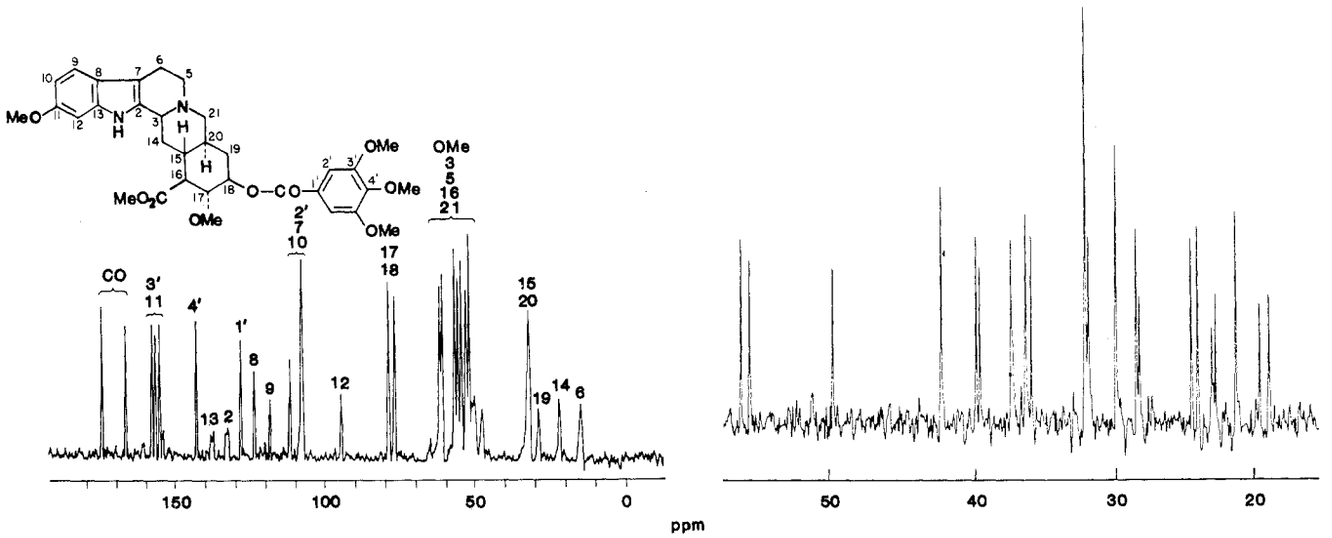

Fig. 3 (left). Carbon-13 solid-state CPMAS spectrum of reserpine at 75.46 MHz obtained with a Bruker CXP-300 NMR spectrometer. [Spectrum courtesy of D. Muller, Bruker Analytische Messtechnik GmbH] Fig. 4 (right). Natural abundance ^{13}C spectrum at 50 MHz of 200 μg of cholesterol obtained in a Tiltmicro probe on a JEOL FX-200 spectrometer. The spectrum was obtained from 50,000 scans (14 hours); the saturated aliphatic region is shown. [Spectrum courtesy of M. J. Albright, JEOL USA]

(these are called CPMAS experiments). Figure 3 (*11*) shows the remarkable resolution obtained in a CPMAS spectrum of solid reserpine.

The discussion of NMR in solids has so far concerned amorphous or powdered samples. Another class of experiments involves the examination of oriented or partially oriented solid or semisolid gel samples and adsorbed species. Experiments of this type include studies of oriented single crystals, or molecules aligned in liquid crystalline phases (*12*) or adsorbed on catalyst surfaces.

Multipulse techniques. The simple repetitive single-pulse FT NMR experiment has been augmented by pulse schemes designed to probe different parameters or phenomena or to improve sensitivity. One example is the two-pulse "inversion recovery" sequence used to determine T_1 values, which in turn yield information about structure and fast molecular dynamics in solution. A newer pulse sequence, designed to increase sensitivity for nuclei in low natural abundance, is used in the CP experiment discussed above. Other pulse sequences (*13*) can be used to reduce line broadening associated with homonuclear dipolar interactions in solids.

Along with specialized pulse sequences developed for solids, there has been interest in alternative sequences for liquid samples. One new sequence, which is gaining widespread application, has been given the acronym INEPT (insensitive nuclei enhanced by polarization transfer) (*14*). It provides significant signal enhancements, particularly for nuclei such as ^{15}N or ^{29}Si, but also provides a means of determining signal multiplicity in proton-decoupled ^{13}C spectra. The pulse sequences discussed so far, and indeed most other sequences used in FT NMR, may be regarded as nonselective in that they excite all spins in a given bandwidth simultaneously. In recent years, a number of new selective pulsed excitation (*15*) methods have emerged which use tailored pulse sequences to excite chosen frequency regions within the spectral bandwidth. Applications include studies of slow chemical exchange, suppression of solvent resonances, and selective inversion of particular resonances in complex multiplets.

Chemically induced dynamic nuclear polarization (CIDNP). This term has been used to describe the enhancement of nuclear polarization observed during certain chemical reactions. The effect is manifest (in NMR spectra of compounds undergoing radical reactions) by the observation of dramatically enhanced absorption or emission (inverted) lines. The CIDNP effect was first noted in 1967 and its applications, particularly in recent years, have increased the utility of NMR as a probe of radical reaction mechanisms and kinetics. The effect allows information to be obtained about chemical events that occur on the time scale of 10^{-8} to 10^{-3} second, and it therefore complements stopped-flow methods, which follow reactions in the millisecond range. Although many CIDNP studies have been performed with ^1H NMR, studies with other nuclei such as ^{19}F, ^{31}P, and particularly ^{13}C have proved of great value in recent years. The CIDNP effect can be induced chemically, thermally, or photochemically, although in the latter case modified probes are required to enable the sample to be irradiated. An application in which a laser was used to initiate CIDNP in protein solutions is described later in this article.

Multiple quantum NMR. The signals observed in normal NMR spectra arise from transitions that obey the selection rule $\Delta m = \pm 1$, where m is the total magnetic quantum number of the spin system. By using special pulse techniques it is possible to excite multiple quantum "coherences" between states where $\Delta m = \pm 2, 3$, and so on. Although not directly observable, these coherences can be converted by pulse techniques into signals that can be detected. In the last few years, studies of multiple quantum transitions (*16*) have been carried out for liquids, liquid crystals, and solids. The method promises to provide information on molecular structures, conformations, and correlated motions. Specifically, the relative simplicity of multiple quantum spectra is useful in determining the structure of molecules aligned in liquid crystals, and relaxation measurements obtained from these spectra are valuable in determining complex anisotropic or correlated molecular motions.

NMR imaging. We use this term to describe a class of experiments in which NMR signals provide information having spatial significance. Most imaging experiments involve the detection of proton signals, although some experiments have been done with other highly sensitive nuclei such as ^{19}F. The instruments

used may be quite different from standard high-resolution NMR spectrometers, but they operate on the same principle: nuclei with a magnetic moment absorb RF energy at a frequency directly proportional to the local external magnetic field. In the imaging technique of zeugmatography, developed by Lauterbur (*17*), a field gradient is superimposed on the main field so that different parts of the sample experience different magnetic fields and hence resonate at different frequencies. The resultant NMR signals provide a linear profile of the distribution of magnetic nuclei across the sample, and by making further scans with gradients in different directions it is possible to reconstruct a two- or three-dimensional image of the sample. Recent developments in field gradient imaging methods are discussed in more detail by Hoult (*18*). Methods that do not involve a static linear field gradient, such as the field focusing technique of Damadian *et al.* (*19*), are also in current use. It is possible to shape the magnetic field to focus on specific volumes within the sample. This technique has been referred to as topical magnetic resonance (*20*), and it enables high-resolution signals to be selectively obtained from small, well-defined sample regions. Many imaging techniques are currently being applied to biological samples including organs and tissues.

Applications in Chemistry

Increased sensitivity has enhanced the utility of NMR as a tool for chemical analysis. It is now possible to obtain natural abundance ^{13}C spectra of moderate-sized organic molecules in large volumes (~10 to 20 milliliters) at submillimolar concentrations, or with several hundred micrograms of material when sample availability is a limiting factor. In the latter case, specially designed probes can be used for optimal results, as shown in the ^{13}C spectrum of 200 μg of cholesterol in Fig. 4. Thus the chemist can use small samples directly from chromatographic separations. Furthermore, NMR is nondestructive and valuable samples can be entirely recovered.

Improvements in sensitivity have been paralleled by advances in the processing and interpretation of NMR spectra. Dozens of new techniques have been developed to aid in the extraction of usable information from complex NMR spectra. Much effort was originally directed toward achieving clear distinctions between closely spaced peaks, and many digital "resolution enhancement" techniques were developed. Two-dimensional FT NMR is a more sophisticated method for separating peaks, and it provides information that helps in assigning peaks to particular atoms in the molecule.

Further assignment aids include pulse sequences that allow the selective inversion of CH, CH_2, or CH_3 resonances in proton-decoupled ^{13}C spectra (*14*), as well as the more routine off-resonance and selective decoupling methods. Multiple quantum methods also promise to be extremely useful for assignment. For example, a combination of two-dimensional spectroscopy and double-quantum NMR allows ^{13}C-^{13}C coupling constants to be readily measured and assigned in natural abundance samples (*21*). With this coupling information it is possible to build up a picture of the connectivity of carbon atoms in a molecule and readily determine the entire structure of the carbon skeleton.

Figure 5 shows the structural information content of a two-dimensional double-quantum spectrum of sucrose (*21*). The spectrum is represented as an intensity contour plot, where the F_1 dimension represents the double-quantum transition frequencies and the F_2 dimension represents the proton-decoupled ^{13}C satellite spectrum arising from the small fraction of molecules containing two ^{13}C nuclei. The strong signals from the larger number of molecules containing only one ^{13}C nucleus have been suppressed. The pairs of coupled ^{13}C nuclei produce four-line intensity patterns (called AB or AX), of which ten are visible in Fig. 5. The F_1 dimension separates these spectra according to their individual double-quantum frequencies (equal to the sum of the chemical shifts of the two carbons with respect to the transmitter frequency), thus identifying them unequivocally.

Polymer chemistry has also benefited from advances in NMR spectroscopy (*22*). The increased dispersion and sensitivity of modern spectrometers allows peaks from individual carbon atoms in complex polymers to be resolved. It is usually possible to accurately determine the local tacticity of polymers through chemical shift differences between carbons attached to different sets of asymmetric centers. NMR has also been used to study side chain and backbone dynamics in polymer systems.

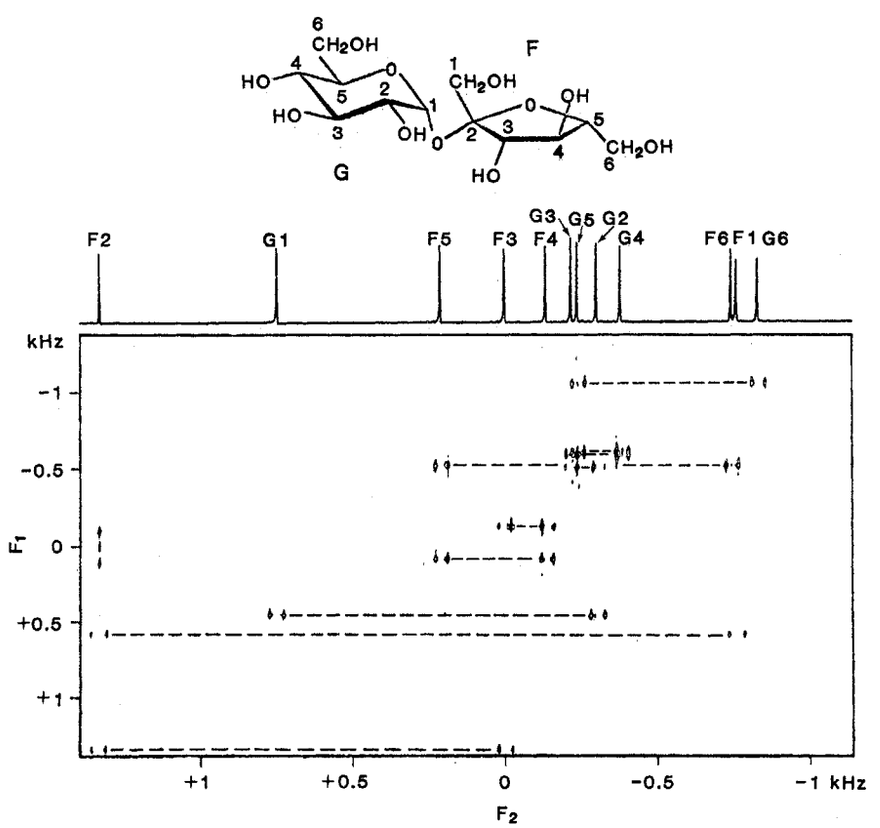

Fig. 5. Two-dimensional double-quantum Fourier transform ^{13}C spectrum of sucrose (*21*). The double-quantum frequencies appear in the F_1 dimension. The F_2 dimension shows AX- or AB-type satellite spectra from molecules containing two coupled ^{13}C nuclei (these four-line patterns are joined by broken lines). The conventional ^{13}C spectrum at the top of the diagram was assigned by noting which resonances have a direct carbon-carbon spin coupling.

Similar advances have been made in the use of ^{13}C NMR for the characterization of fuels. In particular, the techniques discussed above for solid samples have been applied to studies of coal and oil shales. Rapid characterization can be achieved from ^{13}C spectra on the basis of ratios of signal intensities from aromatic and aliphatic spectral regions (*10*).

Other applications in chemistry have arisen from the multinuclear capabilities of modern spectrometers. While ^{15}N studies at natural abundance can be demanding, they provide a unique insight into molecular structure and dynamics (*23*). By virtue of its lone pair of electrons, nitrogen is important in many intermolecular interactions, hence ^{15}N NMR provides an excellent probe for the study of such phenomena as solvent effects, hydrogen bonding, reactivity, and reaction mechanisms.

Metal ions are particularly useful probes in the fields of organometallic and coordination chemistry (*2, 24*). The metal ion is centrally involved in the bonding in organometallic and coordination compounds, and many metals are inherently sensitive monitors of electronic and structural changes. For instance, ^{59}Co chemical shifts cover a range of many thousand parts per million and for this reason have been used to examine structure and bonding in inorganic systems. Examples include the use of ^{59}Co shifts to monitor structural changes in Co(III)–amino acid complexes and to study the solution properties of $Co(NO_2)_6^{-3}$ ions and their decomposition products. Other examples of the value of NMR in inorganic chemistry include ^{103}Rh studies of industrially important $[Rh_{12}(CO)_{30}]^-$ cluster ions and ^{195}Pt studies of the kinetics and hydrolysis of compounds related to the antitumor agent *cis*-dichlorodiamine platinum(II).

In laboratories concerned with the separation and analysis of mixtures it has long been recognized that the direct interconnection of the separation device (for example, liquid chromatography) and the analytical instrument has tremendous advantages in terms of time, labor, and reduction of sample degradation. Gas chromatography–mass spectrometry is already an established analytical technique. Very recently, it has become possible to directly connect liquid chromatography instruments with NMR spectrometers (*25*), largely because of increases in NMR sensitivity. In these instruments ^1H NMR signals are normally detected in flow tubes connected to a high-performance liquid chromatography instrument.

Applications in Biology

The application of NMR spectroscopy to biological or biochemical problems is one of the fastest growing scientific activities. Improved instrumentation has been the main factor responsible for expansion in this area. It is also fair to say that the requirements of biological studies have not just followed instrumental developments, but have in fact dictated ways in which NMR spectrometers have evolved. Probably more than any other physical technique, NMR spectroscopy allows the determination of structure (primary, secondary, and tertiary), interactions, and motion in biological samples (*26*).

Biopolymers. In the past, studies of biopolymers have been somewhat limited by sensitivity. The slow overall molecular motion of biopolymers compared with typical organic molecules produces unfavorable changes in NMR relaxation parameters, effectively decreasing sensitivity and reducing spectral resolution. The instrumentation improvements of the late 1970's made studies of many biopolymers feasible, and both ^1H and ^{13}C NMR methods were applied. One protein that has been extensively studied by ^{13}C NMR is hen egg-white lysozyme, and in several regions of the spectrum of this relatively small enzyme (molecular weight, ~14,000) peaks from individual amino acid residues may be assigned (*27*). In addition to the static or structural nature of biopolymers, NMR allows information on solution dynamics to be obtained; many of the developments in this area were recently reviewed by London (*28*).

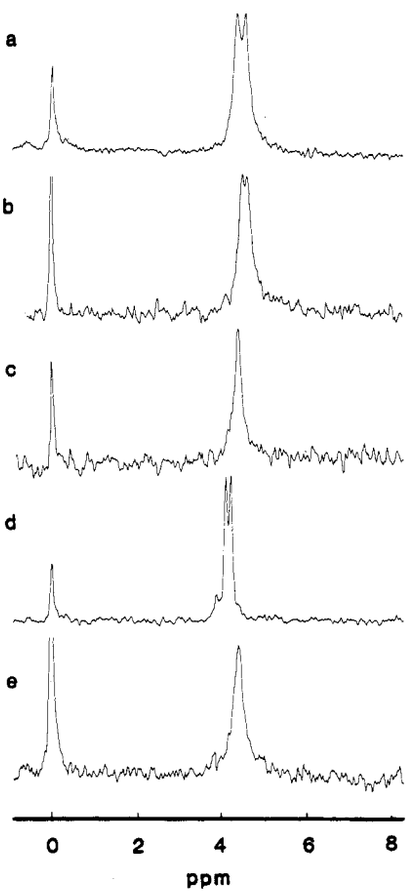

Fig. 6. Phosphorus-31 NMR spectra (*31*) at 109.3 MHz of sonicated alternating purine-pyrimidine deoxynucleotide duplexes: (a) poly(dA-dT)·poly(dA-dT) at 24°C; (b) poly(dA-dbr^{5}U)·poly(dA-dbr^{5}U) at 30°C; (c) poly(dA-dU)·poly(dA-dU) at 31°C; (d) poly(dI-dC)·poly(dI-dC) at 39°C; and (e) poly(dG-dC)·poly(dG-dC) at 23°C. All samples in 0.1*M* NaCl and 0.05 m*M* EDTA, *p*H 6 to 7, except for (b), which was in 5 m*M* tris and 0.1 m*M* EDTA. The peak at 0 ppm is due to a reference compound.

A number of other small proteins have been examined by NMR, including the basic pancreatic trypsin inhibitor (BPTI). For studies of this protein, high-field spectrometers and special techniques such as ^1H two-dimensional FT NMR spectroscopy have proved particularly advantageous (Fig. 2). More esoteric NMR experiments on proteins include high-pressure, high-resolution studies of heme proteins and photo-CIDNP investigations. In the latter case, the irradiation of a protein solution (containing a dye) with a laser beam generates nuclear spin polarization in specific surface residues of the protein. This effect can be used to discriminate between surface and internal residues and to probe protein-substrate interactions (*29*).

Except for small RNA molecules, studies of nucleic acids are more difficult, largely because of their high molecular weights. Proton NMR studies indicated that most transfer RNA molecules in solution have almost identical tertiary structures (*30*). Interactions between tertiary base pairs can be monitored by using resolved N-H resonances in the downfield (deshielded) spectral region of these systems. Proton NMR has also been used to examine the participation of the 2′-OH group in hydrogen-bonding interactions that stabilize particular RNA conformations (*30*).

One difficulty with proton spectra of larger systems is that there are generally many overlapping peaks, even if the experiment is done with a high field. An obvious solution is to probe a nucleus that is not so widely distributed through the molecular system. Phosphorus-31 is an excellent choice, as it is also a nucleus of high inherent sensitivity, and ^{31}P studies have provided much information about structure and bonding in RNA's

and in DNA. Backbone conformations have been determined from high-resolution ^{31}P NMR spectra of medium length (50 to 200 nucleotide pairs), alternating purine and pyrimidine DNA's prepared by sonication of synthetic polymers (*31*). Two of the sequences studied gave singlet ^{31}P resonances (Fig. 6, c and e), while three others yielded two resolved signals of equal area (Fig. 6, a, b, and d), indicating the presence of two distinct alternating phosphodiester backbone conformations. Extensions of such studies may reveal the factors that control the response of backbone conformations to base-pair sequences.

Since the first reports (*32*) of natural abundance ^{13}C NMR spectra of transfer RNA in 1972, there has been much interest in using ^{13}C NMR to probe native nucleic acids (*33, 34*). Recent measurements of both native and denatured DNA (~110 to 160 nucleotide pairs) have demonstrated the ability to resolve peaks for sugar and base carbons (Fig. 7) and to use NMR relaxation parameters to probe motion at these sites (*34*).

The high sensitivity and large chemical shift range of the ^{19}F nucleus make it a potentially useful probe for biological systems (*35*). Its disadvantage is that it must be artificially incorporated into the system being studied. However, this is generally not a major difficulty, as the small size and relatively low reactivity of the fluoro group mean that it does not cause a large perturbation. In fact, the rare occurrence of fluorine in natural biological materials eliminates background peaks, thus allowing specific signals from only the incorporated fluorine to be observed. Most biological ^{19}F studies to date have involved protein systems, particularly enzymes. Examples include insulin, dihydrofolate reductase, ribonuclease, lysozyme, cytochrome c, and hemoglobin. Recent studies have taken advantage of the fact that many microorganisms can incorporate fluorinated amino acids into their proteins.

The structure and function of lipids and biological membranes in general are also of current interest, and ^{13}C and ^{2}H NMR are particularly useful for studying the dynamic behavior of these systems (*36*). For example, ^{2}H NMR has been used to evaluate the order parameter that describes lipid organization, and ^{13}C spin lattice relaxation times have provided information on segmental motion and liberations in lipid chains. Figure 8 shows some recent ^{2}H spectra of selectively deuterated membranes of the microorganism *Acholeplasma laidlawii*. Below the temperature at which the organism grows, 37°C, the spectra begin to broaden due to the formation of gel-state lipid. Information on the fraction of membrane lipid in the liquid crystalline and gel states can be obtained by analyzing such spectra (*36*).

Although ^{1}H, ^{2}H, ^{13}C, ^{19}F, and ^{31}P have been used in most biological studies to date, there have also been investigations with other nuclei. For example, ^{23}Na and ^{35}Cl NMR studies have exploited the presence of Na^+ and Cl^- ions in many biological systems to study ion binding to proteins. Nitrogen-15 NMR has been used in nucleotide binding studies. Metal nuclide NMR has proved of great benefit in studies of metalloenzymes and metal binding in biological systems in general.

Whole cells and organs. NMR spectroscopy of intact cells has predominantly involved the ^{31}P nucleus. Initial studies of erythrocytes showed that ^{31}P peaks could be detected from different types of phosphorus within the cells and that it was possible to distinguish between intracellular and extracellular *p*H by using the slightly different chemical shifts of resonances from inorganic orthophosphate in these two regions. Numerous ^{31}P studies of bioenergetics and metabolism in *Escherichia coli* have also been performed (*37*). Recently, ^{13}C NMR has also been used in whole cell studies. High-resolution ^{13}C NMR spectra of isotopically enriched metabolites in vivo can be obtained in minutes and allow sophisticated studies of, for example, glucose transport and metabolism. In the last few years ^{15}N NMR has also been used to examine intact cells.

Studies of whole organs and tissues have been made possible largely by innovations in instrument design, particularly probe and sample geometries. In the first studies (*38*), reported in 1974, ^{31}P NMR was used to detect major metabolites such as adenosine triphosphate (ATP), phosphocreatine, inorganic phosphate,

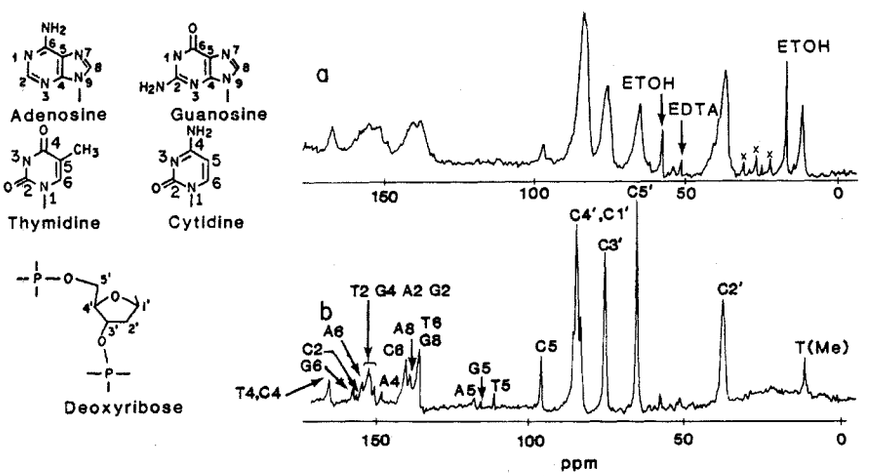

Fig. 7. Carbon-13 NMR spectra (*34*) of (a) double-stranded and (b) single-stranded calf thymus DNA (120 nucleotide pairs) at 100.6 MHz. Conditions were: (a) 80 mg/ml, 32°C, 8000 scans, 20-Hz digital broadening, and 1-second scan interval; (b) 80 mg/ml, 85°C, 3500 scans, 20-Hz digital broadening, and 1.5-second scan interval.

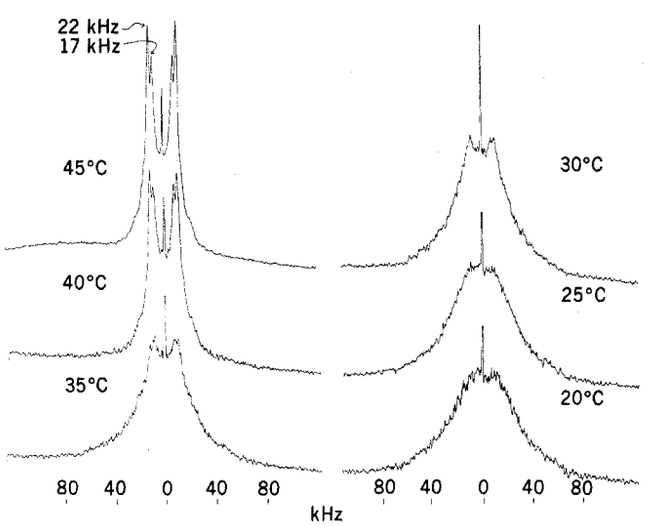

Fig. 8. Deuterium NMR spectra (*36*) (46 MHz) of the membranes of *Acholeplasma laidlawii* enriched to 94 percent in the single fatty acid [2,2-^{2}H]tetradecanonic acid. [Spectra courtesy of I. C. P. Smith]

and sugar phosphate. The ^{31}P spectra as a function of time demonstrated that aging of the muscle resulted in the breakdown of phosphocreatine followed by that of ATP. More recently, these types of studies have been extended to perfused organs such as mammalian hearts and kidneys (*39*).

A very recent development that promises to extend the capabilities of in situ organ and tissue studies is the introduction of surface coils (*40*). These allow greater flexibility than many conventional NMR receiver coils and can be designed and shaped for specific applications. When surface coils are used with a spatial localization technique such as topical magnetic resonance, high-resolution ^{31}P NMR spectra can be obtained from almost any part of an intact laboratory animal. The feasibility of using ^{31}P NMR to study metabolism is already well established. For example, in a recent study (*40*) the metabolic state of skeletal muscle and brain in living rats was determined by using ^{31}P NMR to monitor ATP levels. The application of similar approaches to study human limbs is under way.

A clinical application of the ^{31}P NMR metabolic technique was reported recently (*41*). The case concerned a patient with suspected McArdle's syndrome, a metabolic defect caused by lack of glycogen phosphorylase activity in skeletal muscle. Phosphorus-31 NMR aided in the diagnosis by showing that the patient differed from normal subjects in having no fall in intramuscular *p*H and an excessive reduction in phosphocreatine in response to exercise.

The recent developments in NMR studies of cells, organs, and whole organisms indicate a bright future for NMR in biomedical disciplines. It will be used by the pharmacologist as a structural probe for new drugs, will aid the pathologist in analyses, and, we believe, will eventually become a routine screening or diagnostic device. In another example of the latter application, ^{31}P NMR is being used in preliminary experiments to determine the metabolic state of kidneys and hence their suitability for transplantation (*42*).

NMR imaging. The technique of NMR imaging is one that interests not only scientists but also nonscientists, as it promises to provide a safe, noninvasive, and rapid means of screening for or diagnosing dysfunction or disease in human tissue. Routine clinical whole-body scanning of human patients is some years away. However today, in controlled laboratory environments, it is possible to obtain NMR images of cross sections of human subjects at sufficient resolution (a few millimeters) to yield valuable physiological information.

Many applications of NMR imaging are based on the fact that different tissues or organs have different water contents, and hence a spin density map of the protons in water molecules should provide an image with anatomical detail. The validity of this approach has been demonstrated by NMR-produced images of human hands and limbs and torso and brain cross sections. Graphic photographs and descriptions of the techniques associated with NMR imaging may be found in a recent account of a symposium on NMR of intact biological systems (*43*). In addition to images based on proton spin density maps, it is possible to obtain images that monitor spin lattice relaxation times as a function of spatial location; these are of interest because of the proposal (*44*) that T_1 values of water protons in cancerous or damaged cells are longer than those of protons in normal cells.

References and Notes

1. For a general introduction to NMR spectroscopy, see E. D. Becker, *High Resolution NMR—Theory and Chemical Applications* (Academic Press, New York, ed. 2, 1980).
2. R. K. Harris and B. E. Mann, Eds., *NMR and the Periodic Table* (Academic Press, London, 1978).
3. R. R. Ernst and W. A. Anderson, *Rev. Sci. Instrum.* **37**, 93 (1966).
4. For a review of the instrumental requirements and methods associated with Fourier transform NMR, see D. Shaw, *Fourier Transform NMR Spectroscopy* (Elsevier, New York, 1976); T. C. Farrar and E. D. Becker, *Pulse and Fourier Transform NMR* (Academic Press, New York, 1971); K. Mullen and P. S. Pregosin, *Fourier Transform NMR Techniques: A Practical Approach* (Academic Press, New York, 1976).
5. W. P. Aue, E. Bartholdi, R. R. Ernst, *J. Chem. Phys.* **64**, 2229 (1976); G. Bodenhausen, R. Freeman, R. Niedermeyer, D. L. Turner, *J. Magn. Reson.* **26**, 133 (1977); R. Freeman and G. A. Morris, *Bull. Magn. Reson.* **1**, 1 (1979); D. Terpstra, in *Topics in Carbon-13 NMR Spectroscopy*, G. C. Levy, Ed. (Wiley, New York, 1979), vol. 3.
6. K. Nagayama, P. Bachmann, K. Wuthrich, R. R. Ernst, *J. Magn. Reson.* **31**, 133 (1978).
7. K. Nagayama, A. Kumar, K. Wuthrich, R. R. Ernst, *ibid.* **40**, 321 (1980).
8. M. Mehring, in *NMR Basic Principles and Progress*, P. Diehl, E. Fluck, R. Kosfeld, Eds. (Springer, Berlin, 1976), vol. 11; U. Haeberlen, *Adv. Magn. Reson. Suppl. 1* (1976); J. Schaefer and E. O. Stejskal, in *Topics in Carbon-13 NMR Spectroscopy*, G. C. Levy, Ed. (Wiley, New York, 1979), vol. 3.
9. A. Pines, M. G. Gibby, J. S. Waugh, *J. Chem. Phys.* **59**, 569 (1973).
10. F. P. Miknis, V. J. Bartuska, G. E. Maciel, *Am. Lab.* **11**, 19 (1979); G. E. Maciel, V. J. Bartuska, F. P. Miknis, *Fuel* **58**, 391 (1979).
11. G. C. Levy, R. L. Lichter, G. L. Nelson, *Carbon-13 Nuclear Magnetic Resonance Spectroscopy* (Wiley, New York, ed. 2, 1980), chap. 8.
12. J. W. Emsley and J. C. Lindon, *NMR Spectroscopy Using Liquid Crystal Solvents* (Pergamon, Oxford, 1975).
13. These experiments can be considered an inverse of the MAS technique. Instead of the sample being spun at 54.7° with respect to the external field, the RF field is made to undergo "effective" motion about this angle. Examples are the four-pulse WAHUHA sequence, named after its inventors, J. S. Waugh, L. M. Huber, and U. Haeberlen [*Phys. Rev. Lett.* **20**, 180 (1968)] and the newer MREV-8 (eight-pulse) sequence.
14. G. A. Morris and R. Freeman, *J. Am. Chem. Soc.* **101**, 760 (1979); D. P. Barum and R. R. Ernst, *J. Magn. Reson.* **39**, 163 (1980); D. M. Doddrell and D. T. Pegg, *J. Am. Chem. Soc.* **102**, 6390 (1980).
15. H. Hill, in *Topics in Carbon-13 NMR Spectroscopy*, G. C. Levy, Ed. (Wiley, New York, 1979), vol. 3; G. A. Morris and R. Freeman, *J. Magn. Reson.* **29**, 433 (1978).
16. S. Vega and A. Pines, *J. Chem. Phys.* **66**, 5624 (1977); A. Wokaun and R. R. Ernst, *Chem. Phys. Lett.* **52**, 407 (1977).
17. P. C. Lauterbur, *Nature (London)* **242**, 190 (1973).
18. D. I. Hoult, in *Magnetic Resonance in Biology*, J. S. Cohen, Ed. (Wiley, New York, 1980), vol. 1, chap. 2.
19. R. L. Damadian, L. Minkoff, M. Goldsmith, J. Koutcher, *Naturwissenschaften* **65**, 250 (1978).
20. R. E. Gordon, P. E. Hanley, D. Shaw, D. G. Gadian, G. K. Radda, P. Styles, L. Chan, P. J. Bore, *Nature (London)* **287**, 736 (1980).
21. A. Bax, R. Freeman, T. A. Frenkiel, M. H. Levitt, *J. Magn. Reson.* **43**, 478 (1981).
22. A. E. Woodward and F. H. Bovey, Eds., *ACS Symp. Ser. 142* (1980); J. C. Randall, *Polymer Sequence Determination: Carbon-13 NMR Method* (Academic Press, New York, 1977).
23. G. C. Levy and R. L. Lichter, *Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy* (Wiley, New York, 1980).
24. P. L. Rinaldi, G. C. Levy, G. R. Choppin, *Rev. Inorg. Chem.* **2**, 53 (1980); P. S. Pregosin and L. M. Venanzi, *Chem. Br.* **14**, 276 (1978).
25. E. Bayer, K. Albert, M. Nieder, E. Grom, T. Keller, *Adv. Chromatogr.* **14**, 525 (1979); J. F. Haw, T. E. Glass, D. W. Hausler, E. Motell, H. C. Dorn, *Anal. Chem.* **52**, 1135 (1980).
26. R. G. Shulman, Ed., *Biological Applications of Magnetic Resonance* (Academic Press, New York, 1979); S. J. Opella and P. Lu, Eds., *NMR and Biochemistry* (Dekker, New York, 1978); J. S. Cohen, Ed., *Magnetic Resonance in Biology* (Wiley, New York, 1980).
27. R. S. Norton, A. O. Clouse, R. Addleman, A. Allerhand, *J. Am. Chem. Soc.* **99**, 79 (1977).
28. R. E. London, in *Magnetic Resonance in Biology*, J. S. Cohen, Ed. (Wiley, New York, 1980).
29. R. Kaptein, in *NMR Spectroscopy in Molecular Biology*, B. Pullman, Ed. (Reidel, Dordrecht, 1978).
30. D. R. Kearns, T. Early, P. H. Bolten, in *Frontiers in Physiochemical Biology*, B. Pullman, Ed. (Academic Press, New York, 1977), p. 65.
31. J. S. Cohen, J. B. Wooten, C. L. Chatterjee, *Biochemistry* **20**, 3049 (1981).
32. R. A. Komoroski and A. Allerhand, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1804 (1972).
33. R. L. Rill, P. R. Hilliard, Jr., J. T. Bailey, G. C. Levy, *J. Am. Chem. Soc.* **102**, 418 (1980); M. E. Hogan and O. Jardetzky, *Biochemistry* **19**, 3460 (1980); P. H. Bolton and T. L. James, *ibid.*, p. 1388.
34. G. C. Levy, P. R. Hilliard, Jr., L. F. Levy, R. Inners, R. L. Rill, *J. Biol. Chem.*, in press.
35. J. T. Gerig, in *Biological Magnetic Resonance*, L. J. Berliner and J. Reuben, Eds. (Plenum, New York, 1978).
36. H. C. Jarrell, R. A. Byrd, I. C. P. Smith, *Biophys. J.*, in press; R. E. Jacobs and E. Oldfield, *Prog. NMR Spectrosc.* **14**, 113 (1981).
37. K. Ugurbil, R G. Shulman, T. R. Brown, in *Biological Applications of Magnetic Resonance*, R. G. Shulman, Ed. (Academic Press, New York, 1979).
38. D. I. Hoult, S. J. Busby, D. G. Gadian, G. K. Radda, R. E. Richards, P. J. Seeley, *Nature (London)* **252**, 285 (1974).
39. D. G. Gadian, G. K. Radda, R. E. Richards, P. J. Seeley, in *Biological Applications of Magnetic Resonance*, R. G. Shulman, Ed. (Academic Press, New York, 1979).
40. J. J. H. Ackerman, T. H. Grove, G. C. Wong, D. G. Gadian, G. K. Radda, *Nature (London)* **283**, 167 (1980).
41. B. D. Ross, G. K. Radda, D. G. Gadian, G. Rocker, M. Esiri, J. Falconer-Smith, *N. Engl. J. Med.* **304**, 1338 (1981).
42. P. J. Seeley, P. A. Sehr, D. G. Gadian, P. B. Garlick, G. K. Radda, in *NMR in Biology*, R. A. Dwek, I. D. Campbell, R. E. Richards, R. J. P. Williams, Eds. (Academic Press, London, 1977).
43. R. J. P. Williams, E. R. Andrew, and G. K. Radda, *Philos. Trans. R. Soc. London Ser. B* **289**, 379 (1980).
44. R. Damadian, *Science* **171**, 1151 (1971).

45. Financial support from the National Science Foundation and the National Institutes of Health (G.C.L.) and CSIRO Australia (D.J.C.) is acknowledged. We thank E. D. Becker for helpful criticisms. A. A. Bothner-By, J. Cohen, R. R. Ernst, R. Freeman, P. C. Lauterbur, E. Oldfield, G. Radda, and I. C. P. Smith kindly supplied unpublished materials.