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Publication Date
1956-12-01
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NUCLEAR AND ELECTRON PARAMAGNETIC RESONANCE AND ITS APPLICATION TO BIOLOGY

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NUCLEAR AND ELECTRON PARAMAGNETIC RESONANCE
AND ITS APPLICATION TO BIOLOGY

Power B. Sogo and Bert M. Tolbert

December 1, 1956

Printed for the U.S. Atomic Energy Commission
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NUCLEAR AND ELECTRON PARAMAGNETIC RESONANCE AND ITS APPLICATION TO BIOLOGY

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ABSTRACT

Nuclear and electron paramagnetic resonance is reviewed from a fundamental viewpoint and as applied to biological research. A simplified presentation of the interaction of mass, electric charge, spin and magnetic moment to produce spin resonance is given. Instrumentation is discussed. Biological studies reviewed include naturally occurring unpaired electrons, free radicals produced by chemical reactions and ionizing radiation, solid-state biophysics, structure determination, isotopic analysis, and tracer-study possibilities.
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INTRODUCTION

Each new chemical or physical phenomenon discovered usually finds some medical or biological application. The examination of the new phenomena for such biological possibilities is an interesting study and the authors feel fortunate to be able to review some of the first steps by research workers in the application of electron spin resonance and nuclear magnetic resonance to biology.

The time interval from the discovery of a new physical phenomenon to the practical use of it in biology is becoming increasingly short. The first experiments in electron spin resonance were done eleven years ago, and only ten years haveelapsed since the first successful nuclear magnetic resonance measurements were made. In that amazingly short time, adequate instrumentation has been developed for routine measurements, a variety of chemical and physical phenomena have been measured and interpreted, and theory and observation have been mathematically correlated.

A sufficient amount of this fundamental work has been done to make possible the initial experiments in biology. As yet, no fundamentally new truths have been demonstrated in biology by the use of nuclear magnetic resonance. Electron spin resonance measurements, however, are already beginning to give information from which one can construct a detailed picture of the nature of some biochemical reactions—reactions involving one-electron oxidation-reduction reactions or the so-called free-radical reactions. The discovery, by electron spin resonance, of these free radicals in living material and their correlation with biological activity helps confirm what has been only a hypothesis on certain types of enzymatic reactions. The free radicals produced in biological and organic material by ionizing radiation have helped confirm and make more certain our theories on the primary event in radiation biochemistry. Free-radical activity has been implied in the biochemistry of both carcinogenesis and aging.

Electron spin resonance and nuclear magnetic resonance both involve the absorption of electromagnetic radiation in the radiofrequency range. In this respect they can be considered as extensions of the well-known ultraviolet, visible, and infrared spectroscopy. Electron and nuclear resonance, however, are a unique branch of spectroscopy, for in them one can, in principle, vary the absorption energy levels at will, instead of having to use the energy levels that nature has given. This advantage arises from the fact that one can vary the strength of the applied magnetic field and thus vary the splitting of the

* The preparation of this paper was sponsored by the U.S. Atomic Energy Commission.
electronic nuclear magnetic energy levels. How nice it would be if one could do infrared spectroscopy with visible light! This is virtually what can be done with electron and nuclear magnetic resonance spectroscopy.

The type of information derived from the measurement of this radio-frequency-energy absorption is also similar to known spectroscopy. Thus, ultraviolet and visible spectra are used to detect electron transitions in the over-all molecule, or multiatom segments of it, and infrared spectroscopy is used to determine absorption bands of small groups of atoms, such as C—OH, —CH₃ or —C≡C. The electron spin resonance measurements are used to detect and quantitate unpaired electrons — i.e., free-radical molecules in organic chemistry, or paramagnetic inorganic elements — and to determine the location of these unpaired electrons. Nuclear magnetic resonance is due to energy absorption in specific atoms, and this energy absorption is modified by the nature of the chemical bonds attached to the given atom. It is used to measure and evaluate chemical and molecular forces and distances, nuclear species, and physical states. Thus, these new techniques do not displace existing spectroscopy, but supplement it with information on a different atomic and molecular level.

I. ELECTRON SPIN RESONANCE

The first successful electron spin resonance experiments were carried out by Zavoisky (1945) in Russia. Initially, the applications of spin resonance dealt mainly with solid-state problems in which the energy levels of the paramagnetic ions situated in crystal lattices were studied. The observation of nuclear hyperfine interaction provided a means by which nuclear moments could be measured, and several studies of this type were made. Electron spin resonance has also provided a sensitive tool for the identification and study of free radicals. Recent review articles and books on electron spin resonance include those by: Bleaney (1953), Bleaney and Stevens (1953), Bowers and Owen (1955), Gordy (1956), Gordy, Smith, and Trambarulo (1953), Møllgaard (1955), Shoolery and Weaver (1955), and Wertz (1955).

1. Fundamentals of Spin Resonance

The basic quantities characterizing the electron that are of principal interest in spin resonance are the mass, electric charge, spin, and magnetic moment. The basis of magnetic resonance rests in the interaction of these quantities with applied electromagnetic fields. The electron may interact not only with the magnetic fields that originate from laboratory apparatus, but also with fields arising from the atom and surrounding atoms in which it resides. In fact, it is this internal type of interaction that allows electron spin resonance to be used as a tool in a large variety of physical problems. However, the presentation of the subject is simplified if only the laboratory fields are considered, as a first approximation, followed by a later discussion of the internal interactions.

Mass and electrical charge are familiar quantities, but a short discussion of the spin and magnetic moment of the electron may be worth while. In classical mechanics, a mass of finite spatial dimensions spinning about an axis
possesses an angular momentum which is a vector quantity directed along the axis of rotation. Such a model may be visualized for a quantum mechanical particle, e.g., an electron, with the restriction that the maximum observable magnitude of the angular-momentum vector must be an integral or half-integral multiple of $\hbar/2\pi$, where $\hbar$ is Planck's constant. For an electron, this value for the angular momentum has been found to be $(1/2)(\hbar/2\pi)$. The spin is simply the angular momentum in units of $\hbar/2\pi$, so that the electronic spin is just $1/2$. An important concept in magnetic resonance is the possibility of orienting the spin axis with respect to a particular direction in space. This direction can be defined, for example, by the lines of force of a magnetic field. The results of theory and experiment show that only certain orientations are allowed for a quantum mechanical particle. These allowed inclinations are characterized by the magnitude of the projection of the spin vector along the defined direction in space. The spin projection may have a maximum value equal to the spin itself, with a plus sign to indicate a parallel orientation and a minus sign to indicate an antiparallel orientation. Also, all intermediate projections that differ from the above by integral numbers are allowed. Thus, the electron has only two possible orientations in an applied field, viz., parallel or antiparallel, as shown for $I = 1/2$ in Fig. 1.

The magnetic dipole moment is a vector that is parallel to the spin vector of the electron. The origin of this magnetism probably lies in the circulating currents which are created by the rapid spinning of the charged electron. The magnitude of the intrinsic electronic magnetic moment is almost exactly equal to one Bohr magneton, which is the value of the electronic magnetic moment calculated from the foregoing model. In an applied magnetic field, the magnetic moment behaves like a tiny bar magnet or compass needle, so that it tends to be oriented in a direction parallel to the field. The energy of interaction is given by the product of the magnetic field strength and the projection of the magnetic moment along the field. For the electron, only parallel or antiparallel orientations are allowed, so that we immediately arrive at a pair of energy levels representing the two orientations. These levels are separated by the energy $g\mu_0 H_0$, where $g$ is the so-called $g$ factor or spectroscopic splitting factor and is equal to the magnetic moment in units of the Bohr magneton $\mu_0$ divided by the spin, and $H_0$ is the strength of the applied magnetic field. As $g\mu_0$ is a constant for the electron, we see that the energy difference between the two spin orientations is proportional to $H_0$. Figure 2 shows a plot of the energy levels against magnetic field.

The resonance condition follows immediately from the above discussion when we impose the well-known Bohr frequency relation,

$$h\nu = E_2 - E_1,$$  \hspace{1cm} (1)

where $h$ is Planck's constant and $\nu$ is the frequency of the electromagnetic radiation associated with a transition between the two energy levels $E_2$ and $E_1$. Thus, we obtain the fundamental equation of magnetic resonance:

$$h\nu = g\mu_0 H_0.$$ \hspace{1cm} (2)

The meaning of this relation is clear. If an electromagnetic field (which we shall call $H_1$) oscillating with the proper frequency is applied to a group of electrons that are simultaneously subjected to the constant magnetic field $H_0$,
Fig. 1. Possible orientations of spin vectors in a magnetic field.
Fig. 2. Energy-level diagram as a function of magnetic field.
then energy transitions of magnitude $h \nu$ corresponding to changes in orientation of the electron spin will occur. By inserting numerical values for $h$ and $\mu_0$ into Eq. 2, we obtain

$$\nu (\text{Mc/sec}) = 1400g\nu_0 (K \text{ gauss})$$

For a typical laboratory field of $10^4$ gauss and a $g$ factor equal to 2, the oscillating magnetic field must have a frequency of about 28,000 Mc/sec. This frequency corresponds to a wave length of about 1 cm, and lies in the region where microwave techniques are employed. The energy corresponding to this frequency is about $10^{-16}$ erg or about 2 cal/M.

Let us now consider the more practical case in which we have a macroscopic sample containing $N$ free electrons per unit volume. In an applied magnetic field $H_0$, these electrons will be distributed so that some of them are in the higher energy level $E_2$ and the rest are in the lower energy level $E_1$. The incoming oscillating field $H_1$ will not induce transitions from $E_1$ to $E_2$ corresponding to energy absorption, and transitions from $E_2$ to $E_1$ corresponding to energy emission. These two kinds of transitions occur with equal probability, so that if the two energy levels were initially equally populated with electrons, no net energy change results when the radiation of the proper frequency is applied. Under these conditions, no spin-resonance absorption signal can be observed. Actually, the levels will not be equally populated, but will contain an excess number of electrons in the lower magnetic-energy state. An expression giving the magnetization or magnetic moment per unit volume resulting from this excess population can be calculated with the aid of the Maxwell-Boltzmann distribution law, which describes the relative electron populations at thermal equilibrium. This expression is known as the Curie law of electron spin paramagnetism, and for the special case of electrons with $g = 2$, is

$$M_0 = \frac{N\mu_0^2}{kT} H_0$$

In this equation, $M_0$ is the magnetic moment per unit volume, $N$ is the number of electrons per unit volume, $\mu_0$ is the Bohr magneton, $k$ is Boltzmann's constant, $T$ is the absolute temperature, and $H_0$ is the strength of the applied magnetic field. $M_0$ is a small number when compared with the maximum magnetization $N\mu_0$ that would be produced if all $N$ dipoles were oriented parallel to $H_0$. This difference between $M_0$ and $N\mu_0$ is due to the factor $\mu_0 H_0/kT$, which is approximately the ratio of the magnetic-energy level spacing to the thermal energy of the electron. At room temperature ($T = 300^\circ$K), $kT$ is about $4 \times 10^{-14}$ erg, while $\mu_0 H_0$ for a field of $10^4$ gauss is about $10^{-16}$ erg. Thus, for every 200 electrons in the upper energy state, there will be 201 electrons in the lower energy state. In many problems, one might be justified in ignoring this small excess, but in spin resonance the strength of the signal depends on the magnitude of $M_0$, and when $M_0$ is truly zero no resonance will be observed. In some cases, it is necessary to lower the temperature of the sample, thus enhancing the excess population, in order that the spin resonance signal may be detected. Because the lower energy level contains the excess population, $H_1$ will induce more transitions corresponding to energy absorption than energy emission, and we simply require a suitable absorption cell in order to measure the resulting energy change.
Before we leave the subject of electron spin paramagnetism, it is of importance to introduce another quantity that finds use in magnetic resonance. This quantity is called the spin-lattice relaxation time, and is denoted by $T_1$. In the discussion of bulk magnetization above, we supposed that we started with a sample containing randomly oriented dipoles in a region of a zero applied magnetic field. When a finite magnetic field was imposed, a distribution of electrons among the magnetic energy levels took place such that the resultant energy of the electrons at thermal equilibrium was lower than in the original nonmagnetized system. Thus, it is necessary for magnetic energy to be carried out of the spin system and be deposited in some energy reservoir in order that conservation of energy can be secured. A finite time is required for the completion of this process, i.e., the establishment of thermal equilibrium, and $T_1$ is a measure of this time. The energy reservoir is called the lattice; thus we obtain the name: spin-lattice relaxation time. It is interesting to note that perfectly free electrons with an infinite value for $T_1$ cannot exhibit spin resonance. Sufficient interaction must exist between the electrons and their environment so that the relaxation time is finite.

$T_1$ proves to be important not only in the initial establishment of the bulk magnetization $M_0$, but also in maintaining the excess population when the oscillating field is impressed. $H_1$ attempts to decrease the magnitude of $M_0$ by inducing more transitions to the higher energy level than to the lower state. At the same time, the spin-lattice relaxation mechanism seeks to maintain the original equilibrium value of $M_0$. Thus a competition exists between the two processes. The rate with which $H_1$ produces transitions varies as the square of its amplitude. Thus with very small amplitudes, the spin-lattice mechanism is relatively much stronger than $H_1$, and the energy absorption varies as the square of the amplitude of $H_1$. At larger amplitudes, when the spin-lattice process can no longer retain the initial excess population, the signal intensity ceases to increase with the oscillating-field amplitude and we have a situation that is called saturation. At very high amplitudes, the population excess approaches zero, and the signal intensity actually decreases as the amplitude of $H_1$ is increased.

2. Internal Interactions

The discussion of quasi-free electrons has served to illustrate the basic features of magnetic resonance. The spin-resonance absorption spectrum of such electrons consists of a single, symmetric absorption line with a $g$ value of 2.0023. The unpaired electrons that are observed in more typical experiments are not free, but are embedded in larger systems such as ions, molecules, and crystal lattices. These systems produce electromagnetic fields that interact with the electric-charge distribution or magnetic moment of the electrons. The result is a spin resonance signal which differs from that of a free electron. The change may occur in $g$ value, line shape, and number of component lines. In more severe cases, the spin-resonance absorption may be made unobservable.

The first condition that must be satisfied for the observation of spin resonance is the presence of electrons with uncompensated angular moments, i.e., unpaired electrons. In the atomic state, most of the elements of the periodic table possess such electrons, and their magnetic resonance properties
have been studied, in a few cases, by the method of atomic beams. However, unless an extremely low concentration is maintained, the atoms rapidly combine to form molecules in which the initially unpaired valence electrons become paired in the formation of the chemical bonds. (There are a few exceptions to this rule, such as O₂ and NO.) In this situation, the molecules are diamagnetic and no spin resonance can be observed. However, certain elements contain unfilled inner shells in their electron configurations and remain paramagnetic when the outer valence electrons form chemical bonds.

These "transition" elements consist of those in which electrons enter an outer shell before completely filling inner d or f shells. The five groups of transition elements are: the iron group with unfilled 3d shells, the palladium group with unfilled 4d shells, the rare-earth group with unfilled 4f shells, the platinum group with unfilled 5d shells, and the uranium group with unfilled 5f shells.

In addition to its intrinsic spin, an electron bound to an atomic nucleus possesses an angular momentum arising from its orbital motion. The magnetic moment associated with this motion has a magnitude that is proportional to the value of the orbital angular momentum. An internal interaction, called the spin-orbit interaction, arises from the magnetic coupling of the spin vector with the field of the orbital magnetic moment. The result of spin-orbit interaction is the formation of a resultant angular momentum comprised of the vector sum of the spin and orbit angular momenta together with a resultant magnetic moment. Since a unit of spin angular momentum is twice as effective as a unit of orbital angular momentum in producing a magnetic moment, it is clear that the g value of a bound electron may deviate widely from the value g = 2 of a free spin.

The problem of spin-orbit interaction originally arose in atomic spectroscopy, from which the following equation for the Landé g factor was derived:

\[ g = 1 + \frac{[J(J + 1) + S(S + 1) - L(L + 1)]}{2J(J + 1)} \]  

In this equation, g is the spectroscopic splitting factor for electrons bound to free atoms or ions, while S, L, and J are respectively the quantum numbers of the spin, orbit, and resultant angular momentum.

For atoms or ions in S states (L = 0), a spherically symmetric electron distribution exists and the orbital angular momentum is zero. Then S = J and the g value is 2 as for a free spin. For ions in which L ≠ 0, e.g., D or F states, one would expect g values different from 2 and approaching the free-ion value of Eq. 5. However, in many spin resonance experiments on such ions, one finds a g value surprisingly close to the free-spin value. This fact has been interpreted as the quenching of the orbital angular momentum by the interaction of internal fields with the ion. In these cases, the free ion is not a good approximation for the interpretation of the spin resonance spectra. The internal crystalline or molecular electric fields interact strongly with the nonspherically-symmetric electron-charge distributions that are present when L ≠ 0. The result is the uncoupling of the spin and orbit vectors together with a splitting of the orbital magnetic states. This splitting is usually very large (on the order of 10⁷ Mc/sec), so that only the lowest orbital magnetic state is occupied and the laboratory fields interact only with the spin vector. The crystalline field
may also split the spin levels by a small amount from an indirect interaction that is transmitted through residual spin-orbit coupling. The magnitude of this spin-level splitting depends on the strength of the applied magnetic field and may be distinguished by observing the spin resonance at two different frequencies. This discussion of crystalline field splitting applies mainly to the iron-group transition elements in which the unpaired electrons have very little shielding from the ion environment.

The symmetry of the crystalline field and the orientation of the crystal axes with respect to the externally applied magnetic field also affect the observed g value. For axially symmetric fields, two g values are sufficient to describe the resonance condition, viz., \( g_\parallel \) and \( g_\perp \). Here \( g_\parallel \) and \( g_\perp \) represent the resonance g values for the case in which the axis of symmetry of the crystalline field is parallel and perpendicular respectively, to the direction of the externally applied magnetic field. Measurements of these anisotropic g values should be made on single crystals, because a polycrystalline sample would give a broad, "washed out" resonance due to the random orientations of the crystalline axes. The interpretation of experimentally observed anisotropic g values in terms of crystal-field theory has given valuable information about the bonding of the transition elements in solids. In organic free radicals, the orbital angular momentum is almost completely quenched so that a pure spin electron results. Thus free-radical resonances are relatively sharp and occur at a g value of 2.00.

3. Hyperfine Interactions

Hyperfine interaction results from the magnetic coupling of the unpaired-electron spin with the magnetic nuclei to which it may be bound. The name for the interaction is derived from atomic spectroscopy, in which the same process manifests itself in a relatively tiny splitting of the electronic energy levels. All nuclear species with a nuclear spin (which we will denote by I) greater than zero have a finite magnetic moment. The magnitude of nuclear magnetic moments is about 1/2000th of the magnetic moment of an electron, but it is still large enough, with its close proximity, to provide a significant magnetic interaction with the electron. A nucleus with a spin I will be oriented, when subjected to a magnetic field, so that its projection has one of the \( 2I + 1 \) allowed values: \( I, I-1, \ldots, -I \). Thus the electron is exposed to the applied magnetic field plus an additional field from the nucleus, which can have \( 2I + 1 \) values. Because the nuclear magnetic energy levels are practically equally populated, the electron spin resonance signal consists of \( 2I + 1 \) equally spaced components of equal intensity. Figure 3 shows the hyperfine splitting of the energy levels by a nucleus with \( I = 1 \). A dipole-dipole interaction of this type depends upon the relative orientation of the interacting vectors with a line joining them so that one would expect an anisotropic hyperfine interaction in solids. Such a situation has been verified by experiments on the transition elements. In liquid solution, on the other hand, one would expect that this dipole-dipole interaction would be completely averaged to zero by the rapid tumbling of the molecules with respect to the external field. Experimentally, strong hyperfine interaction is observed in solutions. Even though the long-range dipole-dipole interaction has been averaged out, a residual contact term persists that provides hyperfine splitting. This contact interaction depends on the electron density at the nucleus. Thus the hyperfine splitting
Fig. 3. Hyperfine splitting of energy levels at high fields by a nucleus with spin $= 1$. Constant-frequency transitions at different applied fields are indicated. Selection rules are $\Delta M_s = \pm 1$, $\Delta M_I = 0$. 
provides a useful tool for the determination of the electron-spin-density distribution in the molecule. In many free radicals, the unpaired electron migrates rapidly from atom to atom, producing a hyperfine pattern that arises from a simultaneous interaction with several magnetic nuclei. The spectrum may become quite complicated in this case. However, if only one nuclear species is participating in the interaction and all N of these nuclei are equally coupled to the odd electron, then a relatively simple pattern consisting of $2N + 1$ components of equal spacing results. The intensities will not be equal, but will follow the distribution that is produced from the condition that each nucleus has $2I + 1$ equally probable orientations.

4. Instrumentation

Although the resonance condition (Eq. 2) implies that spin resonance absorption can be observed at any frequency, the majority of the spin resonance spectrometers in operation today work at microwave frequencies. Depending on the type of resonance being observed, several disadvantages may exist in working at radio-frequencies. At a frequency of 100 Mc/sec, for example, the operating magnetic field for a $g = 2$ resonance would be only about 30 gauss, so that resonance absorptions with a line width greater than about 60 gauss would become very difficult to observe. In addition, owing to the small spacing of the magnetic-energy levels, the Boltzmann factor is small relative to its value at higher magnetic fields, resulting in a smaller excess population in the lower state. This fact, together with the decrease in energy change per transition, results in a variation of signal strength that is proportional to the square of the operating frequency. Finally, zero-field splitting of the spin levels by internal crystalline fields may make the resonance unobservable at low frequencies. However, for the observation of narrow resonances such as those originating from many free radicals, operation at radio-frequencies offers the advantages of extreme simplicity and low cost. Air-core Helmholtz coils may be used to provide homogeneous magnetic fields up to 100 gauss or so, and a simple radio-frequency spectrometer of the type used in nuclear magnetic resonance can be used for the detection of the resonances.

In the microwave region, the most commonly used frequencies are 10 kMc/sec (3 cm radiation) and 24 kMc/sec (1.25 cm radiation). These frequencies lie respectively in the X and K bands of the microwave spectrum. Commercial spin resonance spectrometers are available from Varian Associates and Polarad Electronic Corp. The basic components of a simple electron-spin resonance spectrometer operating at microwave frequencies are schematically shown in Fig. 4. A reflex klystron is usually used as the power oscillator for the generation of the oscillating magnetic field that excites the electronic transitions. To enhance its stability, it may be immersed in a thermostated oil bath and surrounded by magnetic and electrostatic shielding. The oscillating field is propagated down a wave guide of proper size into a cavity resonator in which the microwave fields are greatly increased in magnitude. The orientation of the oscillating magnetic field (within the cavity) that interacts with the sample must be perpendicular to the large field $H_0$ produced by the electromagnet. A microwave power monitor consisting of the detector, amplifier, and meter follows the cavity resonator. The cavity resonator is a sharply tuned system and responds only to single
Fig. 4. Simple transmission cavity spectrometer.
frequency. The klystron must be tuned to this frequency if microwave power is to be absorbed by the sample. Because it would be difficult to "track" the frequencies of the klystron and the cavity resonator together, these units are operated at a fixed frequency and the magnetic field is varied to satisfy the resonance condition. Thus the spin-resonance absorption spectrum is obtained by sweeping the magnetic field by varying the current through the electromagnet coils. At resonance, the unpaired electrons in the sample absorb energy from the microwave field within the cavity resonator. This produces a decrease in the power reaching the detector and a corresponding deflection of the meter needle.

The determination of the g value of a resonance requires a simultaneous measurement of the frequency of the microwave field and the strength of the applied magnetic field. Microwave frequencies may be measured with a calibrated cavity resonator, or, more accurately, by heterodyne methods that rely on a radio-frequency oscillator whose frequency is precisely known. A simple nuclear magnetic resonance apparatus is the most widely used device for the accurate measurement of magnetic fields. Nuclear g values are very precisely determined and one needs only to measure the frequency of the nuclear resonance oscillator that lies in the convenient radio-frequency range.

Several types of microwave spin resonance spectrometers differing from the simple transmission system in Fig. 4 have been developed. For example, the microwave power reflected from the cavity may be detected rather than the transmitted power. This system requires only one arm leading to the cavity resonator, and is advantageous when the entire cavity is immersed in a liquefied gas for the attainment of low sample temperatures. Superheterodyne detection utilizing an additional klystron as a local oscillator is used in many high-sensitivity systems. This detection scheme is inherently less noisy than the direct detection scheme of Fig. 4.

Most high-sensitivity spin resonance spectrometers utilize magnetic field modulation with a lock-in detector system for the automatic recording of signals. Spectrometers that utilize this system may be called "differentiating spectrometer" because the first derivative of the absorption curve appears on the recorder chart. A block diagram in which this type of system has been added to the transmission spectrometer of Fig. 4 is shown in Fig. 5. The auxiliary magnetic-field modulation coils are driven by the audio generator at a frequency of about 1000 cps. Thus, the applied magnetic field acting on the unpaired electrons has a small sinusoidally varying component in addition to its large steady value. If the large field $H_0$ is set within the resonance line (see Fig. 6), then the sweeping back and forth by the modulation produces a power absorption that varies at the same rate as the modulation frequency. If the modulation amplitude is small compared to the line width of the resonance, then the amplitude of the signal will be proportional to the product of the modulation amplitude and the slope of the absorption curve. This signal is separated from the average microwave fields by the detector, amplified by the audio amplifier, and injected into the lock-in amplifier. The lock-in amplifier consists of a narrow-band amplifier tuned to the modulation frequency followed by a phase-sensitive detector and filter. The phase-sensitive detector is triggered by the audio generator so that it converts only the signals with the proper frequency and phase into a dc voltage that drives the recorder. A filter between the detector and the recorder determines
the pass band for the entire system, and response times as long as a minute (corresponding to a pass band of about 0.003 cps) have been employed. Since random noise is proportional to the square root of the pass band, the lock-in amplifier provides a convenient way of obtaining high signal-to-noise ratio. To insure an undistorted presentation of the signal a period of about 20 response times is required for recording the signal. The maximum response time that can be effectively used is limited by the over-all stability of the apparatus. Thus temperature control, vibration-proof mountings, and electronic regulation are necessary prerequisites for the attainment of high sensitivity.

For biological studies, a primary limiting factor appears to be sensitivity. Biological specimens approximating in vivo status contain substantial amounts of water, which, with its large electric dipole moment, produces a large dielectric loss in the cavity resonator. The effect is a decrease in the microwave power stored in the cavity and a loss of sensitivity. At a wavelength of 3 cm, only about $10^{-5}$ to $10^{-4}$ liter of an aqueous solution may be used advantageously as a sample. About $10^{12}$ unpaired electrons are required to produce a perceptible signal for a one-gauss-width line. Thus the concentration of unpaired electrons in an aqueous medium must be about $10^{-6}$ mol/l. In non-lossy solvents such as benzene, about 10 times as much sample can be used, so that minimum concentrations of about $10^{-7}$ mol/l are required. For lines wider than one gauss, a correspondingly larger number of electrons is required, because signal detectability depends upon the height of the absorption curve, whereas the area under the absorption curve is proportional to the number of unpaired electrons.

5. Applications of Spin Resonance to Biology

The possible role of free radicals in biochemistry, and in biochemical oxidation-reduction reactions in particular (Michaelis (1951)), has long been of interest to biologists. During the past two or three years, increased interest in these theories has arisen following the development of electron spin resonance as a technique that can actually detect such intermediate states. The several recent observations by such methods of unpaired electrons, or free radicals, in biological systems now lends support to these free-radical theories. Free radicals have been observed in many compounds of biological interest and as oxidation products of biological material. Paramagnetic resonance has been observed in biological material after heating after subjecting it to ionizing radiation, and after exposing it to ultraviolet or visible radiation. The free radicals that are produced either normally or abnormally have been suggested as carcinogenic agents and as a prime factor in the again process.

Although it may be true that all physical or chemical experiments are biologically interesting in a fundamental sense, we have attempted to select only those experiments reported in the literature that deal specifically with biological materials or that are of immediate biological interest. It should be emphasized that many of the experiments reviewed are not "finished" in the sense that an unambiguous biological interpretation can be made. Usually, owing to the complexity of the substances dealt with, only tentative hypotheses concerning the nature or biological role of the spin resonance absorbing centers
Fig. 5. High-sensitivity differentiating spectrometer.
Fig. 6. Magnetic-field modulation of absorption curve.
can be made. Thus, as far as biological significance is concerned, the work reported here should be considered as work in progress rather than work completed.

Excellent reviews of the many experiments of a more chemical or physical nature may be found in Bleaney (1953), Bleaney and Stevens (1953), Gordy, Smith, and Trambarulo (1953), Bowers and Owen (1955), Ingram (1955), Shoolery and Weaver (1955), and Wertz (1955).

a. Naturally Occurring Unpaired Electrons

Commoner, Townsend, and Pake (1954) have observed spin resonance absorption in a large variety of biological materials. The samples were lyophilized to eliminate the large dielectric losses that are produced by water at microwave frequencies. The resonances are relatively narrow--about 5 to 10 gauss wide--and their $g$ values all lie at about 2.00. The area under the absorption curve was compared with a standard to evaluate the free-radical concentration in the various substances. The biological materials studied included leaves, roots, seeds, fungus, frog eggs, insects, and various organs and tissues from rabbits and mice. In every case, a spin resonance absorption was obtained. The estimated free-radical content varied from about $10^{-8}$ to $10^{-6}$ M/g of dry weight. Etiolated barley seedlings exhibited only a very small signal when compared with leaves which were grown in the light. However, after the seedlings were illuminated for 24 hr, the absorption had grown to the same size as that exhibited by the nonetiolated seedlings. Similarly, ungerminated digitalis seed showed no absorption, while germinating seeds exhibited a signal corresponding to about $10^{-7}$ M/g of dry weight. These experiments indicate a relationship between biological activity and free-radical concentration.

Most biological material shows an electron spin resonance after heating. Charred glucose, charred natural and artificial cellulose, charred anthracene and glycerine, charred vegetable root and other complex natural organic materials, charcoals formed below 600°C, and deposits from luminous flames all show paramagnetic resonance with a $g$ value of approximately 2.003. Even coal, naturally occurring from the ground, exhibits such resonances (Bennett, Ingram, and Tapley (1955), Ingram and Bennett (1954a), Uebersfeld, Etienne, and Combrisson, (1954), and Ingram, Tapley, Jackson, Bond, and Murnaghan (1954)), and the amplitude of the line decreases from the oldest coal to the youngest one. It seems that paramagnetic resonance often appears when organic material is damaged, either by nature, as in coals, or by artificial heating, as in charred material.

The direct association of electron spin resonance with biological activity in plant material has only recently been demonstrated by Commoner, Heise, and Townsend (1956). Chloroplasts, which are the biologically active nuclei of plant cells, were isolated from green tobacco leaves and put in flat cells, and their spin resonance was measured at 9000 Mc/sec. The samples were illuminated as needed through a 2-mm hole in the cavity resonator. In the dark, a small spin resonance absorption was observed. Upon illumination there was an obvious increase in the intensity of the paramagnetic resonance. The response to illumination is rapid, readily reversible, and recurrent. It takes about 1 min for the light-induced resonance to decay away. The decay
and growth curves are approximately straight lines when plotted on semilog paper. The half time for appearance of resonance after illumination is about 12 sec, and for decay the half time is about 32 sec.

The resonance signal increases with relative light intensity and exhibits a light-saturation effect. Commoner, Heise, and Townsend (1956) suggest that two classes of unstable paramagnetic species may exist. One class includes a triplet state of chlorophyll and the related proposed chlorophyll-containing lattice in which light induces the formation of conduction electrons and their corresponding holes. The second class of possible paramagnetic substances includes components of the oxidation-reduction transport system, such as DPN. The experiment described does not distinguish between these two classes.

Naturally occurring unpaired electrons can be detected from metal ions, as well as from purely organic material. Among the more important of such elements that have been detected are manganese, copper, vanadium, and iron. Shields, Ard, and Gordy (1956) have investigated some of these naturally occurring spin resonance absorptions in several substances. In this case, the materials were not lyophilized, but consisted of fresh or naturally dried plant substances. An absorption spectrum consisting of 6 lines with a spacing of 95 gauss and a total spread of 475 gauss was observed in several leaves. This signal was attributed to the transition element manganese, which is known to play a role in the biological activities of plants. The 6-line spectrum arises from the hyperfine interaction between the unpaired electron and the Mn$^{55}$ nucleus, which has a spin of 5/2. Because the multiplet spacing of a manganese ion embedded in a crystal would depend on the crystalline field, it was concluded that the Mn$^{++}$ ions were dissolved in the water within the leaves. In addition to the manganese-ion resonance, a narrow line with a $g$ value of 2.00 was observed in pine cones, pine needles, fallen oak leaves, naturally dried ivy stems, and other apparently dead plant materials. It was suggested that this sharp single resonance absorption may arise from bound or semibound oxygen. A third type of absorption was found in the ribs of cotton leaves and the stems of oak leaves, which consisted of a broad line of 500-gauss width with a $g$ value of 2.06. The tentative assignment of this resonance to cupric ions was made, the $g$ value agreeing very well with that obtained by Ingram and Bennett (1954b) for the copper derivative of chlorophyll.

Melamine has also been shown to exhibit an electron spin resonance by Commoner, Townsend, and Pake (1954). This is an interesting result, because melamine formation can be induced by ultraviolet and ionizing radiation, which, in themselves, are also capable of forming free radicals in biological material.

b. Free Radicals Produced by Chemical Reactions

Many chemical oxidation or reduction reactions have been studied that produce more or less stable free-radical intermediates. Some of these purely chemical reactions involve compounds found in biological systems. The mechanisms of the reactions are probably characteristic of the corresponding biochemical steps. In addition, a number of organic compounds are known which exist as free radicals. Related compounds that are present in biological systems need to be examined for possible spin resonance.
The most extensively studied chemical reaction leading to a semistable free-radical intermediate is oxidation of hydroquinones to semiquinones and thence to quinones:

This general reaction has been studied by Wertz and Vivo (1955), Venkataraman and Fraenkel (1955), and Blois (1955). On oxidation of pyrogallol with oxygen, a free-radical intermediate is formed, which shows a complex spin resonance spectrum characteristic of semiquinones (Hoskins and Loy, 1955). This intermediate is fairly stable at low temperatures.

Diarylamines, such as diphenylamine, react with oxygen in an alkaline toluene-alcohol solution to produce stable free radicals (Hoskins, 1956). Hoskins postulates that this reaction forms diphenylnitric oxide, \((C_6H_5)_2NO\). The spectroscopic splitting factor for the observed spin resonance is \(g = 2.0062\). Triphenylmethyl free radicals, which are usually formed by the reaction of an alkali metal on the triphenylmethyl halide,

\[
(C_6H_5)_3CCl + Na \rightarrow NaCl + (C_6H_5)_3C, 
\]

are quite stable in solvents such as benzene, and exhibit a strong spin resonance signal (Jarrett, Sloan, and Vaughan (1956), and Walter, Codrington, D'Adamo, and Torrey (1956)).

Many derivatives of triphenylmethyl and related compounds show such stable free radicals. In triphenylmethyl, hyperfine structure shows there is no planarity of the rings. Naphthalene negative ion in tetrahydrofuran or dimethoxyethane also shows spin resonance (Tuttle, Ward, and Weissman (1956), and deBoer (1956)). In a closely related study, Bruce, Norberg, and Weissman (1956) have studied the electron-exchange rate between N,N,N',N'-tetramethyl-p-phenylenediamine and Wurster's Blue. Wurster's Blue is the product derived from the first compound by a one-electron oxidation. The spin resonance line broadening showed an electron-exchange rate for which \(k = 2.5 \times 10^4\) liter mole\(^{-1}\)sec\(^{-1}\). This technique is useful for electron-exchange reactions for which \(k > 3 \times 10^3\) liter mole\(^{-1}\)sec\(^{-1}\).

Gibson and Ingram (1956) have observed free electrons, not associated with the iron, for met-hemoglobin. In these experiments a concentrated solution of acid-met-hemoglobin or myoglobin was placed in a small cup at the bottom of the cavity resonator, potassium periodate or hydrogen peroxide was added as an oxidizing agent, and the entire resonator was cooled in liquid air. The oxidized hemoglobin and myoglobin showed spin resonance with a \(g\) value of 2.003. Very similar absorption lines were observed from intermediate oxidation states of metal-free tetraphenylporphin and metal-free phthalocyanine.
c. Free Radicals Produced by Ionizing Radiation

A free-radical mechanism for the primary steps in radiation damage to organic material has long been accepted. In the solid state these free radicals are often trapped in a semistable environment and may be detected by spin resonance absorption. At Duke University, Gordy and co-workers have begun an extensive study of the effects of ionizing radiations on biological substances by observing the spin resonance absorption from biological materials that have been subjected to X irradiation. The initial experiments were carried out by Gordy, Ard, and Shields (1955) on proteins such as hair, toenail, feather, cattle hide, raw silk, skull bone, and fish scale; amino acids such as glycine, alanine, valine, leucine, glutamic acid, threonine, serine hydrochloride, and cystine; and carboxylic and hydroxy acids including acetic, formic, propionic, palmitic, glycolic, citric, pyruvic, and lactic acids. Ordinarily, these substances do not exhibit spin resonance absorption, but, after irradiation with 50-kv X rays, free radicals are formed which are quite stable. The resonances spectra that were observed in the above samples have a g value of 2.0, are relatively sharp, and usually consisted of several components. In some cases, the fine structure was ascribed to hyperfine interaction between the unpaired electron and the magnetic nuclei in the free radical and a tentative identification of the free radical could be made on the basis of the number of hyperfine components and their relative intensities. In other resonance absorptions, the component separation was found to depend on the strength of the applied magnetic field, and the splitting was ascribed to crystalline-field interactions. The signals obtained from hair, toenail, and feather were similar to that obtained from cystine, and it was concluded that the resonance observed in these proteins arises predominantly from their cystine constituent. It was proposed that the odd electron in cystine is on one of the sulfur atoms or is shared between the two S atoms in a three-electron bond. Cattle hide, raw silk, and fish scale all presented a doublet structure in their spectra with a spacing of about 12 gauss. The same type of doublet was also found in a signal from irradiated glycyglycine. This splitting was ascribed to a direct dipole-dipole interaction between the odd electron which is presumably localized on an oxygen atom, and the bridging proton.

Recently, Gordy and Shields (1956) have extended the study of the spin resonance spectra of X-irradiated proteins to include histone, insulin, hemoglobin, and albumin. In every case, two types of patterns are obtained, either separately or in combination. One pattern is that characteristic of irradiated cystine, and the other is a doublet similar to the one obtained from irradiated glycyglycine. McCormick and Gordy (1956) have studied the spin resonance of a series of X-irradiated peptides and polypeptides, partly as an aid in the interpretation of the results on the more complex proteins. The substances studied include glycyglycyglycine, DL-alanyl-DL-alanine, acetyl DL-alanine, glycyl-DL-valine, acetyl DL-leucine, DL-alanylglutamic acid, and DL-alanylglycyglycine. Electron spin resonance absorption has been observed in X-irradiated sugars and cellulose fibers by Shields, Ard, and Gordy (1956). The work has been extended to X-irradiated hormones and vitamins by Rexroad and Gordy (1956). Progesterone, para-thyroid, vitamin A, vitamin K, biotin, and ascorbic acid exhibited spin resonance absorption, but hexestrol gave no detectable resonance after prolonged irradiation. X-irradiated nucleic acids such as DNA, RNA, thymidine, guanosine, guanylic acid, adenosine, cytidine, and inosine were found by Shields and Gordy (1956).
to exhibit characteristic spin resonance absorption. The hope in this research is the determination of the mechanism of radiation damage to the cell nucleus. Van Roggen, Van Roggen, and Gordy (1956) have observed spin resonance absorption in an X-irradiated single crystal of D-alanine. An interesting variation of the resonance pattern with respect to the relative orientation of the crystal axes with the external magnetic field occurs. This orientation dependence indicates that the motions of the radical in the crystal are highly restricted. Gordy and Shields (1956) find that the spin resonance patterns of X-irradiated glycine, valine, and leucine become more complex and less resolvable when observed at liquid N\textsubscript{2} temperatures than when observed at room temperature. Also, a noticeable difference in the effects of ionizing radiations has been detected for different isomeric forms of leucine.

d. Solid-State Experiments

The study of the spin resonance absorptions from transition elements in inorganic salts has yielded a large amount of information about the metal bonding in these systems. A review of this type of work is given by Bleaney and Stevens (1953) and Bowers and Owen (1955). The experiments are performed on single crystals of the salt being investigated. In many cases, it is necessary to dilute the crystal with an isomorphous, diamagnetic substance and observe the spin resonance at low temperatures in order that a sufficiently narrow signal can be obtained. The variation of the $g$ value with different orientations of the crystalline axes with respect to the magnetic field may be quantitatively interpreted in terms of the spatial distribution of the unpaired electrons. If nuclear hyperfine interactions are observed, the localization of the unpaired electrons is further established. Thus, a detailed knowledge about the chemical bonds of the transition elements can be obtained if a proper theoretical interpretation of their spin resonance spectra is made.

Recently, several metal-organic compounds of biological interest have been studied; viz., chelate, phthalocyanine, and hemoglobin derivatives. Bowers and Owen (1955) mention the unpublished results of Bogle and Owen on the Ti\textsuperscript{+3} oxalate: KTi(C\textsubscript{2}O\textsubscript{4})\textsubscript{2}·H\textsubscript{2}O. The observations were made at temperatures of 90\degree K and 20\degree K. The $g$ values are $g_\parallel = 1.86$ and $g_\perp = 1.96$. No nuclear hyperfine structure was observed, but the spectrum showed that there were two distinct magnetic complexes per unit cell. Spin resonance absorptions in several trichelate complexes of Cr\textsuperscript{+3} have been investigated by Singer (1955). From single-crystal measurements on chromic acetylanetone the relative orientations of the electric axes were determined. The $g$ value of 1.983 for this compound was interpreted as an indication of considerable covalent character in the Cr\textsuperscript{+3} bonds. Powdered samples of chromic acetylanetone, oxalate, and ethylenediamine complexes were also examined, as were liquid solutions of chromic acetylanetone, potassium trioxalate chromate, and potassium chrome alum. The magnitude of the crystalline field of axial symmetry was found to be very large in the trichelates, and it was proposed that the dipole moments associated with the carbonyl groups are the important factors in producing the large field. By a comparison of the resonances in the liquid and powdered samples, it was found that the electric-field interaction is a property of the isolated individual molecules in the trichelates, while in the alums it is a property of the whole crystal.

McGarvey (1956) has investigated the spin resonance of copper chelates. The $g$ values for a single crystal of Cu\textsuperscript{+2} acetylacetonate were found to be
$g_{\parallel} = 2.254$ and $g_{\perp} = 2.075$. These $g$ values were interpreted as indicating the existence of strong $\pi$ bonding of covalent character and weak $\sigma$ bonding. The solutions of several copper chelates exhibit an interesting asymmetric hyperfine interaction with the copper nucleus. The splitting varies with the choice of solvent, and a correlation was indicated between the strength of the solvated complex and the hyperfine splitting, suggesting that the bonding solvent pulls the electron away from the copper.

Ingram and Bennett (1954b) have begun a study of the spin resonance absorptions in phthalocyanine, chlorophyll, and hemoglobin derivatives. The central structure of these molecules is similar, and the detailed single-crystal spin resonance data are of value for determining the nature of the chemical bonding of the transition element. Single crystals of copper and cobalt phthalocyanine were studied, as well as several polycrystalline samples. The results are summarized below, with the more recent work by Bennett and Ingram (1955) on copper phthalocyanine included.

A dilute single crystal of copper phthalocyanine was observed at temperatures of $20^\circ$K and $270^\circ$K. The $g$ values were found to be $g_{\perp} = 2.165$ and $g_{\parallel} = 2.045$. The maximum copper hyperfine splitting was 210 gauss, while the minimum line width obtained by dilution was 50 gauss. The cobalt, iron, manganese, vanadium, nickel, and metal-free derivatives of phthalocyanine all exhibited paramagnetic resonance with line widths of several hundred gauss. Polycrystalline samples of the aluminum, lead, zinc, and magnesium phthalocyanines showed no resonance absorption. Four different derivatives of copper chlorophylls all gave an absorption line 100 gauss wide with a $g$ value of 2.05. Human hemoglobin showed only a faint signal.

Bulk magnetic-susceptibility measurements of the ferric hemoprotein complexes suggested that the iron is essentially held by ionic bonds in these structures. The ferric ion has an orbital singlet state with $L = 0$, so that a $g$ value of 2.0 was expected for the spin resonance absorption. However, Ingram and Bennett (1954b) and Bennett, Ingram, George, and Griffith (1955) found that polycrystalline samples of hemin, acidic hemoglobin, and its fluoride complex gave spin resonance absorption with a $g$ value very close to 6.0. The measurements were repeated at five different wave lengths and the same $g$ value was obtained each time, which ruled out the possibility that zero-field splitting was responsible for the large observed $g$ value. The experiment clearly demonstrates that the electronic state of the iron in these complexes is quite different from that of the free $Fe^{3+}$ ion, and must include considerable covalent character. Measurements were also made on the azide complex of ferrihemoglobin; in which the bonding of the iron is classified as essentially covalent. The results show that a significant difference exists in the bonding of iron in a covalent inorganic coordination complex such as the ferricyanide ion.

George, Bennett, and Ingram (1956) have extended the measurements on hemoglobin derivatives to single crystals of acidic ferrymyoglobin. The observed $g$-value variation, as a function of the angle between the crystal axes and the applied magnetic field gives accurate values for the orientation of the heme groups with respect to the external crystal axes. The measured $g$ values are $g_{\parallel} = 2.00$ and $g_{\perp} = 6.00$. Measurements at very short wave
lengths (6 mm) still give a $g_\perp = 6.0$, indicating that the zero-field splitting of the ground state is considerably greater than $2 \text{ cm}^{-1}$, which is much larger than encountered in ionically bound ferric salts.

The orientation of the heme group of crystalline myoglobin and hemoglobin can be determined rather accurately by measurements of electron spin resonance. Bennett and Ingram (1956), Ingram and Kendrew (1956), and Ingram, Gibson, and Perutz (1956) have studied these compounds, and discussed some of the possible arrangements of the four heme groups present in the hemoglobin molecule. Ingram and Kendrew (1956) show that the polarization dichroism of the myoglobin crystals can be predicted from these electron spin resonance measurements.
II. NUCLEAR MAGNETIC RESONANCE

1. Fundamentals of Nuclear Magnetic Resonance

The first nuclear magnetic resonance absorptions in condensed systems were observed by Bloch, Hansen, and Packard (1946) and Purcell, Torrey, and Pound (1946). Recent reviews of the application of nuclear magnetic resonance include: Andrew (1955), Gordy (1956), Grivet (1955), Gutowsky (1954), Ingram (1955), Pake (1950), Purcell (1954), Shoolery and Weaver (1955), and Wertz (1955). The stable magnetic nuclear species have spins ranging from $I = 1/2$ to $I = 6$ and a magnetic dipole moment which is about $1/2000$ that of the electron. The fundamental unit of nuclear magnetism is the nuclear magneton, which is the electronic Bohr magneton multiplied by the ratio of the electron mass to the proton mass. The fundamentals of nuclear magnetic resonance are essentially the same as outlined for spin resonance. For nuclei with $I = 1/2$, the treatment would be identical. Nuclei with $I$ greater than $1/2$ have $2I + 1$ equally spaced energy levels in an applied magnetic field. Transitions occur between adjacent levels, with the resonance condition given by

$$\hbar \nu = g \mu_n H_0 \quad (6)$$

where $\hbar$ is Planck's constant, $\nu$ is the frequency, $g$ is the nuclear $g$ factor, $\mu_n$ is the nuclear magneton, and $H_0$ is the magnetic field at the nucleus. Because of the smallness of its magnetic moment, the resonance frequency of a typical nuclear species exposed to a magnetic field of $10^4$ gauss is about $10^4$ Mc/sec, which is to be compared with the electron frequency of $28,000$ Mc/s. Also, the excess population in the lower nuclear energy level is only about one in $10^6$ at room temperature in a field of $10^4$ gauss. The result is that the magnetic resonance absorption from a given number of nuclei is smaller by a factor of about $10^7$ than that obtained from an equal number of electrons.

The application of nuclear magnetic resonance to chemical problems rests in the fact that different nuclear species have different resonance frequencies in the same magnetic field. The nuclear $g$ values are constant and can be changed only by a nuclear transition, which would require much higher energies than are normally available in a chemical experiment. Thus a $g$-value measurement makes it possible to immediately identify the origin of an observed nuclear resonance absorption. Although nuclei themselves do not participate directly in chemical processes, the electrons that surround them are directly involved, and the nuclear resonance may be used as a tool to determine characteristic features of the electronic state. The primary action of the electrons surrounding the nucleus is a shifting of the resonance frequency in a given laboratory field from that which would be observed for a bare nucleus. The electrons contribute a small internal field at the nucleus, so that the nucleus sees the sum of the electronic field and the externally applied field. The magnitude of the frequency deviation depends sensitively on the type of bonding of the atom in which the nucleus resides as well as on the substitutents that are attached to the bonds. This type of interaction is called the chemical shift. Thus, not only can the nuclear species be identified by nuclear magnetic resonance, but also the chemical environment about it can be determined. A given chemical compound then exhibits, at least in
principle, a characteristic nuclear magnetic resonance spectrum that distinguishes it from all other compounds.

2. Instrumentation

In principle, nuclear magnetic resonance spectrometers do not differ markedly from the electron spin resonance spectrometers previously discussed. Magnetic field modulation and lock-in detection provide high sensitivity for the recording of the nuclear absorption. The practical difference in the two systems arises from the difference in their respective resonance frequencies. Two schemes are widely used for the observation of nuclear resonance. In the nuclear-induction system, two mutually perpendicular coils are coupled to the sample. One of these coils is driven by a radio-frequency oscillator, and serves as an exciter of transitions among the nuclear energy levels. The other coil serves as a pickup coil by observing the radiation that is emitted by the nuclei when they make a transition. The pickup coil is tuned to the same frequency as the radio-frequency oscillator and is coupled to a low-noise radio receiver. The output of the radio may be coupled to an oscilloscope or to the lock-in amplifier. The second method of detecting nuclear resonance is a single-coil device. The sample is coupled to a coil which is driven by an oscillating detector. At resonance, the sample draws energy from the oscillating field in the coil. This causes a reaction in the oscillating detector, which may be fed into a radio receiver. Commercial spectrometers are available from Varian Associates and Nuclear Magnetics Corp.

3. Applications of Nuclear Magnetic Resonance to Biology

The application of nuclear magnetic resonance to biological problems has been much slower than for electron spin resonance. This seems to be not only because of the complex instrumentation required and inherently low sensitivity of nuclear resonance, but also the great difficulty in interpreting the data. However, many physical and physical-chemical studies which are of direct interest to the biological sciences have been made with nuclear resonance techniques. Among the results obtained are new physical constants, structure determinations, and elucidation of the nature of solvation processes. As an analytical tool for such elements as oxygen and nitrogen the technique of nuclear magnetic resonance holds great promise and is very interesting in view of the absence of useful radioactive isotopes of these elements.

a. Studies with Complex Biological Material

Several studies of the nuclear magnetic resonance absorption by protons in biological material have been made. Shaw, Elsden, and co-workers (1950, 1951, 1952) have observed proton magnetic resonance in a large variety of proteins, carbohydrates, and vegetable tissue. A narrow line was found which was shown to originate from the water within the sample. Superimposed on this sharp resonance was a broad line of about 6-gauss width, which arose from the protons in the large molecules.

Proton magnetic resonance studies of water have been made with aqueous solutions of sodium desoxyribonucleate, hemocyanin, soluble starch, and egg albumin by Jacobson, Anderson, and Arnold (1954). They have shown
an extensive bonding of the water to the desoxyribonucleate molecule. For the hemocyanin, starch, and albumin in 1.6% solutions, the proton resonance lines were almost identical with pure water, but in 1.6% sodium desoxyribonucleate solution the proton absorption was decreased 17%. A 60% solution reduced the resonance absorption area by 85%. Addition of an electrolyte (NaCl) partially reversed this process. Although the dielectric method for studying water structure changes is more sensitive, it is disturbed by electrolytes, which in moderate concentrations do not interfere with the proton resonance method.

Odeblad and Lindstrom (1955) have reported some preliminary observations on the proton magnetic resonance absorption in biological materials. Signal intensities and relaxation times were observed. A sharp signal was identified as arising from the water content of the sample. A large, broad peak was also found. Odeblad and Bryhn (1956) have studied the change in the proton magnetic resonance spectra of human cervical mucus during the menstrual cycle. The postmenstrual signal consists of a single, sharp line, while the midcycle resonance exhibits an additional broad component.

Odeblad has reported on a number of studies in progress of the measurement of proton magnetic resonance in biological tissue, including the crystalline lens of the eye, the adrenal glands, and red blood cells. Heavy-water exchange experiments with these tissues indicate there are two main types of protons—those that can exchange rapidly with heavy water, and which give rise to a sharp signal, and the other, a nonexchangeable group which gives a broad peak comprising most of the signal area.

Oshima and Kusumoto (1956) have studied the proton resonance of stretched and unstretched rubber at various temperatures. Stretching affects the proton resonance both in line width and in transition temperature. This would be an interesting type of experiment to perform with muscle. Banas, Mrowca, and Guth (1955) have studied proton magnetic resonance in synthetic as well as natural rubber. For unvulcanized material, a sharp drop in line width with increasing temperature was found at 125°K. This transition is considered due to an increased rate of rotation of CH₃ groups about the C₃ symmetry axis.

In general, direct measurements of proton resonance signals from complex biological materials have been disappointing. Except for the water peak, a broad resonance line is observed, corresponding to the many different types of protons present in the sample. As in most biochemical studies, it will probably be necessary to perform some type of chemical separation before the biological sample can be profitably examined by proton magnetic resonance.

b. Organic Structure Determinations

Many organic compounds have been studied by proton magnetic resonance, and some of the results are of considerable interest to the biologist. One of the most important of these projects has been the investigations by Kromhout and Moulton (1955) and Moulton and Kromhout (1956), who have studied and interpreted the resonance absorption of urea, glycine, hydroxylamine hydrochloride, cyanamide, acetamide, α- and β-alanine,
benzidine, and other compounds. They obtained further evidence for the
-NH₂⁺ configuration of glycine in the solid state. (See also Shaw (1952).)
By the use of line widths as a function of temperature they were able to show
the onset of rotation of amino groups. Benzidine shows a transition at -40°C,
which was assigned to the onset of rotation of the amino group. Acetamide
showed no transition from -196°C to 64°C, and thus may have a rigid amino
group. In glycine, a proton resonance transition at -85°C correlates with a
change in dielectric constant at this same temperature and is also presumed
to be related to the rotation of the amino group. DL-α-alanine and L-α-
alanine were the same, and showed a presumed rotational transition at -50°C;
b-α-alanine showed a transition at -25°C, also probably due to the rotation of
the amino group.

Phillips (1955) has shown a restricted rotation of the amino group in
N,N-dimethylacetamide, which confirms existing infrared and Raman spectra
data. The effect of temperature on methylaniline has been studied by Gutowsky
and Pake (1950), and Andrew (1953) has demonstrated the planarity of the urea
model.

By use of phosphorus chemical shifts, Van Wazer, Callis, and Shoolery
(1955) have been able to give fundamental support to the idea that there is a
difference between isolated, end, middle, and branching phosphate groups.
Each of these four kinds of phosphate groups gives a separate resonance peak.

Nuclear magnetic resonance spectra on many other compounds have been
published. A few of importance to the biologist include: 12 conjugated aromatic
hydrocarbons (Bernstein and Schneider (1956a)); pyridine and related compounds
(Bernstein and Schneider (1956b)); alkyl derivatives (Glick and Bothner-By (1956));
conjugated aromatic hydrocarbons (Bernstein and Schneider (1956c)); and
trifluoroacetic acid (Hood, Redlich, and Reilly (1955)).

The hydrogen-bond shift of the proton magnetic resonance has been
measured by Huggins, Pimentel, and Shoolery (1956) for phenol, o- and
p-chlorophenol, o-cresol, and acetic acid in various concentrations in carbon
tetrachloride solution and also for acetic acid in acetone solution. The proton
resonance was correlated qualitatively with known hydrogen-bonding properties
and infrared-spectra changes for these compounds.

Instruments are not yet sufficiently well standardized, and development
work has not progressed to the point where standard manuals of proton
magnetic resonance spectra are available. In time these will be available,
and then such data will be an excellent aid in analytical chemistry or bio-
chemistry.

c. Isotopic Analysis

The quantitative and qualitative analysis of unknown substances for
certain isotopes containing magnetic nuclei is of great potential in biology.
Such analysis can serve not only as an analytical tool, but also as a tracer
technique. For oxygen and nitrogen, which possess no radioactive isotopes
of sufficient lifetime to be useful for tracer studies, this possibility is very
interesting. Among the isotopes of particular importance to biology, and which
possess a magnetic nucleus, are hydrogen, with spin = 1/2; deuterium, with
spin = 1; carbon-13, with spin = 1/2; nitrogen-14, with spin = 1; nitrogen-15, with spin = 1/2; phosphorus-31, with spin = 1/2; and oxygen-17, with spin = 5/2. These isotopes, if present in sufficient concentrations and amount, can be detected and assayed by nuclear magnetic resonance. This can be done even in the presence of many other substances.

Not only can such isotopes be detected and measured, but also something about their chemical bonding can be determined by the measurement of the chemical shifts of the particular isotope under consideration. These chemical shifts, which are very small compared with nuclear shifts, are displacements of the resonance frequency caused by chemical binding of other atoms to the one in question. Weaver, Tolbert, and Laforce (1955) have measured the O\textsuperscript{17} chemical shifts for a number of organic compounds. The data are shown in Table I. Holder and Klein (1956a) have measured the nitrogen-14 chemical shifts for a number of nitrogen-containing compounds and these data are shown in Table II. The chemical shifts for N\textsuperscript{15} are similar to those for N\textsuperscript{14}. The chemical shifts for a number of silicon-containing compounds have been measured by Holzman, Lauterbur, Anderson, and Koth (1956); these measurements show the possibility of tracer studies with silicon-29. The Si\textsuperscript{29} shifts covered a range of about 1.5 gauss in a 10,000-gauss field. This is somewhat less than the O\textsuperscript{17} and N\textsuperscript{14} chemical shifts.

All the isotopes just mentioned may easily be used in a tracer fashion, because they are the rare isotope of a given element. Chemical shifts of other elements, while useful as analytical tools, are not easily adapted to tracer biology.

An extensive compilation of phosphorus-31 chemical shifts has been listed by Muller, Lauterbur, and Goldenson (1956). Shifts vary from -1.1 to +2.3 gauss in a 10,000-gauss field with the reference selected as H\textsubscript{3}PO\textsubscript{4}.

The proton chemical shifts, of course, can also be used in many cases to measure and analyze mixtures of substances. As for phosphorus-31, the abundance of the H\textsuperscript{1} isotope precludes the possibility of using the measurements in a tracer technique. Perhaps the best compilation of proton chemical shifts is that of Gutowsky (1954), but it is expected that in time more extensive listings will become available.

Observations on the chemical shielding and spin coupling of carbon-13 nuclei in various chemical compounds have been made by Holm (1956). The chemical shifts of C\textsuperscript{13} for a number of compounds are given in Table III and cover a range of about 2.8 gauss in a 10,000-gauss field.

The primary considerations in the general application of nuclear magnetic resonance absorption to biological materials is the relatively low sensitivity of this technique, coupled with a practical restriction on sample size. This restriction on sample size arises primarily from the necessary requirement of a reasonably homogeneous magnetic field throughout the sample volume. For a magnet with a 12-inch pole diameter and a 2-inch gap, a field inhomogeneity of 0.1 gauss at 10\textsuperscript{4} gauss over a sample volume of 10 ml is a reasonable figure. On the basis of an active sample volume of 10 ml the concentrations required of several biologically interesting nuclei for the
Table I

Chemical shifts of oxygen-17 (Weaver, Tolbert, and Laforce, (1955))

<table>
<thead>
<tr>
<th>Compound</th>
<th>Shift in a $10^{12}$ gauss field (gauss)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0</td>
</tr>
<tr>
<td>Tetramethylorthosilicate</td>
<td>-0.2</td>
</tr>
<tr>
<td>Octamethylcyclotetrasiloxane</td>
<td>-0.8</td>
</tr>
<tr>
<td>N-butynitrite</td>
<td>-1.7</td>
</tr>
<tr>
<td>Hydrogen peroxide (30%)</td>
<td>-1.9</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>-2.2</td>
</tr>
<tr>
<td>Formic acid</td>
<td>-2.7</td>
</tr>
<tr>
<td>Sodium formate</td>
<td>-3.0</td>
</tr>
<tr>
<td>Formamide</td>
<td>-3.2</td>
</tr>
<tr>
<td>Ethynitrate</td>
<td>-3.4</td>
</tr>
<tr>
<td>N-butynitrite</td>
<td>-3.8</td>
</tr>
<tr>
<td>Sodium nitrate</td>
<td>-4.3</td>
</tr>
<tr>
<td>Ethynitrate</td>
<td>-4.7</td>
</tr>
<tr>
<td>Sulfur dioxide, liquid</td>
<td>-5.4</td>
</tr>
<tr>
<td>Acetone</td>
<td>-6.0</td>
</tr>
<tr>
<td>Nitroethane</td>
<td>-6.4</td>
</tr>
<tr>
<td>Sodium nitrite</td>
<td>-6.9</td>
</tr>
</tbody>
</table>
Table II

Chemical shifts of nitrogen-14 (Holder and Klein (1955))

<table>
<thead>
<tr>
<th>Compound</th>
<th>Shift in a 10^4 gauss field (gauss)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄⁺</td>
<td>+6.02</td>
</tr>
<tr>
<td>(C₃H₇)NH, (CH₃-CH₂)₃N</td>
<td>+5.75</td>
</tr>
<tr>
<td>N₂H₄</td>
<td>+5.66</td>
</tr>
<tr>
<td>(CH₃)₄NBr</td>
<td>+5.52</td>
</tr>
<tr>
<td>NH₃</td>
<td>+5.44</td>
</tr>
<tr>
<td>O=C(NH₂)₂</td>
<td>+5.36</td>
</tr>
<tr>
<td>NH₂OH·HCl</td>
<td>+5.20</td>
</tr>
<tr>
<td>CH₃-C=O</td>
<td>+4.98</td>
</tr>
<tr>
<td>NH₂</td>
<td></td>
</tr>
<tr>
<td>(SCN)⁻</td>
<td>+4.06</td>
</tr>
<tr>
<td>CH₃-CN</td>
<td>+3.85</td>
</tr>
<tr>
<td>CN⁻, CH₃SCN</td>
<td>+3.80</td>
</tr>
<tr>
<td>C(NO₂)₄, C₂(NO₂)₆</td>
<td>+3.00</td>
</tr>
<tr>
<td>C₅H₅N (pyridine)</td>
<td>+2.76</td>
</tr>
<tr>
<td>N₂</td>
<td>+2.68</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>+2.54</td>
</tr>
<tr>
<td>C₆H₅NO₂</td>
<td>+2.52</td>
</tr>
<tr>
<td>n-C₃H₇NO₂</td>
<td>+2.28</td>
</tr>
<tr>
<td>NO₂⁻</td>
<td>0</td>
</tr>
</tbody>
</table>
Table III

<table>
<thead>
<tr>
<th>Compound</th>
<th>Shift in a 10^4 gauss field (gauss)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diiodomethane</td>
<td>1.90</td>
</tr>
<tr>
<td>Methyl iodide</td>
<td>1.53</td>
</tr>
<tr>
<td>Methyl bromide</td>
<td>1.26</td>
</tr>
<tr>
<td>N-alkanes</td>
<td>1.27 to 1.07</td>
</tr>
<tr>
<td>Methylamine(^{a})</td>
<td>1.16</td>
</tr>
<tr>
<td>Dibromomethane</td>
<td>1.11</td>
</tr>
<tr>
<td>Dimethyldisulfide</td>
<td>1.09</td>
</tr>
<tr>
<td>Cycloalkanes</td>
<td>1.05 to 0.95</td>
</tr>
<tr>
<td>Methyl chloride</td>
<td>1.07</td>
</tr>
<tr>
<td>Ethylene oxide</td>
<td>0.92</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.81</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>0.76</td>
</tr>
<tr>
<td>Dioxane</td>
<td>0.55</td>
</tr>
<tr>
<td>Nitromethane</td>
<td>0.53</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0.52</td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td>0.35</td>
</tr>
<tr>
<td>Nitriles</td>
<td>0.14 to -0.02</td>
</tr>
<tr>
<td>Aromatic and olefinic hydrocarbons</td>
<td>0.10 to -0.0</td>
</tr>
<tr>
<td>Benzene</td>
<td>0.00</td>
</tr>
<tr>
<td>(\text{H}_2\text{O} \text{OR})(^{\text{a}})</td>
<td>-0.31</td>
</tr>
<tr>
<td>(\text{ROCOR})</td>
<td>-0.36</td>
</tr>
<tr>
<td>Formic acid</td>
<td>-0.37</td>
</tr>
<tr>
<td>(\text{RCNR}_2)</td>
<td>-0.45</td>
</tr>
<tr>
<td>(\text{KCN}(^{b})</td>
<td>-0.49</td>
</tr>
<tr>
<td>(\text{RC-OH})</td>
<td>-0.52</td>
</tr>
<tr>
<td>(\text{R-C-R})</td>
<td>-0.79</td>
</tr>
<tr>
<td>(\text{a-hydroxy acid (carbonyl carbon)})</td>
<td>-0.90</td>
</tr>
</tbody>
</table>

\(\text{R}\) denotes any alkyl groups

\(^{a}\) 40% aqueous solution

\(^{b}\) saturated aqueous solution enriched in \(\text{C}^{13}\).
detection of their nuclear resonance absorption with a typical high-sensitivity spectrometer are estimated below (Table IV). Working concentrations of these isotopes should be 10 to 100 times the values in the table. The calculations assume a line width of about 0.1 gauss, room temperature, a magnetic field of $10^4$ gauss, and optimum values for the relaxation times and instrument variables.

It can thus be seen from the calculations that the use of the isotopes as tracers will require large samples and fair concentrations of the given isotope.

The fundamental equations describing nuclear magnetic resonance signals show the linear dependence of signal amplitude upon nuclear concentration as well as the complicated dependence of signals upon relaxation times and radio-frequency field intensity. Holder and Klein (1955) have used this dependency to carry out a determination of the absolute abundance ratio of two isotopes by integrating the areas under their respective absorption curves. Such a method has been routinely applied to the determination of hydrogen and deuterium in water. Usually the proton signal is observed for samples of low hydrogen concentration, and the deuteron signal is used for samples with low deuterium concentrations. Using such techniques, Holder and Klein (1955) have measured $\text{Li}^6/\text{Li}^7$ ratios as well as $\text{H}^2/\text{H}^1$ in paramagnetically relaxed water solutions to an accuracy of 1%.

d. Determination of Physical States and Constants

Many questions about the exact physical state of organic and inorganic compounds can be answered by nuclear magnetic resonance studies. Most work of this type has thus far been done with simple chemical systems, but the technique and results can and will be extended to biological systems. Representative examples are given below.

Water and its relation to dissolved solutes is an important problem in all biological systems. By proton magnetic resonance, Morgan, Murphy, and Noole (1956) have measured the extent of hydrogen bonding by Cr+++ ions in water-glycerine mixtures. Various diamagnetic salts in aqueous solutions have been studied by Shoolery and Alder (1955). They have shown that in the solvated hydrogen-bond structure, exchange occurs faster than $10^4$ times/sec for most salts. Exceptions were Al+++ and concentrated ZnCl$_2$ solutions. The latter two exceptions seem to be all or partially due to viscosity phenomena.

The absorption of water on TiO$_2$ has been studied by Mays and Brady (1956) and Fuschillo and Aston (1956). They have shown that at a fraction of a monolayer, ice clusters are not formed at $-77^\circ$K, and yet there is a mobility of the proton.

Wertz and Jardetzky (1956) have studied the shifts of various concentrations of Na$^+$ ion in water. No shifts were observed, but a definite difference in line widths was observed, probably due to complex formation. Proton magnetic resonance studies have been used by Grunwald, Lowenstein, and Meiboom (1956) to study the protolysis of methylammonium ion in water and determine the rate constant for the reaction. Hydration effects have been studied by Wertz (1956) in the chlorine-35 ion by magnetic resonance.
Table IV

Concentrations of several nuclei for observation of nuclear resonance absorption

<table>
<thead>
<tr>
<th>Isotope</th>
<th>$H^2$</th>
<th>$C^{13}$</th>
<th>$N^{14}$</th>
<th>$N^{15}$</th>
<th>$O^{17}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resonance frequency (Mc/s)</td>
<td>6.5</td>
<td>10.7</td>
<td>3.1</td>
<td>4.3</td>
<td>5.8</td>
</tr>
<tr>
<td>Minimum concentration (Mol/liter)</td>
<td>$6 \times 10^{-5}$</td>
<td>$5 \times 10^{-5}$</td>
<td>$6 \times 10^{-4}$</td>
<td>$6 \times 10^{-4}$</td>
<td>$2 \times 10^{-5}$</td>
</tr>
<tr>
<td>Natural abundance (%)</td>
<td>0.016</td>
<td>1.1</td>
<td>99.6</td>
<td>0.4</td>
<td>0.04</td>
</tr>
<tr>
<td>Minimum concentration of natural element (Mol/l)</td>
<td>0.4</td>
<td>$5 \times 10^{-3}$</td>
<td>$6 \times 10^{-4}$</td>
<td>0.15</td>
<td>0.05</td>
</tr>
</tbody>
</table>
The measurement of dissociation constants of strong acids by conductivities and Ostwald's dilution law has been shown to give values too low, and new values, derived from nuclear magnetic resonance data, have been determined in some cases. Hood, Redlich, and Reilly (1955) have used proton and fluorine magnetic resonance of trifluoroacetic acid in water and obtained a dissociation constant of $K = 1.8$. The old value was $K = 0.588$. Using proton magnetic resonance, Hood, Redlich, and Reilly (1954) have studied nitric, perchloric, and hydrochloric acids in water. Dissociation constants of $K = 22$ for nitric acid and $K = 38$ for perchloric acid were determined. In an earlier work, Gutowsky and Saika (1953) studied the proton resonance of nitric, perchloric, sulfuric, and acetic acids, and of sodium and potassium hydroxide. They noted proton shifts in all cases which were dependent on the yield of hydrogen-containing ions. Saturated solutions of sodium and potassium chloride, or potassium nitrate or sulfate caused no shifts of proton resonance from pure water.
BIBLIOGRAPHY


Ingram, D. J. E. and Bennett, J. E. 1954a. Phil. Mag. 45, 545.


Nuclear Magnetics Corp., 154 Boylston St., Boston 16, Massachusetts.

Odeblad, E. and Bryhn, U. 1956. Private communication; to be published in Acta Radiol.


Polarad Electronics Corp., 43-20 34th St., Long Island City 1, New York.


