REPORT

A BRIEF HISTORY OF NUCLEAR MAGNETIC RESONANCE

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NMR in bulk materials was first reported in 1946 by Bloch, Hansen, and Packard at Stanford (*1*) and by Purcell, Torrey, and Pound at Harvard (*2*). By 1952 the importance of their discovery was recognized when the Nobel Prize in Physics was awarded to Bloch and Purcell.

In the past 40 years NMR spectroscopy has bloomed into a major tool in analytical chemistry, a valuable method for studying physical phenomena from kinetics to superconductivity, a technique in structural biology that rivals X-ray crystallography, a routine procedure for imaging in diagnostic radiology, a way to study metabolic function in humans and animals, and an evolving method in materials science.

Continuous improvements in NMR instruments and techniques are fostering new and important applications. One recent recognition of the importance of NMR methods was the selection of Richard Ernst as the 1991 Nobel Laureate in Chemistry for his work in developing FT-NMR spectroscopy and its derivatives.

In this REPORT the overall development of NMR and some of the major mileposts in its history are discussed. In the limited space available, I cannot be comprehensive in presenting the topic or give credit to all of the investigators who have pioneered advances in NMR methods and applications to physics, chemistry, biology, and other sciences. I have included names of some 40–50 individuals to personalize the history of NMR, but many other names could equally well have been selected. Some references to innovative publications are given, but many of the literature citations refer to books or review articles. (A far more complete history is planned as part of the *Encyclopedia of NMR*, currently being prepared for publication in 1995 [*3*]). Although the development of NMR is, of course, continuous, it is useful to divide the history into decades, beginning with the first publications in 1946. But we should start with a bit of background on the early years.

Prehistory: 1926–45

The concepts of electron spin and the magnetic moment of the electron had been firmly established in the early 1920s by a number of studies, especially the Stern–Gerlach experiment, in which beams of atoms were separated in an inhomogeneous magnetic field according to the orientation of the electron magnetic moment. In the mid-1920s it became apparent that many features in atomic spectra could be accounted for only if certain atomic nuclei likewise possessed spin and a magnetic moment. Refinements of the Stern–Gerlach experiment verified this concept by 1933.

In 1939 Rabi et al. (*4*) made a major improvement in beam techniques by sending a stream of hydrogen molecules through not only the inhomogeneous magnetic field required for deflection, but also through a homogeneous magnetic field, where they were subjected to radio frequency (rf) electromagnetic energy. Energy was absorbed by the molecules at a sharply defined frequency, and the absorption caused a small but measurable deflection of the beam. This was the first observation of NMR, and Rabi received the Nobel Prize in 1944. However, such studies were limited to nuclei in small molecules under very high vacuum in a molecular beam, the deflection of which served to detect the resonance.

The contribution of nuclear magnetic moments to bulk magnetic susceptibility had been demonstrated in 1937 for hydrogen at low temperature (about 2 K), but this approach had limitations (*5*). In 1936 Gorter unsuccessfully attempted to observe magnetic resonance in solid LiF and other inorganic salts by detecting the heat produced when resonant rf energy was absorbed. In 1942 he tried again, this time looking for an anomalous dispersion of the rf field (*6*). The failure of these attempts was largely attributable to the unfortunate choice of LiF, which has a long relaxation time, as the sample.

Decade of discovery: 1946–55

Bloch took a different approach. He knew that, by applying rf energy, the macroscopic nuclear magnetization could be rotated away from its equilibrium position parallel to the applied magnetic field (Figure 1). From the laws of physics, he knew that this displaced magnetization would then precess about the magnetic field at a well-defined frequency. Bloch reasoned that this precessing magnetization would induce an electrical signal in an appropriately placed copper coil at this frequency, which is in the rf range.

Bloch, Hansen, and Packard (*1*) tried the experiment with a sample of water. It worked, and NMR (or nuclear induction, as Bloch called it) was born. Meanwhile, Purcell, Torrey, and Pound had been able to directly measure the small absorption of rf energy by the proton magnetic moments in a block of paraffin (*2*). Although their experiment was quite different from Bloch's, the same phenomena are involved and the two approaches worked equally well. Interestingly, Bloch and Purcell had never met each other at the time that their papers appeared, just a few weeks apart.

The early days of NMR must have been exciting—basic principles were elucidated and applications of the new method were explored. The construction of magnets that were sufficiently homogeneous and stable to permit observation of reasonably narrow nuclear resonances in liquids was a tour de force. Likewise, major effort was put into the design and construction of electronic circuits from the primitive components then available to detect the weak NMR signal in the presence of unavoidable, thermally generated electrical noise. The rapid development of NMR owes much to the early decision of Russell Varian to produce a commercial system based on a homogeneous electromagnet. Researchers could buy a basic system and, although they might have to modify it, they did not have to build magnets and amplifiers from scratch.

Early work by Bloembergen, Purcell, and Pound (*7*) explained the concepts of nuclear relaxation and showed why NMR signals from solids are orders of magnitude wider than those from liquids, where rapid molecular Brownian motion causes nuclear magnetic dipole–dipole interactions to average to zero. As magnet homogeneity improved, the resonance lines from liquids became narrower and narrower, thus permitting more precise measurement of the resonance frequencies.

The basic NMR relationship is

$$\omega = \gamma B_{\text{nucleus}} \qquad (1)$$

where the resonance frequency ω depends on the magnetogyric ratio γ (a property of the nucleus) and the magnetic field applied to the nucleus B_{nucleus}. It was anticipated that a given nucleus would show the same frequency at a fixed value of the applied magnetic field, regardless of which chemical compound the nucleus resides in. In 1949 and 1950, however, observations of the signals from ^{19}F and ^{31}P showed variations in frequency that were beyond the (still rather large) experimental error. Thus it was postulated that the magnetic properties of the electrons surrounding the nucleus provide a shielding σ of the applied magnetic field B_0

$$\omega = \gamma B_0(1 - \sigma) \qquad (2)$$

where the value of σ depends on the density and configuration of electrons. This shift in the resonance frequency from what had been anticipated was called the chemical shift. It was initially an annoyance to the physicists who found that chemical shifts limited the accuracy of their measurements of magnetogyric ratios but, as it turns out, it provided the cornerstone for applying NMR to chemistry.

The chemical shift for ^1H was demonstrated only after further improvements in the homogeneity and stability of magnetic fields, because—as we now know—the range of shieldings for protons is orders of magnitude smaller than the range of shieldings for other nuclei. In 1951 the dramatic demonstration of the ^1H chemical shift in ethanol (*8*) (Figure 2) first made it clear to chemists what NMR spectroscopy might do as an analytical method.

Meanwhile, further improvements in resolution revealed that even chemically shifted resonances were, in many instances, collections of separate resonance lines. When analyses by Gutowsky and McCall (*9*) indicated that the spins of neighboring nuclei are responsible for these multiple lines, a new mechanism had to

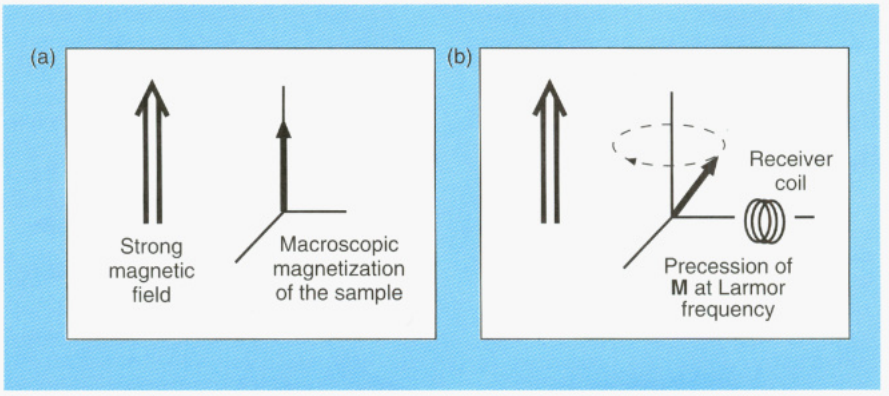


Figure 1. Tipping macroscopic nuclear magnetization away from (a) its equilibrium position parallel to the applied magnetic field **M** and (b) the resulting precession of **M** that induces an electrical signal in the receiver coil.

be constructed, because it was known that magnetic dipolar interactions average to zero in rapidly tumbling molecules. Thus the concept of indirect spin–spin coupling or scalar coupling was conceived (*10*). Not long after, it was found that some spin coupling (as in the OH group of ethanol) failed to produce the expected multiplets and the idea of chemical exchange was developed.

The standard procedure for observing a resonance line was to vary the frequency (or, more commonly, the strength of the magnetic field) through the resonance condition, display the deflection on an oscilloscope, and record it. This continuous wave (cw) method remained the standard for many years because it permits sequential observation of each of the many resonance lines in a spectrum. Faithful representation of the lineshape, however, requires a slow passage through resonance, thus necessitating improved stability of the instrument and several minutes to complete a scan.

Bloch suggested an alternative method of excitation using a short rf pulse (*11*). In 1949 Hahn showed that this procedure did indeed produce a free precession signal. Moreover, he showed that sequences of pulses could be used to generate additional information in the form of a spin echo (*12*). Pulse methods came into use, largely by physicists, to study systems with a single line, such as the broad line of a solid sample. However, for many years the method was of little use to chemists because of the complexity of the free induction decay (FID) signal obtained following the excitation pulse.

Decade of chemical applications: 1956–65

By the mid-1950s the basic physics of NMR and its potential value in chemistry had been elucidated, and commercial instruments were available. The instruments were very primitive by today's standards. In 1956 the observation frequency for ^1H NMR spectroscopy was only 40 MHz, fixed by a crystal at one specific frequency. The field of the electromagnet was stabilized independently of the rf by large vacuum tubes that controlled the current and by a feedback loop from the newly invented "super stabilizer." The magnet had to be adjusted for optimum homogeneity by placing thin metal shims behind the pole pieces and tightening the assembly with a huge wrench. (The term "field shimming" still persists and is understood to mean optimization of homogeneity. Today, however, magnet homogeneity is adjusted by varying electric currents in coils that are placed in conjunction with the magnet.)

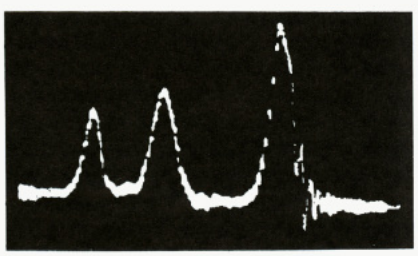
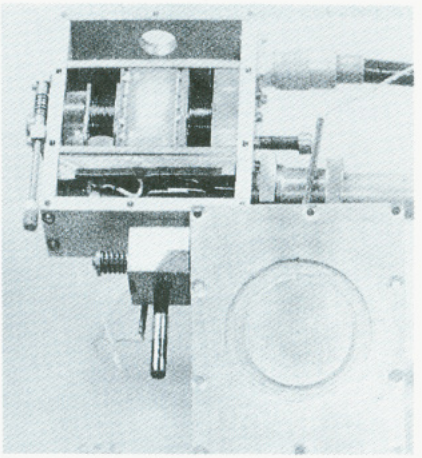


Figure 2. ^1H NMR spectrum of ethanol showing separate resonance lines for the OH, CH_2, and CH_3 protons (left to right).
(Adapted with permission from Reference 8.)



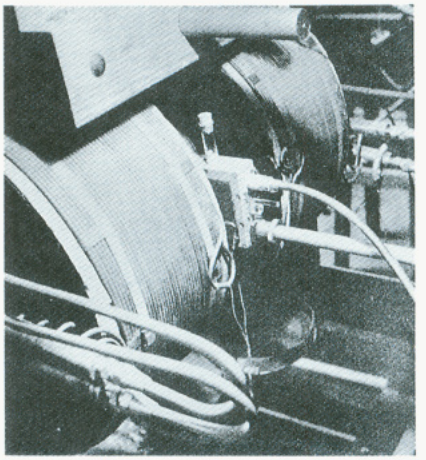


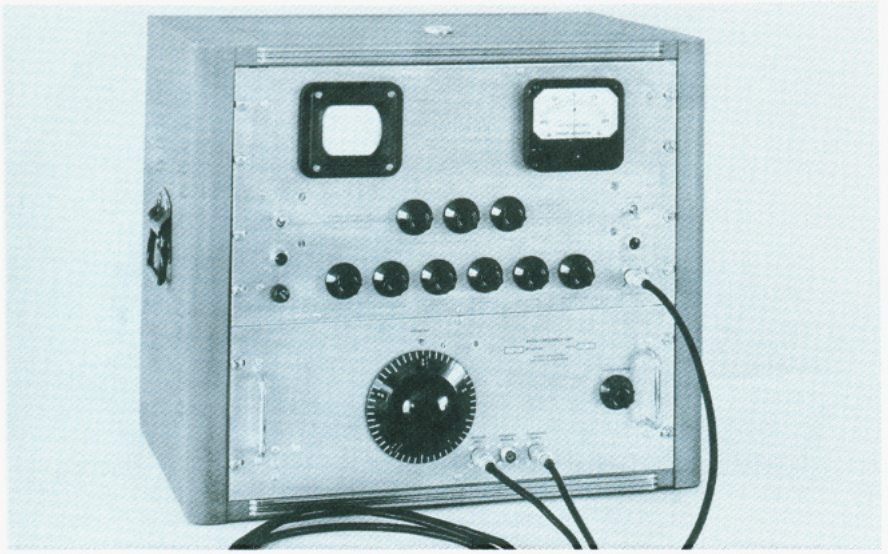
Photo of Bloch's probe with the cover plate removed (left) and electromagnet with the probe containing a sample tube about to be inserted into the magnet gap (right). (From Bloch's Nobel Prize address, entitled "The Principle of Nuclear Induction," reprinted with permission from *Science*, **1953**, *118*, 425.)

Scanning the magnetic field through the range of ^1H resonances usually took about 5–10 min—long enough to avoid serious lineshape distortions. The scan could take no longer, because random drift of the magnetic field might become a dominant factor. In fact, because of field drift, each spectrum had to be calibrated separately and often the average of several scans was used to improve precision. Obtaining NMR spectra was a time-consuming job for an instrumental specialist.

But the rewards for getting a good spectrum were great. Organic chemists soon found that NMR spectroscopy was an ideal technique for elucidating or verifying the structure of moderate-sized molecules. Almost every compound on the shelf gave new and interesting data. Each month, Varian published a series of advertisements called "NMR at Work" in the *Journal of the American Chemical Society*, demonstrating new applications for NMR spectroscopy. Organic chemists and the new breed of NMR spectroscopists awaited these ads as eagerly as they anticipated the research articles.

The classic paper by Shoolery and Rogers (*13*) in 1958 demonstrated the usefulness of NMR spectroscopy in the study of steroids. Even at 40 MHz, ^1H NMR spectra showed that the chemical shifts of angular methyl groups are of diagnostic value. Other work at about the same time showed the wealth of information that could be obtained from NMR spectra of alkaloids, sugars, porphyrins, and other compounds. Soon Karplus (*14*) demonstrated that vicinal three-bond scalar couplings depend on bond angles, and studies of conformation—especially in sugars—became popular. Jackman's book (*15*) in 1959 summarized a wealth of material on correlations between NMR spectra and structural features within a molecule, and Roberts' book (*16*) gave a simple account of basic principles that could easily be grasped by most chemists.

Also during this period, great strides were made in understanding the origin of the complex spectra that were increasingly being observed, where simple considerations of multiple spins interacting to give uncomplicated multiplets are not valid. Quantum treatments of spin interactions and symmetry considerations clearly showed what we should expect, and computer programs were developed to assist in the analysis of complex spectra. The treatise by Pople, Schneider, and Bernstein (*17*) brought this material together, along



Varian F6 nuclear fluxmeter (1949), the first commercially available instrument to use the NMR principle, was used by physicists to measure the magnetic properties of isotopes. (Courtesy of Varian Associates.)

with much theoretical background written from the perspective of chemists.

During this decade improvements in magnet design and construction permitted an increase in field strength so that the ^{1}H NMR frequency rose from 40 MHz to 60 MHz to 100 MHz. Along with advances in electronics, the increased magnetic field strength resulted in enhanced sensitivity and in spectra that were, in some cases, easier to interpret. However, the major advance in instrumentation was the Varian A-60 spectrometer.

This instrument incorporated a simple and practical means for using an NMR signal to maintain a constant relationship between the magnetic field and the rf applied to the sample being studied. Thus, the ever present random drift of the magnetic field was compensated for, and each spectrum could be obtained at a known scan rate. Suddenly, preprinted NMR charts became feasible! Moreover, because of design improvements, the magnet, its power supply, and cooling water unit were reduced in size—the instrument could now fit into an ordinary chemistry laboratory, and the cost decreased. The A-60 spectrometer really brought NMR spectroscopy to the masses (of chemists); more than 1000 instruments were sold worldwide before the market gave way in the late 1960s to instruments based on solid-state electronics.

Two other especially significant developments in NMR methodology deserve mention. One is double resonance, where two rf fields of different frequencies are applied simultaneously—one to observe a signal while the other perturbs the spin system. The concept of double resonance had been known for many years, but it was not until the 1960s that the theory was worked out in detail, principally by Baldeschwieler (then at Harvard) and by Freeman and Anderson (then at Varian). Accessories were developed to permit double-resonance studies with commercial instruments. Numerous applications were undertaken, ranging from simple decoupling of spin multiplets to detailed investigations of energy levels by spin tickling to studies of molecular conformation by the nuclear Overhauser effect (NOE), first introduced to organic stereochemistry in 1965 (*18*).

The other major development was ^{13}C NMR spectroscopy. Because of its low inherent sensitivity relative to ^{1}H and ^{19}F and ^{13}C's natural abundance of only 1.1%, the first study of ^{13}C NMR in a series of organic compounds did not appear until Lauterbur's classic paper (*19*) in 1957. He demonstrated that ^{13}C chemical shifts span a large range and provide potentially useful information. Even then, little could be done in a practical way until double-resonance methods were brought to bear to collapse spin multiplets and introduce NOE enhancements, which together increased signal intensities to the point that practical observation was feasible. The most extensive work in developing the systematics of ^{13}C NMR spectroscopy and applying it to organic problems came from the laboratories of Strothers (University of Western Ontario), Roberts (California Institute of Technology), and Grant (University of Utah) who obtained specialized instruments optimized for the study of this "less receptive" nucleus. Wenkert (now at University of California–San Diego) pioneered ^{13}C NMR spectroscopy for structure determination in more complex molecules.

Decade of technology: 1966–75

Superconducting magnets. With the development of 100-MHz instruments, the technology of iron-core electromagnets had been pushed about as far as it could go, because iron saturates near the field strength of 2.35 T. Further increases in field strength could come only from low-temperature superconducting solenoids. Although the technology of superconducting materials was well known, great efforts were needed to produce a magnet of high field strength that was capable of constant operation with little or no drift, had superb homogeneity, had a volume large enough for sample tubes, and was housed in a Dewar capable of retaining liquid helium and liquid nitrogen for adequate periods. Several such magnets were constructed in individual laboratories, but the first successful commercial magnet was the basis for the Varian HR-220 in 1966.

Since then, a steady stream of improvements in magnet technology, largely spearheaded by Richards (Oxford University) in collaboration with Oxford Instruments and Bruker Instruments, permitted increases in ^{1}H NMR frequency to 270 MHz, 360 MHz, and 400 MHz. A 500-MHz instrument was introduced around 1978. A 600-MHz instrument using a nonpersistent magnet was developed at Carnegie Mellon University in 1978 and, in 1987, a commercial 600-MHz instrument using a persistent magnet was introduced. Currently, 750-MHz systems are being developed commercially.

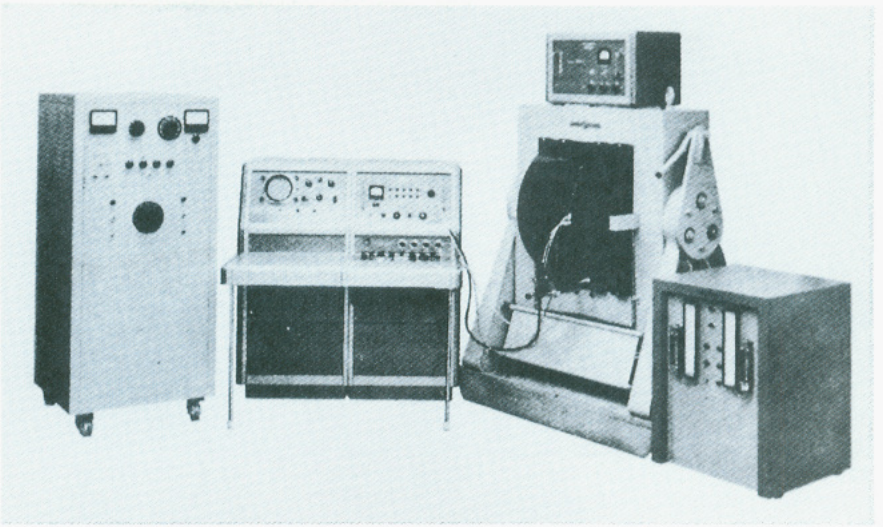
Superconducting magnets allowed researchers to spread out the spectrum expressed in frequency units and distinguish chemical shifts in large molecules that might nearly be coincident at lower magnetic fields, thereby facilitating the interpretation of spectra from complex organic compounds. This increased chemical shift dispersion, together with continuing increased instrumental sensitivity, permitted a serious attack on peptides, oligonucleotides, and

other biochemical molecules. Certainly, molecules such as amino acids and nucleotides had been studied at low field strength by Jardetzky (Stanford), Cohn (University of Pennsylvania), and others in the 1950s. The first NMR spectrum of a protein (a 40-MHz spectrum that showed only a broad envelope from the expected 800+ proton resonances) was reported by Saunders, Wishnia, and Kirkwood in 1957 (*20*). Although spectra of denatured proteins showed sharp lines, little useful structural information could be obtained. Thus most of the early work on biochemical systems was aimed at obtaining the necessary NMR parameters for amino acids, small peptides, mononucleotides, and simple sugars.

The first spectra of proteins obtained at high magnetic field strength using a superconducting magnet were reported in 1967 by McDonald and Phillips (*21*). They demonstrated that improved resolution could be obtained by using higher field strength, which meant that much more detailed information could be obtained from such spectra. Chemical shift changes were found to be associated with conformational changes in proteins and with ionization of protons as pH varied. However, many of these studies were limited primarily to an examination of histidine resonances, because they are highly deshielded and lie in a relatively uncluttered spectral region. Further advances in biological NMR spectroscopy would have to await two technological improvements.

FT-NMR spectroscopy. Adequate sensitivity has always been a problem in NMR spectroscopy. As investigators have studied samples that are dilute or of limited amount, and as they have extended NMR spectroscopy to less sensitive nuclei (such as ^{13}C and ^{15}N), the signal-to-noise ratio often becomes the limiting factor in determining what can be studied. In the early 1960s coherent time averaging was applied to NMR spectroscopy. Because N consecutive scans recorded in digital memory and coherently added produce a signal that is N times as large as one scan, whereas random noise is only $N^{1/2}$ times as large, the signal-to-noise ratio can be improved by the expenditure of more time.

The problem is that cw methods require a sequential frequency scan at a rate slow enough to avoid distortion of the spectral lines. This is an inefficient process, because only a narrow region of the spectrum is studied at a time. Particularly with the increased frequency dispersion accompanying higher magnetic fields and the extension of NMR methods to nuclei such as ^{13}C with a large chemical shift range, many minutes could be required to obtain a spectrum. Although acceptable for a single scan, it is not feasible to do 10,000 such scans to improve the signal-to-noise ratio by a factor of 100.



An early Varian high-resolution spectrometer (ca. 1958) comprises (from left to right) the magnet power supply, spectrometer console, magnet with super stabilizer (on top), and water-cooling heat exchanger.

Clearly, a method to excite the entire spectrum simultaneously was needed. In optical and IR spectroscopy, excitation and the resulting multiplex (Felgett's) advantage were obtained by using an interferometer and subsequent Fourier transformation of the time response. For NMR spectroscopy, the corresponding excitation was found in a short rf pulse.

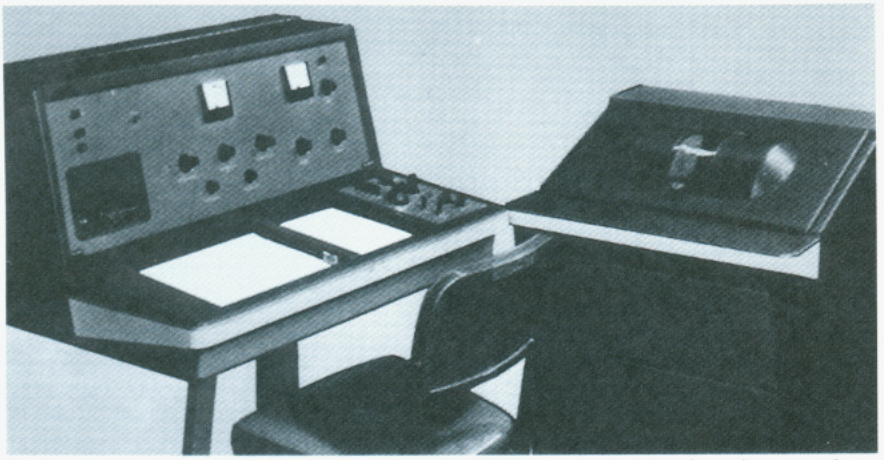
Lowe and Norberg (*22*) showed in 1957 that the FID following an rf pulse could, in principle, be transformed into the spectrum that would have been obtained by a slow scan. But it wasn't until publication of the seminal paper by Ernst and Anderson (*23*) in 1966 that the process could be made to work in practice. Their initial studies involved lengthy data processing, in which FIDs were coherently added and then converted by paper tape, magnetic tape, and punched cards into a form that could be processed on a large digital computer at a remote site.

Fortunately, minicomputers that could be interfaced directly to the spectrometer were being developed. Progress in FT-NMR spectroscopy owes much to these computers and fairly user-friendly software.

The development of FT-NMR methods has truly revolutionized the field. Not only could sensitivity be enhanced by time averaging in a practical manner, but the speed of the pulse FT method could be exploited alternatively to study fast processes such as chemical reactions and time-dependent NMR phenomena (e.g., relaxation). Pulse sequences, such as that for the spin echo that had been used only for single-line systems, could now be applied to chemically interesting molecules with many resonances. The study of less receptive nuclei, such as ^{13}C, became commonplace.

In addition to advances in NMR spectroscopy of liquids, new developments in understanding the interactions in solids, especially by Waugh (MIT) and Pines (University of California–Berkeley), permitted the development of new pulse methods to artificially narrow the inherently broad lines in solids. FT methods permitted the study of solids with chemically shifted lines. Magic angle spinning, discovered in 1959, could now be used in conjunction with new techniques that transfer magnetization from one nuclear species to another (cross polarization) to obtain high-resolution spectra of ^{13}C and other nuclei in solids.

2D NMR spectroscopy. During the last years of the decade, the most exciting new area was 2D NMR spectroscopy, in which nuclear magnetizations are allowed to precess during an initial time period, various pulse sequences are applied, and an FID is recorded. Two-dimensional Fourier transformation of the two independent time domains results in a spectrum that can be displayed along two



Varian A-60 (ca. 1961). Notice that, instead of several pieces of equipment, the instrument now comprises only two.

orthogonal frequency dimensions.

Jeener (Free University of Brussels) originated the idea of 2D NMR spectroscopy in 1971, but Ernst was the key figure in developing it into a practical and useful method during the mid-1970s (*24*). Over the years, literally hundreds of different 2D methods and improvements on earlier methods have been devised, each aimed at correlating resonances at different frequencies on the basis of some interaction between the nuclei responsible for the resonances. Two-dimensional NMR spectroscopy is a very powerful method for assigning lines in complex spectra and for studying interactions mediated by cross relaxation, chemical exchange, or other physical factors (*25*).

Decade of biological applications: 1976–85

A vast armamentaria of equipment and techniques had been developed in the first 30 years of NMR. Along with powerful spectrometers using high-field magnets, computer hardware and software tailored for applications, well-developed FT techniques, and evolving methods for 2D NMR and line narrowing in solids, NMR spectroscopy was used during this decade to solve problems in many areas of science.

Structures of complex organic molecules that had been elucidated only with tedious application of double-resonance methods could now be determined systematically and efficiently by 2D methods. Problems in solid-state chemistry and physics that had defied attack by NMR spectroscopy could now be solved with combinations of line-narrowing and 2D methods. However, the most far-reaching advances occurred in applications to a variety of biological systems. Not only could high-resolution studies of biopolymers in solution be carried out much more effectively than before, but advances in techniques permitted the investigation of NMR in living systems.

Macromolecular structure. Studies of biopolymers received a significant boost with the introduction of high-field magnets that permitted separation of spectral lines caused by chemically distinct nuclei. However, the spectra presented an embarrassment of riches because interpretation depends largely on assignment of each of the hundreds of lines to the nucleus responsible for that resonance. In smaller molecules, double-resonance methods played a major role in working out chemical bonding schemes via spin-coupling connectivities that could be investigated by selective spin decoupling; in favorable cases, NOE measurements gave valuable information on internuclear distances.

Such methods were applied to proteins and other biopolymers, but with only limited success. Hundreds of separate, tedious experiments were required, and interpretation of the results was not always straightforward. Probably the greatest success came with hemoproteins, where the large shifts caused by paramagnetic ions and aromatic ring currents considerably simplified the interpretation of large parts of the spectra.

The real breakthrough came when Nagayama, Wüthrich, Bachmann, and Ernst showed that 2D NMR methods could be applied to biopolymers (*26*). Correlated spectroscopy (COSY) and its offshoots permitted the establishment of spin-coupling connectivities to facilitate spectral assignments, and nuclear Overhauser enhancement spectroscopy (NOESY) allowed relaxation effects to be used to estimate internuclear distances. NOESY helped in making spectral assignments, but more importantly, it provided a way to estimate distances between nonbonded protons, giving NMR spectroscopy a general method by which to determine large numbers of internuclear distances in 3D space.

In principle, the information obtained from COSY and NOESY experiments could have been extracted from a large number of sequential 1D spin-decoupling and NOE measurements. However, the 2D approach permits simultaneous measurements throughout the entire spectrum, providing the same sort of general improvement in efficiency that FT-NMR spectroscopy had given to 1D spectral acquisition.

Interpretation of this vast array of data in terms of a 3D molecular conformation required the development of sophisticated methods of data analysis and coordination with molecular dynamics programs that assess the relative energies of various conformations. As in the early development of FT-NMR spectroscopy, major advances in computer speed and capacity as well as sophisticated programs occurred about this time and proved to be essential for the application of NMR methods (*27*).

By 1980, three years after the first 2D spectra of a protein had been obtained, solvent signal suppression methods had been developed to the point whereby spectra of proteins in water could be recorded, permitting peptide NH resonances to be included in the spin-coupling and NOE pathways. With this crucial additional information, it was possible to determine the complete 3D structures of small proteins, and in 1985 the structure of a 57-residue protein was published (*28*). The size of molecules that could be studied was still very small by protein standards, but NMR spectroscopy was established as an alternative to X-ray crystallography for biopolymers.

In vivo NMR spectroscopy. While traditional high-resolution NMR methods were refined and applied to ever larger and more complex, individual, well-defined molecules, some investigators were exploiting the speed and sensitivity of FT-NMR methods to look at the complex mixture of small molecules in living cells, organisms and, eventually, whole animals and humans. In some ways, such studies were not new. Bloch jokingly commented that he had carried out the first in vivo

NMR experiment in 1946, when he put his finger in the probe and obtained a signal from the water. Over the years NMR studies were carried out on water in blood cells, sodium in muscle and blood cells, and water in a mouse. Most investigations were limited to abundant substances that give large signals, principally water, and only a limited amount of information was obtainable.

In 1973 Moon and Richards (*29*) observed separate ^{31}P NMR signals from the intracellular constituents of reticulocytes, and in 1974 Hoult et al. (*30*) showed that metabolic changes in a living, excised muscle could be followed by observation of the signals from adenosine triphosphate as well as creatine phosphate, inorganic phosphate, and other phosphates.

During the 1976–85 decade, many groups made forays into metabolic studies by NMR spectroscopy. Methods were invented for keeping cells alive and growing them in NMR tubes to permit studies in cellular systems (*31*). Excised organs were perfused with nutrient solutions in NMR tubes and their metabolism followed. Surface coils were developed to obtain signals from localized volumes near the skin of experimental animals. Depth pulses were designed to tailor rf excitation and define more precisely the volume of interest, and rf coils were implanted in animals to examine internal organs.

Radda (Oxford University) and Shulman (Yale) pioneered the noninvasive NMR study of metabolism in humans with large superconducting magnets. Most of these studies relied on ^{31}P NMR spectroscopy, but enriched ^{13}C samples added a dimension to the study of metabolism. Some use was made of ^{1}H NMR spectroscopy, but the large water signal seriously interfered with studies of metabolites at millimolar levels.

NMR imaging. A separate line of development that would have profound effects on in vivo NMR spectroscopy began in 1973 when Lauterbur (*32*) showed that 2D images could be obtained by imposing magnetic field gradients across a sample. With such a gradient, the Larmor equation is modified and becomes

$$\omega = \gamma B_o(1 + Gr)(1 - \sigma) \qquad (3)$$

where G is the gradient in direction r. Thus the NMR frequency becomes a measure of position along the gradient. By repeating the measurement with gradients in different directions, it is possible to reconstruct a 2D image. In 1975 Kumar, Welti, and Ernst (*33*) showed that the then-new

Superconducting magnet for a Bruker 600-MHz spectrometer. (G. Clore, left, and A. Bax of NIH)

2D NMR technique provided a more efficient way of obtaining an image and, with some further modifications, this rapidly became the method of choice for 2D (and later 3D) imaging.

It was immediately apparent that NMR imaging had great potential for investigating human and animal anatomy. Because of differences in water content and relaxation times, biological tissues can be distinguished with suitable NMR pulse sequences, and images with exquisite discrimination between normal and pathological tissues can be readily obtained. Commercial development of NMR imaging, shortened to magnetic resonance imaging (MRI), began in the late 1970s. In the early 1980s practical diagnostic instruments began to appear in radiology departments, and the MRI market grew exponentially.

NMR imaging methods also provide the most general method for localizing a volume of interest for spectroscopy. By 1985 techniques were being developed to integrate imaging and spectroscopy in living animals and humans.

Current decade—medicine, structural biology, and materials science: 1986–95

With a third of the current decade remaining, it is too soon to characterize what ultimately may be the major thrusts of NMR. However, it is safe to highlight three different areas in which advances have been so rapid and profound that they will rank in the forefront of the contemporary developments in NMR.

Medical diagnosis. By 1987 MRI had become sufficiently widespread to engender a Consensus Conference at the National Institutes of Health to address questions about its safety and efficacy as a diagnostic tool for a wide range of diseases. In many ways, MRI was determined to be significantly superior to other imaging modalities such as X-ray computed axial tomography (CAT) and, in many other ways, it was on the verge of excelling. Today, it is difficult to find any large hospital in the United States in which MRI is not available. Clearly, at $2 million to $3 million per instrument, MRI has become the tail that wags the NMR dog!

In the area of biomedical research, MRI is undergoing further advances. Echo-planar imaging (EPI) provides an alternative method of obtaining NMR images in a fraction of the time currently needed (50 ms vs. ~ 5 min) (*34*). Although it is only now being developed commercially, EPI stems from a method of imaging introduced by Mansfield (University of Nottingham) soon after Lauterbur's invention. In EPI, motion artifacts can be eliminated and resolution improved for many internal organs.

Functional imaging, in which the emphasis is on some type of function rather than merely anatomy, is becoming more useful in research and diagnosis (*35*). Studies of blood flow and diffusion of water and metabolites in tissues use spin–echo methods that have long been understood and applied in high-resolution NMR spectroscopy. Magnetic susceptibility effects from paramagnetic deoxyhemoglobin permit the study of localized brain function in regions where oxygen is being consumed. With the development of excellent techniques to suppress the water signal and to localize a small volume of interest, ^{1}H NMR spectroscopy is beginning to provide valuable information on metabolic function in several organs, including the brain.

Structural biology. Dramatic advances have been made recently in the application of high-resolution NMR spectroscopy to the determination of the 3D structure of biopolymers, especially proteins. Earlier studies had been restricted to proteins of molecular weight 5000–10,000 Da, because the complexity of the 2D spectra and the linewidths increase rapidly with molecular size.

Extension to larger proteins required the development of ingenious new methods that depend on the availability of proteins uniformly enriched in ^{13}C and/or ^{15}N, which can often be prepared inexpensively by recombinant DNA techniques.

These new methods transfer ^{1}H magnetization through the peptide link from one amino acid residue to another and permit sequence assignments to be made by 3D and 4D NMR methods (*36*). The latter are logical extensions of the commonly used 2D techniques, but they require large amounts of data acquisition time and intensive computational capabilities.

Complete structures of proteins of molecular weights close to 20,000 Da have been reported, and current methods appear to be applicable up to about 30,000 Da. The precision of the NMR methods is now approximately as good as that of X-ray crystallography, and NMR spectroscopy has the advantage of determining the structure in solution, where dynamic processes important to protein functioning can be examined (*37*). The structures of protein complexes and nucleic acids are also being determined by NMR spectroscopy. Of course, the NMR approach has some limitations in that certain proteins show inadequate chemical shift dispersion, and linewidths for larger proteins preclude their analysis by current methods. Moreover, the NMR approach is still very labor intensive for analyzing multidimensional spectra.

Materials science. Until line-narrowing methods were developed, NMR spectroscopy provided only limited information about solid materials. Now, not only can relatively high-resolution spectra be obtained in solids, but the imaging methods—developed primarily for biomedical applications—can be combined with line-narrowing techniques to image solids. NMR spectroscopy thus provides a new approach to the investigation of detailed structure in heterogeneous materials such as polymer ceramics and their composites (*38*).

Other advances in sample spinning techniques have permitted narrow lines to be obtained for quadrupolar nuclei, thereby opening up high-resolution NMR studies in many inorganic solids. New methods have been developed for studying the structure of solids with probe gases, such as xenon. Thus NMR spectroscopy is beginning to have a real impact on the rapidly developing field of solid-state chemistry.

Other aspects

In this brief history, I have been unable even to mention other areas in which NMR has played an important role. NMR has been used in solid-state physics since its inception to investigate a wide range of phenomena in metals, semiconductors, and other materials. Geologists use NMR magnetometers; oil explorers use NMR in well-logging (the NMR probe fits inside the sample). The food industry uses it to measure moisture content, and agricultural studies have focused on the noninvasive nature of NMR to look at seeds and plants. These and other applications of NMR continue to expand.

It is estimated that overall there are some 15,000 NMR instruments of one sort or another worldwide. Of these, about 5000 are in the medical field and account for a large fraction of the money spent on NMR instrumentation. However, even with all the developments in other areas, the largest number of NMR spectrometers (on the order of 8000) is still devoted to the application that began almost 40 years ago: analytical chemistry and structural studies of organic molecules.

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