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— *Review Article* —

Electron Spin Resonance Spectroscopy

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THE YEARS since World War II have brought a continual and almost geometric increase in the sophistication of physical measurements. While the chemist or pharmaceutical chemist of three decades ago depended for his routine work upon pipets, burets, and balances with the occasional use of the refractometer, pH meter, and the single-beam spectrophotometer, his modern counterpart has at his disposal a profusion of complex electronic instruments. Not only have the old instruments been refined into self-scanning automatic recording systems, but also new atomic and optical principles have been discovered and have led to new types of measurement.

Two new branches of spectroscopy of particular interest in this review are commonly known as nuclear magnetic resonance (NMR) and electron spin resonance (ESR). They are mentioned together because they represent applications of the same principle to two different types of elementary particles. The former, NMR, concerns energy levels due to orientation of the *atomic nucleus* in a magnetic field, while the latter, ESR, has the same relationship with respect to the *electron*. Though there are many differences in the practical details of the instrumentation, there are also many common features, particularly from a viewpoint of the theory. The principal concern of this review is electron spin resonance, but we shall have occasion to compare it with NMR, an instrument probably more familiar to the reader.

The purpose of this article is to set down

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enough of the physical principles and applications of electron spin resonance to make the contemporary literature readable, with particular emphasis on those applications currently being made in chemistry and biology. For more detailed discussion of the theory or specific applications, a wealth of review and research literature is available (1-12).

There is not a unanimity in the use of the name "electron spin resonance." The name used depends on the particular application and the habits of the research worker (5, 12). From a broad instrumental point of view, "electron spin resonance," "electron paramagnetic resonance," and just plain "paramagnetic resonance" are all synonymous, and only the first mentioned will be used here.

While the spectroscopy of nuclear magnetic resonance requires the presence of nuclei possessing magnetic moments, the mere presence of electrons does not lead to an ESR spectrum. Only an atom or molecule possessing an *unpaired* electron will exhibit ESR absorption. Thus, most chemical compounds are inactive in this respect because their electrons are paired; *i.e.*, every possible electron orbital either is empty or is filled with two electrons of opposite spin.

The following types of substances are susceptible to study in ESR spectroscopy: (a) paramagnetic ions of transition group elements, (b) free radicals, (c) triplet-state molecules, often formed by excitation from the (paired electron) ground state in phosphorescent molecules. A triplet-state molecule has two unpaired electrons; the oxygen molecule is an example, though an atypical one. (d) Odd-electron elements and

molecules in the gaseous state, and (e) metals and other solids containing conduction electrons and holes.

This review is devoted to those of the above substances which exist in detectable quantity in liquid solution at ordinary temperatures—principally items *a* and *b*. Item *c* is given only brief mention because of the experimental problems involved in obtaining a spectrum (13).

PHYSICAL PRINCIPLES

It is well known (14)¹ that the electron possesses an axis about which it rotates with an invariant angular momentum, referred to as its spin angular momentum or *spin*. The magnitude of the spin is expressed as $p_s = \frac{1}{2}(h/2\pi)$, where the *spin quantum number*, $\frac{1}{2}$, stands in place of an integer in a similar equation for the orbital angular momentum of an atomic electron. Planck's constant, h , has the value 6.62×10^{-27} erg second. The spinning charge comprises an electric current which produces a magnetic dipole moment, μ_s , also an invariant of the electron, whose value is related to the unit of orbital magnetic moment, μ_o , the Bohr magneton, by

$$\mu_s = \frac{1}{2} g \mu_o \quad (\text{Eq. 1})$$

In this equation, g is a factor determined by the charge structure of the electron and is called the Lande g factor or the spectroscopic splitting factor; for an electron with no orbital angular momentum, g has the value 2.0023. For the purpose of a simple image not structurally meaningful, it is convenient to imagine the electron consisting of a tiny magnet of pole strength 9.27×10^{-13} electromagnetic units and with north and south poles separated by 1 Å. This is a way of illustrating the fact that the spin moment is 9.27×10^{-21} erg/gauss.²

Consider a volume of a dilute gas contained within the pole gap of a magnet with parallel pole faces, *i.e.*, within a homogeneous magnetic field. The rule of spatial quantization permits the electron magnets to occur only in two orientations, as illustrated in Fig. 1. This is a consequence of the fact that the spin quantum number S is $\frac{1}{2}$ and the general rule according to which there are $2S + 1$ permitted orientations. The two permitted states are characterized by a set of quantum numbers representing the orien-

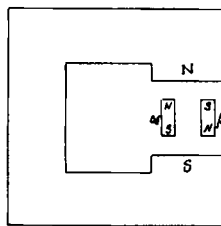


Fig. 1.—Schematic representation of the two permitted electron orientations in a magnetic field. The orientation α is the excited state; β is the ground state.

tation of the electron magnet in the magnetic field, $M_\alpha = +\frac{1}{2}$, $M_\beta = -\frac{1}{2}$. The only possible transitions are those for which $\Delta M = M_\alpha - M_\beta = 1$ and $\Delta M = M_\beta - M_\alpha = -1$. The orientation, α , which is parallel to the magnetic field, is mechanically unstable because of the repulsion of like poles; while the antiparallel orientation, β , is stable. The work required to rotate the electron magnet from condition β to condition α is proportional to the magnetic moment and the magnetic field strength H (gauss). According to Eq. 1, the energies of the states relative to the condition of zero magnetic field strength can be computed by

$$E_\alpha = \mu_s H = \frac{1}{2} g \mu_o H \quad (\text{Eq. 2})$$

$$E_\beta = -\mu_s H = -\frac{1}{2} g \mu_o H$$

The energy of the transition $\Delta M = +1$ is thus

$$\Delta E = E_\alpha - E_\beta = g \mu_o H \quad (\text{Eq. 3})$$

Equations 2 and 3 are expressed graphically in Fig. 2, in which the length of the arrow corresponds to ΔE at a given externally applied field.

As applied to electrons in free atoms, one will find in books on spectroscopy (*e.g.*, Reference 15) an equation for g which takes into account the orbital angular momentum as well as the spin.³ Since the orbital motion in molecules is intimately connected with the physical and chemical bonds, it is not possible, except in gaseous systems, to effect reorientation of the orbital moments without absorbing large energies. As expressed in the literature (3), orbital orientation is quenched.⁴ The electron spin, however, is not involved in the molecular structure; accordingly, spin orientation readily occurs with energy levels almost identical to those of the free electron. Small variations from the free-spin value due to forces between the orbital and the spin magnetic moments are expressed by adjustment of the value of g as used in Eq. 1. This effect is referred to by the term "spin-orbit coupling."

¹ The concepts fundamental to this discussion are found in numerous books on introductory atomic physics or quantum mechanics. Most of the vocabulary is in a succinct form in Reference 14.

² In electrodynamics, a distinction is made between the magnetic field strength (gauss) and the magnetic induction (oersted). While the latter is logically appropriate here, common usage dictates the former. Computation results are the same either way since the materials commonly used have unit magnetic permeability.

³ The equation for g referred to would yield precisely 2 for the free electron and must be corrected for a polarization effect arising in quantum electrodynamics to yield the value 2.0023 referred to above.

⁴ An exceptional class of solid compounds occurs in the lanthanide and actinide rare earth salts, in which the unpaired electrons are deep within the atomic kernel (5, 6, 16).

As in other branches of spectroscopy, absorbed electromagnetic energy lifts the energy level of a molecule from a ground state to an excited state. The ground state energy is represented by the point on curve β of Fig. 2 which corresponds to the applied magnetic field strength. Absorption will occur only if a photon of the electromagnetic radiation matches the energy required to lift the system to the value on curve α at the same H value, illustrated by the length of the arrow in Fig. 2. A given value of field strength H requires a definite electromagnetic frequency, ν , which is calculated in cycles per second by combining Einstein's equation, $\Delta E = h\nu$, with Eq. 3 to yield

$$\nu = \frac{g\mu_0 H}{h} \quad (\text{Eq. 4})$$

It would appear that there is an infinite range of frequencies to choose from, for one need only choose a value of the magnetic field which corresponds to a convenient frequency according to Eq. 4. In practice, the difficulties and cost of generating a steady and homogeneous magnetic field increase in a geometric manner with the field strength. Other desiderata, such as sensitivity and resolvability of the spectrum, are improved by higher frequency and thus higher field strength, so that a compromise is necessary. Though for some purposes frequencies in the 10 to 100 megacycle range may be used, the most common frequency now used is about 9 gigacycles (Gc.) (9000 megacycles) per second, which is in the microwave region of the radiofrequency spectrum. Calculation from Eq. 4 shows that the corresponding magnetic field strength is of the order of 3000 gauss.

INSTRUMENTAL

In gross schematic structure, the ESR spectrometer consists of the magnet, the microwave circuit, and electronic equipment for regulation, detection, and recording. A block diagram of a simplified spectrometer is shown in Fig. 3. The microwave source is a reflex klystron oscillator which sends 9 Gc. per second power by way of a pipe of rectangular cross section called a wave-

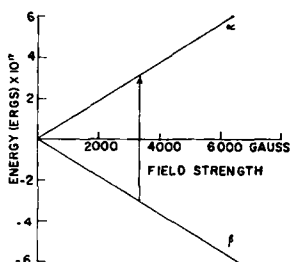


Fig. 2. — Energies of the two states of a free electron as a function of magnetic field strength.

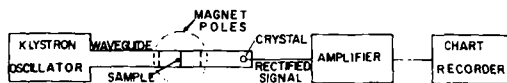


Fig. 3.—Schematic diagram of a simplified ESR spectrometer.

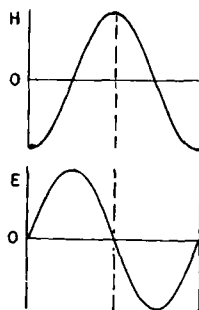


Fig. 4.—Standing - wave amplitudes in a full-wavelength resonant cavity as a function of position along the axis of the cavity. The ordinate of the upper curve is the magnetic field strength; that of the lower curve is the electric field strength.

guide into a resonant cavity through a circular iris of about 0.5-cm. diameter. The cavity may be a walled-off section of waveguide, but it is often an especially constructed chamber with built-in field-modulation coils, special fittings for support of the sample, etc. The source frequency must be carefully adjusted to be exactly resonant with the cavity—a condition such that there are an integral number of half-wavelengths in the cavity. A second iris through the far wall of the cavity permits a fraction of the power to escape and be detected by a silicon crystal rectifier, and the magnitude of the exit power is registered on a recording voltmeter. Because of the strict frequency requirement for resonance⁵ in the cavity, it is not possible to obtain an ESR spectrum by varying the frequency. According to the conjugate nature of frequency and magnetic field strength (Eq. 4), the same purpose is served by varying the magnetic field at a fixed frequency.

A practical spectrometer varies in numerous respects from the above, but it has as its main characteristics the following components: an electromagnetic oscillator, a resonant circuit containing the sample within the magnetic field, and provisions for detecting, amplifying, and indicating the power absorbed by the sample.

The position of the sample is a critical factor in the sensitivity of the spectrometer. Ideally, one desires to absorb as much magnetic energy in the sample as possible with no power lost in other ways. Thus, the most favored position for the sample is at the maximum (loop or antinode) of the magnetic standing wave in the cavity. Because the electric and magnetic waves are 90°

⁵ The word resonance is used in this article in two separate senses. In this section, simple instrumental resonance is used as resonance between the source frequency and one of the natural frequencies of the cavity. As used in the name of the phenomenon the word resonance implies the condition that the photons of the source frequency have the exact energy to effect the desired magnetic transition.

out of phase, the antinode of the oscillating magnetic field is at the same position as the node of the electric field, as shown in Fig. 4. As the dielectric loss in matter due to oscillating electric dipoles is proportional to the oscillating electric field strength, maximum magnetic absorption and minimum electric absorption occur at the same position.

Of course, the position of the node is merely a mathematical point, so that any actual physical sample would overlap less ideal positions. For many substances, a small amount of overlap is no disadvantage. However, some samples, particularly aqueous solutions, are so lossy that they tend to kill the oscillation in the cavity and must be inserted in capillary tubes or in thin flat quartz cells carefully aligned in the electric nodal plane.

The presentation of the spectrum may take many forms. It could be presented as a graph of the point-by-point d.-c. voltage output of the crystal rectifier as a function of the magnetic field. An automatic device for linear sweeping of the magnetic field together with a recording voltmeter would produce such a graph, as shown in Fig. 5A.

To increase the signal-to-noise ratio, many ESR spectrometers use a device known as a phase-sensitive (lock-in or coherent signal) detector. This device is capable of eliminating extraneous signals and noise voltages by accepting only an alternating voltage of the same frequency and phase as some constant-frequency reference voltage.

An alternating magnetic field is superimposed on the slowly swept magnetic field by means of auxiliary coils parallel to the pole faces of the magnet, referred to as field modulation coils. The frequency of the field modulation is arbitrary, and spectrometers exist in which it is as low as 30 cps. and as high as 1 megacycle. The voltage producing this field is connected to the reference input of the phase-sensitive detector and operates a gate to turn the detector on and off in synchrony with the alternating output voltage from the crystal detector. The output of this device is illustrated in Fig. 5B for the sample whose absorption spectrum is shown in Fig. 5A. As the external magnetic field is slowly swept through the absorption curve of Fig. 5A, the field modulation imposes an alternating field, indicated by the sinusoidal wave form. The crystal receives a d.-c. voltage according to the height of the curve, upon which is superimposed an alternating voltage whose magnitude depends upon the steepness of the curve. The phase-sensitive detector responds only to the alternating voltage, which is

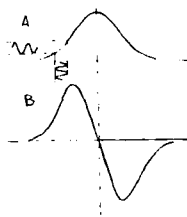


Fig. 5.—Key: A, ESR absorption spectrum of a substance showing no hyperfine structure; B, ESR derivative spectrum resulting from use of field modulation and phase-sensitive detection. The abscissa is magnetic field strength; the ordinate is voltage output at the recording voltmeter. The vertical sine wave represents the field modulation of the magnetic field; the horizontal sine wave is the resulting alternating output voltage.

then rectified. The voltage recorded at each point of the abscissa has a value proportional to the derivative of Fig. 5A at the same H value, as shown in Fig. 5B. This curve is often referred to as a derivative spectrum.⁶

Though the novice may find it difficult to interpret such a curve, with experience it yields more information to the eye than does the original absorption curve, at least in complicated spectra with overlapping of individual lines. This is because subtle changes of the slope are exaggerated in the derivative curve. Some workers have carried this procedure one step farther to the second derivative and claim even better interpretability (17).

A limiting factor in any sensitive instrument is the amount of interference arising from extraneous sources. Electronic instruments utilize grounding, shielding, and regulating techniques to reduce stray pickup; yet every element which emits or carries electric power is an internal source of thermal noise. The klystron oscillator is the most important noise source in a modern ESR spectrometer, and the sensitivity is primarily determined by this tube (18). Since the noise should average out to zero, an average signal taken over a time long enough should give a spectrum of any desired signal-noise ratio, and most electronic instruments are equipped with a low-pass filter of variable time constant for this purpose. To extend the time constant beyond a few seconds, however, has many practical disadvantages and requires an instrument of unattainable stability. An ingenious technique used by Commoner and co-workers (19) takes advantage of the averaging effect by recording the signal from identically prepared samples on the same chart 10 to 15 times. Though in a single sweep it is difficult to distinguish the signal from the noise, the repetition produced a composite

⁶ Strictly, the derivative spectrum is found only in the limit as the field modulation approaches zero.

pen track of breadth equal to the noise voltage upon which the signal appears unambiguously.

Electronic devices based on the same principle store the information from successive sweeps, reproducing the average after completion of some hundreds of sweeps (20, 21). Figure 6 compares an ESR spectrum by Klein and Barton (20) of a dilute solution of manganous ion in one sweep with an average of 5000 sweeps over 7 hours.

CHARACTERISTICS OF A SPECTRUM

The absorption spectrum of a free electron appears as a single spike centered at field strength corresponding to that calculated from Eq. 4, with $g = 2.0023$. Spectra of unpaired electrons in atoms and molecules vary from this for several reasons.

Line Shift and g Value.—As discussed in a previous section, interaction with the orbital angular momentum results in a variation of g by spin-orbit coupling. To borrow a term from NMR, this is a kind of chemical shift, as the deviation from the free spin value depends upon magnetic effects in the environment.

Line Broadening.—For precision in the information to be obtained from a spectrum, the line width must be as small as possible. There are several causes of broadening in lines, a knowledge of which is necessary for rational study of ESR spectra.

Some line broadening arises from purely instrumental causes. Lack of homogeneity in the magnetic field, producing a different field strength at different parts of the sample, would produce broadening for obvious reasons. Another common cause of broadening is the use of large values of field modulation alternating field strength. It is clear from inspection of Fig. 5 that if the amplitude of the field modulation is of the order of magnitude of the line width itself, an apparent broadening of the line will occur. Sometimes this may be necessary, however, as increase of the field modulation is one way of increasing the sensitivity; and if the sample is very dilute in paramagnetic solute, a compromise is necessary between detection of the free radical and characterization of its true spectrum.

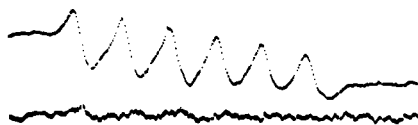


Fig. 6.—ESR spectrum of an aqueous solution containing 8×10^{-11} moles of manganous ion in the spectrometer cell. The lower curve is the direct output of the spectrometer; the upper curve is an electronic average of the same sample as a result of 5,000 traverses.

The presence of neighboring paramagnetic molecules alters the local magnetic field strength. This broadens the spectral line in part because of the statistical deviations of the field owing to momentarily different environments. This effect is called "dipole broadening" or "spin-spin broadening." Dilution of the solution will result in narrower lines by increasing the average distance between molecules.

In complicated organic free radicals at low concentration, the ESR spectrum will often be a single broad line. This broadening could be caused by failure to resolve hyperfine splitting (see *Hyperfine Structure*). If the spectrum consists of a series of narrow lines, large field modulation may wash out this structure by the mechanism discussed above.

The breadth of an ESR spectrum line is actually the result of a complex combination of causes, both intramolecular and environmental. The ultimate line width is related to the lifetime of a magnetic state. Any effect which facilitates energy coupling between the spin center and the environment leads to broadening; any effect that tends to isolate it leads to narrowing. For further information, one should consult the literature on the subject of the spin-relaxation process (3).

Fine Structure.—Students of atomic spectroscopy will recall that fine structure pertains to the separation of electronic energy levels of the same principal quantum number and different angular momentum quantum number (14). Fine structure appears in ESR spectra only in the gaseous and crystalline states. For further discussion, one should refer to the pertinent literature (5, 10–12).

Hyperfine Structure.—An important characteristic of an ESR spectrum arises owing to the presence of nuclei having magnetic moments within the orbital of the unpaired electron. Like that of the electron, the spin of a hydrogen nucleus can be oriented in only two directions. In one such orientation, it adds its field to that of the external field, while in the other it subtracts. Thus, if the electron lies very close to this nucleus, an applied field measurably smaller (or larger) than for a free electron is required to bring the electron to its resonance value. The energy diagram of Fig. 2 may be amended to contain this information. A single proton acting as a magnet will add or subtract a definite amount to the applied field, regardless of the value of the latter. Figure 7 shows two values of H at which resonance occurs at a given frequency—one lower and one higher than that expected in the absence of the proton. Since there will be an

approximately equal number of protons in each of the two states, it will give rise to a doublet symmetrically disposed around the basic line position. This is the simplest case of hyperfine splitting and is analogous to spin-spin splitting in NMR.

It was noted above that two orientations are permitted the hydrogen nucleus. This is a consequence of the nuclear spin quantum number $I = 1/2$, exactly analogous to the rule outlined for the electron with $S = 1/2$. Other nuclei having other values of the nuclear spin quantum number will conform to the rule that the only nuclear orientations permitted are those in which $M_I = \pm I$ and values smaller in magnitude by integers, where M_I is the projection of I along the magnetic field direction. For nitrogen-14, for which $I = 1$, M_I is permitted the values $+1$, 0 , -1 , leading to a three-peak spectrum. Since one-third of the atoms in a given sample will be in each such state, the three lines will be of equal intensity.

A striking example of such a spectrum is that found for the free radical divalent anion, peroxyamine disulfonate, obtained as the potassium salt, as shown in Fig. 8. The splitting is caused by the nitrogen-14 in the molecule.

Values of the nuclear spin quantum number depend upon both the atomic number and the atomic weight, *i.e.*, both on the number of protons and neutrons. Different isotopes of the same element will thus have different I values. Some values for common nuclides are listed in Table I.

Elements with even mass numbers (total of protons and neutrons) have integral values of I , those with odd mass numbers have half-integral values, and nuclei with even atomic number and even atomic mass number have zero spin. The latter is a result of the pairing of the spins of the respective protons and neutrons.

Table I shows that in an organic compound containing ordinary carbon, hydrogen, and oxygen, only the hydrogen will produce hyperfine splitting. However, splitting due to the small naturally occurring fraction of carbon-13 is sometimes observed (see below).

PRACTICAL AND THEORETICAL APPLICATIONS

Quantitative Analysis.—The use of ESR as a quantitative measure of free radical concentration is based on the fact that the integral of the absorption spectrum is proportional to the number of spin centers within the cavity (3). For spectrometers with first-derivative presentation, this proportionality may be utilized only by performing a double integration on the raw

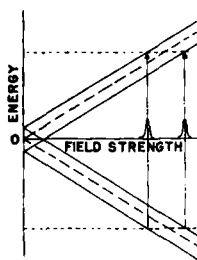


Fig. 7.—Energies of the two magnetic states of an electron as a function of magnetic field strength showing hyperfine splitting by a nucleus with $S = 1/2$. The scale of the splitting is exaggerated.

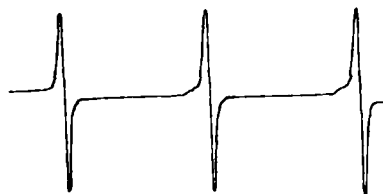


Fig. 8.—ESR spectrum of potassium peroxyamine disulfonate dissolved in an aqueous ammoniacal solution.

spectrum and comparing it with a similar integration for a standard of known titer. Such computations are time consuming and, for reasons listed below, are often of questionable accuracy. Electronic integrators (22) and other computational aids (23) may be used to facilitate the integration, but even these yield only order-of-magnitude results. The inaccuracy of all integration processes as applied to ESR arises from at least two basic causes: (a) the instability of the spectrometer, which appears as baseline drift of the integrated spectrum and (b) the difficulty of resolving hyperfine structure in a time sufficiently short to obtain an accurate integral. A third cause of inaccuracy occurs, particularly in lossy solutions, if the sample must be removed from the cavity to insert the standard whose dissipative character may be quite different. This difficulty may be avoided by using a double cavity capable of accommodating the two samples and displaying their spectra at the same time (24).

For many purposes only relative concentration of a single substance is required. In this case, the maximum height of the derivative signal serves as a proportional measure of concentration (25).

A standard substance useful and almost indispensable in ESR spectroscopy is 1,1-diphenyl-2-picrylhydrazyl (DPPH) which can be prepared as a polycrystalline solid in the free radical form. In this form, its spectrum is a single peak of about 3 gauss width and a g value of 2.0036 (3). Its line width varies somewhat with crystal size, evidently connected with the surface adsorption of paramagnetic oxygen (26–28). It is stable for months in the solid state but undergoes a few

per cent decay in a year. In dilute solution in benzene and other solvents, it exhibits a spectrum of five lines with line separation of about 10 gauss, a result of hyperfine splitting by the two nearly equivalent nitrogen nuclei.

Other standards of free spin which have been proposed and used include manganous ion (29), ruby crystal (30), and carbonaceous material (31); these are useful for particular applications but have certain practical disadvantages.

Qualitative Analysis and Chemical Structure.—As discussed earlier, there are three important characteristics of an ESR spectrum by which it can be identified—*viz.*, its g value, its line width, and its hyperfine structure. Each of these can play a part in the qualitative identification of a paramagnetic entity and furnish clues to its chemical nature.

As a simple example, manganous ion in an aqueous solution can be readily identified by observation of its six-line spectrum of 100-gauss separation (32) (see Fig. 6). With methods beyond the scope of this discussion, the electronic structure of coordinated complexes and chelates of transition-group ions may be deduced by study of the details of the hyperfine structure (33).

The free radical produced by electric discharge through methane was identified as the methyl radical by its ESR spectrum, consisting of four symmetrical hyperfine lines of intensity ratios 1:3:3:1 (34). Four lines would be expected from a free radical with three equivalent protons from the multiplicity rule, $2I + 1$, since the total nuclear spin of three aligned protons would be $I = 3/2$. The relative intensities of the lines are computed from the statistical weights of the four possible combinations. There is only one permutation for which all of the nuclear magnets point to the north (or the south) pole of the ex-

ternal magnet, which corresponds to $M_I = \pm 3/2$. Reversal of any one of the three protons will yield the result $M_I = \pm 1/2$. Since each permutation is equally probable, the statistical weight of $M_I = 1/2$ is three times that for $M_I = 3/2$. While it is true that most organic free radicals do not have the structural simplicity of the methyl radical, their spectra sometimes do. From the fact that the ESR spectrum of a free radical is a symmetrical triplet of intensity 1:2:1, it is deduced that the unpaired electron is localized on a group containing two equivalent protons (35).

The methods of analysis illustrated above can be extended only in principle to more complex organic and biological free radicals. However, like the infrared spectrum, common features in two spectra are to be associated with similar structural features. Ingram (3) cites at length the work of Gordy *et al.* (36), who showed the similarity of the ESR spectra of free radicals from a number of animal proteins with that of cystine. Schieser (37) has recently pointed out common features in the spectra of free radicals formed in the Marquis test (and other color tests) on various alkaloids. He concludes that the site of the free radical is a phenolic moiety common to all the compounds studied.

Electron Density in π -Radicals.—Free radicals formed by oxidation or reduction of aromatic molecules owe their stability to the participation of the unpaired electron in the aromatic resonance. In the language of the quantum theory, it is in a π -orbital. This is confirmed by the ESR spectra of such radicals, which show hyperfine splitting by all of the ring hydrogens. Moreover, hyperfine splitting is also contributed by the protons of methyl groups attached directly to the ring, which is a direct verification of hyperconjugation (3).

A quantum mechanical analysis of the aromatic ring shows that the hyperfine splitting by a ring proton is directly proportional to the unpaired electron density (the square of the wave function) at the carbon atom to which it is attached (38). Hyperfine splitting calculated from this theory is in good agreement with experimental values (39). Electron densities of both positive and negative radical ions of a number of aromatic hydrocarbons have been computed (40).

Hyperfine splitting in aromatic radicals attributed to the 1% of carbon-13 in natural carbon has been observed in certain spectra with narrow well-resolved lines (41). Figure 9 shows the ESR spectrum of dibenzo-*p*-dioxin (42). The five principal lines of intensity 1:4:6:4:1 arise from hyperfine splitting by one set of four equiv-

TABLE I.^a—NUCLEAR SPIN QUANTUM NUMBERS OF SEVERAL COMMON NUCLIDES

Element	At. No.	Mass No.	I
Hydrogen	1	1	$1/2$
Hydrogen-2 (deuterium)	1	2	1
Helium	2	4	0
Carbon-12	6	12	0
Carbon-13	6	13	$1/2$
Carbon-14	6	14	0
Nitrogen	7	14	1
Oxygen-16	8	16	0
Oxygen-17	8	17	$5/2$
Oxygen-18	8	18	0
Fluorine	9	19	$1/2$
Sodium	11	23	$3/2$
Vanadium	23	51	$7/2$
Manganese	25	55	$5/2$

^a Sullivan, W. H., "Trilinear Chart of Nuclides," Oak Ridge National Laboratory, 1957. See this or other sources for a complete list.

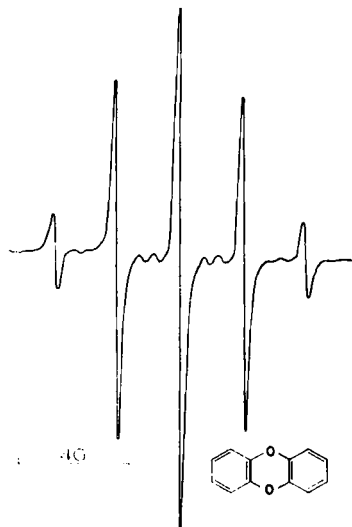


Fig. 9.—ESR spectrum of dibenzo-*p*-dioxin, showing hyperfine splitting by four equivalent protons together with C^{13} splitting by two sets of four equivalent carbon atoms. The C^{13} is that naturally present in 1% abundance.

alent protons. The satellite lines are of the proper intensity to be ascribed to splitting by two sets of four equivalent carbon-13 atoms in their natural abundance. These observations are related to the local electron densities in a way more complicated than the proton splittings (43).

Reaction Kinetics.—Limited as it is to the detection of free radicals and other paramagnetic substances, ESR cannot by itself give direct results of kinetics and mechanisms of reactions. Used with other spectrographic instruments, it is a powerful auxiliary for study of those reactions involving free radicals of reasonably long lifetimes. Its use in this connection may be enhanced by the development of the following accessory techniques: low-temperature measurements, methods for electrolytic generation of free radicals within the instrument, and rapid mixing and flow techniques. All of these have the effect of facilitating the observation of free radicals of short lifetimes.

In direct application to kinetic problems, ESR is used to measure the free radical titer according to the rules outlined in *Quantitative Analysis*. Used in this way, it has had few applications in simple organic systems. This is in part owing to the short lifetimes of most structurally simple free radicals at their usual reacting temperatures.

Of more than casual interest to pharmaceutical chemists, phenothiazine and its derivatives form long-lived free radical intermediates upon oxidation. Borg and Cotzias (44) have carried out studies on chlorpromazine and related sub-

stances in the presence of certain inorganic ions by optical spectroscopy, supplemented by ESR data. Tuck and Tozer (45), using ESR supplemented by optical spectrophotometry, studied the decay in acidic solutions of the free radical of phenothiazine and a number of its substituents and have analyzed the mechanism of the decay process. Piette *et al.* (46) have studied the rate of peroxidase catalyzed oxidation of chlorpromazine.

In the field of polymerization, in which the free radicals are stabilized by trapping in solid or viscous media, ESR promises to play an important part in kinetic studies, as suggested by Whiffen (47). In some recent studies it has been used as an auxiliary technique in polymer rate studies (48).

Rates of rapid electron exchange reactions involving a free radical may be estimated by a broadening effect on its spectrum when mixed with the other reactant. Ward and Weissman (49) showed that the complex spectrum of the naphthalene free radical ion lost its resolution as a result of an increase of line width when mixed with unreduced naphthalene. In 0.8 *M* solution in tetrahydrofuran, they estimated the half-life of the individual free radicals to be of the order of a microsecond. This is analogous to the well-known proton exchange effect observed in NMR spectra of substances undergoing keto-enol tautomerism (50).

Biological Applications.—Early in its development, Commoner *et al.* (51) pioneered the application of ESR to biological systems when they packed their rather insensitive instrument full of lyophilized samples of some 22 varieties of vegetable and animal life and proved that they contained paramagnetic matter. As the sensitivity of their instrument was improved, more difficult problems were tackled. The year 1957 brought two exciting developments: Commoner and his staff (19, 52) showed the presence of free radicals in aqueous media containing enzymes, and Calvin and Sogo (53) showed that the free radical concentration in photosynthetic processes responded to the intensity of light. Other workers were close behind (54). Since that time, both of these areas have had continued activity (8, 55, 56), some of which could well have been included in the section on *Reaction Kinetics*.

It must be mentioned here that ESR spectra of enzymic and other biological material (as well as those of polymeric radicals) usually depart from the precise symmetry found in simpler molecular compounds. This is to be associated with two important causes—*viz.*, the simultaneous existence in solution of two or more free radicals

with different g values and the colloidal nature of macromolecular systems, such that the molecular tumbling which averages out spectral anisotropy is inhibited.

Of fundamental importance in biology and nuclear medicine are studies of the effects of ionizing radiation on biochemical substances. The laboratory of Gordy has produced many such studies concerning the effects of X-rays and gamma-rays on native proteins (57) and on other polyamino acids (58). These studies have shown that free radicals are formed as the primary effect of irradiation, and the detailed spectra give direct information about the structure of the free radical and its orientation. Studies of the effect of radiation on biological systems have been carried out (59).

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